Biota Neotropica 19(2): e20180625, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Plasticity in the timing of activity in the Red-rumped Agouti, *Dasyprocta leporina* (Mammalia: Rodentia), in the Atlantic Forest of southeastern Brazil

Laura Martins Magalhães[1] & Ana Carolina Srbek-Araujo[1,2]*

[1]Universidade Vila Velha, Programa de Pós-Graduação em Ecologia de Ecossistemas, Rua Comissário José Dantas de Melo, 21, Boa Vista, 29102-920, Vila Velha, ES, Brasil
[2]Instituto SerraDiCal de Pesquisa e Conservação, Belo Horizonte, MG, Brasil
**Corresponding author: Ana Carolina Srbek-Araujo, e-mail: srbekaraujo@hotmail.com*

MAGALHÃES, L.M., SRBEK-ARAUJO, A.C. **Plasticity in the timing of activity in the Red-rumped Agouti, *Dasyprocta leporina* (Mammalia: Rodentia), in the Atlantic Forest of southeastern Brazil**. Biota Neotropica. 19(2): e20180625. http://dx.doi.org/10.1590/1676-0611-BN-2018-0625

Abstract: Timing of activity is a consequence of adaptations to daily and seasonal changes in the environment and examining these patterns is important to better understand the temporal niches of the species. Here we examine temporal activity in the Red-rumped Agouti (*Dasyprocta leporina*) in two fragments of Atlantic Forest and those factors that influence the circadian rhythm in the study areas. Camera traps were used to gather data in two protected areas (one montane and other coastal) in the state of Espírito Santo, southeastern Brazil. A total of 49 photos were taken in the mountains and 152 in the coastal area. Activity patterns were diurnal and bimodal, and animals were active for 14–15 hours each day. Activity peaked in the morning soon after sunrise and then before and during dusk. Activity patterns were different in the two study areas (W = 6.77, p = 0.034). There was a longer peak in the morning in the coastal area, and a higher peak in the evening in the mountains, where activity starts later and becomes less intense earlier. The difference between activity patterns in the two locations suggests plasticity in agouti behavior. Because the two study areas are at about the same latitude, we suggest that the behavioral plasticity is due to different temperature regimes associated with a local effect of altitude and topography of the terrain on the incidence of solar rays inside the forest. The influence of other factors is also discussed. In addition to a better understanding of the temporal niche of the Red-rumped Agouti, the behavior patterns we describe here can be useful to optimize strategies for *D. leporina* conservation in southeastern Brazil once the species is more susceptible to poaching at times when animals are most active.

Keywords: *Dasyproctidae, abiotic factors, activity patterns, circadian rhythm, photoperiod.*

Plasticidade comportamental no horário de atividade de cutia, *Dasyprocta leporina* (Mammalia: Rodentia), na Mata Atlântica do sudeste do Brasil

Resumo: O horário de atividade reflete adaptações e respostas das espécies às variações diárias e sazonais do ambiente, sendo o entendimento destes padrões importante para uma melhor compreensão do nicho temporal das espécies. Este trabalho objetivou caracterizar o horário de atividade da cutia (*Dasyprocta leporina*) em dois remanescentes de Mata Atlântica e estabelecer os fatores que podem interferir no ritmo circadiano da espécie considerando peculiaridades das áreas amostradas. Foram analisados dados obtidos a partir de armadilhas fotográficas em duas áreas protegidas, sendo uma na região serrana e outra próximo à costa, ambas no estado do Espírito Santo, sudeste do Brasil. Foram obtidos 49 registros na região serrana e 152 registros na área costeira. A espécie apresentou padrão de atividade diurno e bimodal, mantendo-se ativa por 14-15 horas. Foi detectado um pico de atividade matutino, logo após o nascer do sol, e outro vespertino. O padrão de distribuição diária dos registros foi diferente entre as áreas amostradas (W = 6,766; p = 0,034), ressaltando que o pico de atividade matutino apresentou maior duração na área costeira, enquanto o pico vespertino foi proporcionalmente mais representativo na região serrana, onde as atividades se iniciam mais tarde e se tornam menos intensas mais cedo. Estas diferenças evidenciam a ocorrência de plasticidade comportamental em *D. leporina* no que se refere ao padrão de atividade local. Entretanto, a diferença entre as áreas estudadas não pode ser atribuída à latitude, uma vez que a distância entre as localidades é de menos de um grau. Sugere-se que a variação do horário de atividade de cutias nas áreas amostradas seja devido a diferenças na temperatura ambiente, associadas a variações locais decorrentes do efeito da altitude e da topografia do terreno na incidência de raios solares no interior da floresta. A influência de outros fatores também é discutida. Além do melhor entendimento do nicho temporal de cutias, os padrões comportamentais descritos no presente estudo podem ser úteis para otimizar estratégias de conservação de *D. leporina* no sudeste do Brasil considerando que a espécie é mais suscetível à caça quando os animais estão mais ativos.

Palavras-chave: *Dasyproctidae, fatores abióticos, fotoperíodo, padrão de atividade, ritmo circadiano.*

Introduction

Circadian rhythms are intervals of approximately 24 hours during which the vital biological processes that maintain organisms occur (Goldman 1999) and are part of the suite of adaptations of species to their environments. These rhythms are physiological and behavioral adjustments in response to changes in environmental conditions that are consequence of the day-night cycle (Beltran & Delibes 1994, Goldman 1999). Thus, patterns of sleep-vigilance, variable body temperature, hormone and enzyme secretions and regulation of cell cycles (among others) are consequences of circadian rhythms (Kronfeld-Schor & Dayan 2003, Pita et al. 2011). These patterns are due to as biological clocks that provide the organisms with the mechanisms to anticipate and prepare for daily changes that occur regularly in the environment (Goldman 1999).

Timing of activity is one of the expressions of the circadian rhythm and is part of a species' natural history and their manifestations have ecological and evolutionary significance (Kronfeld-Schor & Dayan 2003). This is due to the fact that patterns of activity are molded by selective forces (e.g., ecological interactions) and evolutionary constraints (e.g., functional and morphological constraints, genetic variability), that are the basis of time partitioning among species and that, in turn, may allow or not species coexistence (Kronfeld-Schor & Dayan 2003).

While daily activity patterns tend to be ubiquitous in terrestrial mammals, some plasticity is often found in response to environmental variations. Activity patterns may be influenced by abiotic factors, such as light (photoperiod), moon cycle, temperature, precipitation and latitude, and by biotic factors, such as food availability, interactions with competitors and risk of predation (e.g. Bronson 1988, Beltran & Delibes 1994, Goldman 1999, Kenagy et al. 2002, Kronfeld-Schor & Dayan 2003, Wagner et al. 2008, Pita et al. 2011, Suselbeek et al. 2014, Sassi et al. 2015). Thus, examining how organisms respond to daily and seasonal variation in the environment and plasticity in behaviors allows us to better understand patterns of community structure, species coexistence, resource partitioning and predator avoidance (Kronfeld-Schor & Dayan 2003).

Agoutis (genus *Dasyprocta* Illiger, 1811) are medium-sized terrestrial rodents whose diet mainly comprises fruits, seeds and roots (Oliveira & Bonvicino 2006). They often collect and bury fruits and seeds in holes they excavate, and thus store for later consumption. Because they do not always recover stored seeds and fruits, they are also important in seed dispersal (Galetti et al. 2006, Oliveira & Bonvicino 2006). Agoutis are threatened in some localities due to illegal hunting (Cullen Jr. et al. 2000), and their population decline results in reduced seed dispersal and negative consequences for plant species whose seeds are dispersed by agoutis (Galetti et al., 2006).

Here, we examine daily activity patterns of the Red-rumped Agouti, *Dasyprocta leporina* (Linnaeus, 1758), in two Atlantic Forest fragments in southeastern Brazil. Also, by comparing patterns in the two fragments, we infer factors that may generate plasticity in the timing of agouti activity patterns.

Material and Methods

1. *Study area*

The study took place in two protected areas in the state of Espírito Santo. One in the mountainous central-southern region of the state, the Santa Lúcia Biological Station (Estação Biológica de Santa Lúcia - EBSL, 19º57'-19º59' S and 40º31'-40º32' W), in the municipality of Santa Teresa. The other, in the northeastern coastal region of the state, the Vale Natural Reserve (Reserva Natural Vale - RNV, 19º06'-19º18' S and 39°45'-40º19' W), in the municipality of Linhares (Figure 1).

The EBSL comprises ca 440 ha and with adjacent private lands they form a forest fragment of 900 ha (Srbek-Araujo & Chiarello 2005). This fragment includes primary forest that is predominantly Ombrophilous Dense Forest (Thomaz & Monteiro 1997). It is a mountainous region and elevation varies from 550 to 950 m (Srbek-Araujo & Chiarello 2005). Annual temperature averages 19.9ºC (Thomaz & Monteiro 1997), varying between an average minimum of 16.5ºC and average maximum of 22.8ºC (Álvares et al. 2013). Annual rainfall is 1,868 mm (Thomaz & Monteiro 1997). Climate, following Köppen's classification, is humid subtropical with temperate summer (Cfb, Álvares et al. 2013). A network of trails allows access to different areas in the reserve.

The RNV comprises 22,711 ha and is connected to the Sooretama Biological Reserve (Reserva Biológica de Sooretama; 27,859 ha) and two other smaller, private, protected areas (Reserva Particular do Patrimônio Natural – RPPN Recanto das Antas and RPPN Mutum-preto; Srbek-Araujo & Chiarello 2017). Together they form a remnant of natural habitat of ca 50,000 ha (Linhares-Sooretama Block) and comprise more than 10% of the remaining Atlantic Forest in the state (based on data available in FSOSMA & INPE 2017). Most of the RNV is dense lowland forest (Tabuleiro forest), classified as Perennial Seasonal Forest (Jesus & Rolim 2005). Here the topography is relative flat, varying from 28 to 65 m above sea level (Jesus & Rolim 2005). Average annual temperature is 24.3°C, with average minimum of 18.7ºC and average maximum of 29.9ºC (Kierulff et al. 2015). Annual rainfall is 1,214 mm and varies widely year to year (Kierulff et al. 2015). Climate here, following Köppen's classification, is tropical with a dry winter (Aw; Alvares et al. 2013). The RNV has a network of unpaved roads that permit access to various parts of the reserve.

2. *Data collection*

To record the activity of *D. leporina*, all data were gathered using camera traps (hereafter, simply 'camera'). Because animals have to be active to trigger photographs, we assumed that the capture is indicative of the activity of the species being photographed, then capture rate is proportional to the level of activity in each place (Srbek-Araujo & Chiarello 2013) as well as at each time of day (Rowcliffe et al. 2014). While the model of camera may differ in sampling efficiency (Srbek-Araujo & Chiarello 2007), we also assumed that difference will not affect the timing of the photographs obtained for the same species.

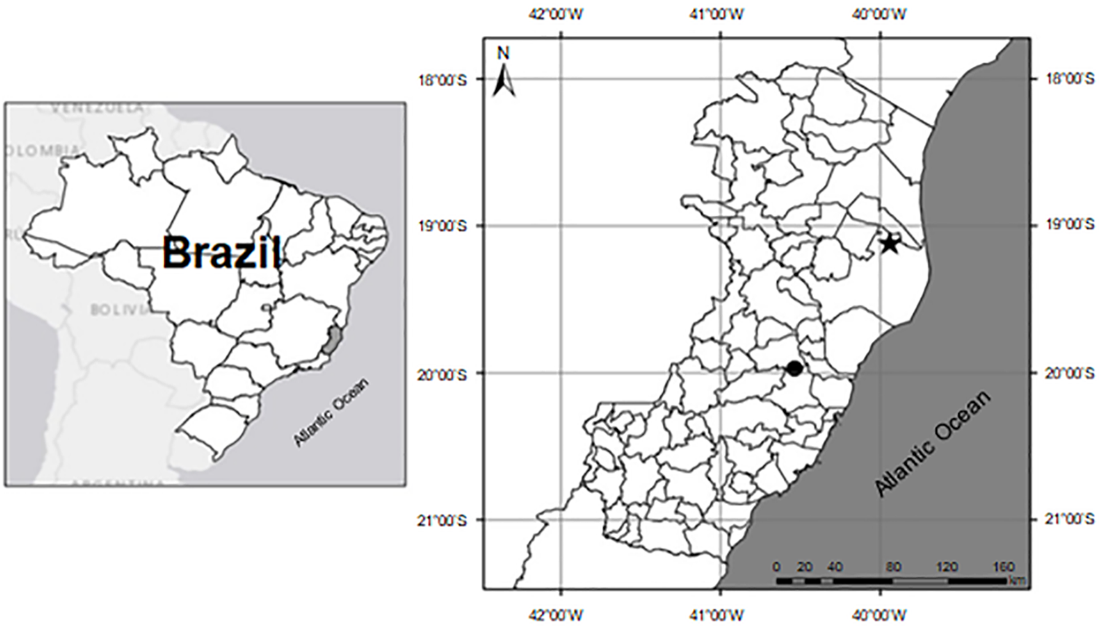


Figure 1. Map illustrating the locations of the study areas in southeastern Brazil, with a circle indicating Santa Lúcia Biological Station and a star indicating Vale Natural Reserve, in the state of Espírito Santo.

In the EBSL, cameras were in the field from February 2002 to January 2004 (~24 months). From February to November 2002 we used three Wildlife Pro Cameras (Foresty Suppliers Inc., USA), after which we used an additional three Deer Cam – Scouting Cameras (Non Typical Inc., USA) for a total of six cameras during that time interval. Cameras were setup on trails and over a total of 20 independent and widespread points within the EBSL. Cameras were moved monthly between sampling points.

In the RNV, cameras were installed from August 2007 to October 2008 (~14 months). At this time, we used 10 Tigrinus cameras (conventional model; Tigrinus Research Equipment, Brazil). Cameras were setup along trails 500 m from the nearest internal road. Cameras were placed in 10 independent and distant sampling points in different parts of the RNV and all 10 were sampled throughout the study period.

All cameras were set to function 24 hours/day, with an interval of 20 s between triggered events, and were loaded with 200 ISO 35 mm color negative films with 36 exposures. The cameras were set to stamp the date and the time of the record on each photo. Solar time was always used for the time of day. We checked cameras once each month for general maintenance and collecting records. We did not use bait and placed cameras about 45 cm above the ground attached to tree trunks. For details of camera protocol see Srbek-Araujo & Chiarello (2005, 2013).

3. *Data analysis*

To avoid double counting of photographs related to the same capture event and insure independence of records, at each sampling point we counted only the first capture of *D. leporina* when more than one record was obtained within 1 hour interval (= independent record). Thus, a new, independent observation was separated from the previous observation by at least one hour (following Srbek-Araujo & Chiarello 2013).

We summed independent records in hourly intervals to characterize the activity pattern at each reserve. We then calculated the percent of records obtained at each one-hour interval to highlight differences related to the pattern of records distribution, rather than differences resulting from the distinct number of records obtained per locality. We defined an activity peak as when the percentage of captures in any given hour was greater than half that of the hour with the greatest percent of captures.

To compare activity patterns between the two study areas, we used Mardia-Watson-Wheeler test (circular statistics). This test uses the exact time of each capture as an independent input for the analyzes. We used the program Oriana (version 4.0; Kovach Computing Services 2009), and alpha for tests was set at 0.05 (Zar 2010).

Results

In a total of 201 independent agouti captures, 49 were from EBSL and 152 from RNV. Animals were active during about 15 hours throughout the day and are clearly diurnal (Figure 2). The first capture was at 04:52 h and the last at 18:50 h (Table 1). Activity was bimodal, with the first activity peak in the morning, between 05:00 h and 08:00 h, and the second peak in the afternoon to dusk, from 14:00 h to 17:00 h (Table 1, Figure 2).

Agoutis had a total of about 14 hours of activity in the EBSL, most of which occurred during two peaks. The morning peak was at 06:00 h and the afternoon peak at 14:00 h–15:00 h (Table 1, Figure 2). No agoutis were captured here between 08:56 and 10:32 h (Figure 2). Agouti activity lasted 15 hours, also with two peaks, at RNV. The morning peak was between 05:00 h–08:00 h and the evening peak between 16:00 h–17:00 h (Table 1, Figure 2). In both areas, activity declined greatly

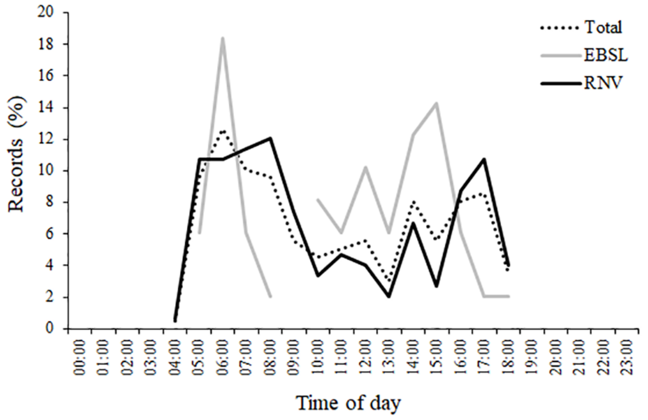


Figure 2. Hourly records of the Red-rumped Agouti (*Dasyprocta leporina*) indicating the percent of the total activity and that at the Santa Lúcia Biological Station (EBSL) and the Vale Natural Reserve (RNV) in southeastern Brazil.

Table 1. Timing of activity of the Red-rumped Agouti (*Dasyprocta leporina*) overall (Total) and by study area (Santa Lúcia Biological Station – EBSL, Vale Natural Reserve – RNV) in southeastern Brazil.

Event	Total	EBSL	RNV
First record	04:52	05:01	04:52
Last record	18:50	18:50	18:49
First peak activity - morning	05:00-08:00	06:00	05:00-08:00
Second peak activity - afternoon	14:00-17:00	14:00-15:00	16:00-17:00

after the morning peak and the afternoon peak was preceded by two small groupings of records (Figure 2). Agoutis were only captured three times at night (19:48 h, 20:06 h, 23:53 h) all in the RNV.

Agouti activity patterns differed between the two study areas (W = 6.77, p = 0.034). The morning peak was longer in the RNV, while the afternoon peak was higher in the EBSL, where activity starts later and becomes less intense earlier (Figures 2 and 3).

Discussion

The Red-rumped Agouti has a very clear diurnal and bimodal pattern of activity in both study areas. The two intervals with the most activity, after sunrise (morning) and then before and during dusk, are similar to the daily activity patterns of agoutis in different parts of the Americas (Oliveira & Bonvicino 2006, Lambert et al. 2009, Norris et al. 2010, Suselbeek et al. 2014, Cid et al. 2015, Ferreguetti et al. 2018). This pattern differs from that observed in Ecuador (Blake et al. 2012) and Panama (Duquette et al. 2017), where the activity pattern was unimodal. While the general bimodal pattern of activity seems to be more common for the genus *Dasyprocta*, some plasticity in the timing of activity is evident, such as the differences between EBSL and RNV.

Latitude is one of the main abiotic factors that can influence the daily and seasonal activity in mammals because the incidence of light changes with latitude, changing light intensity and daylength (Bronson 1988), and altering the physiological response of the species due to variations in the duration of day and night (Goldman 1999, Wagner et al. 2008). In this study, the two study areas are less than one degree apart in latitude, and therefore latitude is unimportant as a factor to explain the difference of agouti activity patterns between EBSL and RNV.

We suggest that variation in patterns of agouti activity between the study areas may be a consequence of the variation in luminosity due to the differences in altitude (mountainous versus coastal) associated with topography (shady slopes versus flat plains) between the study areas. These factors result in differences in the incidence of light (solar rays) inside the forest. In this sense, we emphasize the EBSL is at a minimum of 500 m higher and has more complex topography than the RNV. The relief on the EBSL area is composed of slopes with accentuated soil declivity and small intermontane floodplains overlapping rivers and streams, being the reserve subdivided by a deep valley in the direction northwest to southwest in which runs the Timbuí river (Mendes & Padovan 2000).

Additionally, temperature also affects different aspects of the biology of the organisms, controlling physiological adjustments to those conditions, such as variation in metabolic rate, thermoregulation, and coat properties (e.g. Walsberg 1988, Kenagy et al. 2002, Sassi et al.

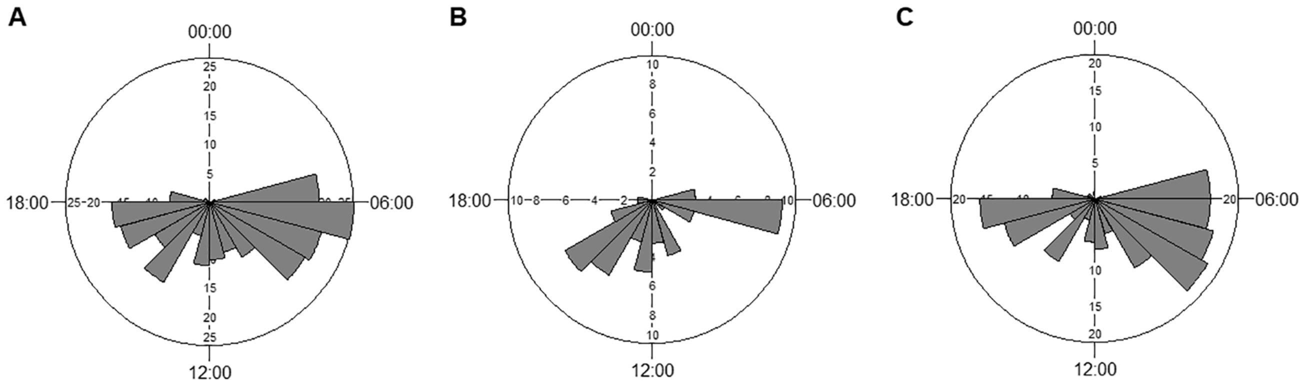


Figure 3. Rose diagrams showing activity patterns of the Red-rumped Agouti (*Dasyprocta leporina*) with the total (A), for the Santa Lúcia Biological Station (B) and for the Vale Natural Reserve (C) in southeastern Brazil.

2015), highlighting that behavior can also be influenced by variation in solar radiation (Hinze & Pillay 2006). Even though mammals thermoregulate, it is clear that climate regimes (mainly low or high external temperatures) have consequences for energetics and therefore can moderate the level of activity of both diurnal and nocturnal species (Beltran & Delibes 1994, Kenagy et al. 2002). The influence of temperature on agouti daily activity have already been proposed for Central American Agouti (*Dasyprocta punctata* Gray, 1842) in Panama (Lambert et al. 2009) and Azara's Agouti (*Dasyprocta azarae* Lichtenstein, 1823) in Brazilian Pantanal (Cid et al. 2015). In these areas, the agoutis may use behavioral strategies to reduce the exposure to heat during the hottest times of the day (Lambert et al. 2009), and the activity pattern change across the temperature gradient during the day (Cid et al. 2015). Nevertheless, the agoutis keep the daily activity range constant, with similar amount of time performing their activities regardless the temperature during the morning and in the afternoon (Cid et al. 2015). Thus, we suggest agouti activity patterns in study areas may also be influenced by differences in the ambient temperature, especially lower temperatures at the beginning and at the end of the day in EBSL, perhaps explaining why activity is different in the mountainous region with a milder climate compared to the hotter coastal region (more than 4°C greater on average at RNV, noting that the daily thermal amplitude may be even higher in mountainous regions). Behavioral plasticity in response to environmental heterogeneity was also recorded to the small rodent *Phyllotis xanthopygus* (Waterhouse, 1837), for which groups of individuals collected at different altitudinal sites showed different rate and pattern of activity under the influence of different experimental temperatures (Sassi et al. 2015).

In addition to the abiotic factors cited, environmental perturbations, such as habitat fragmentation, may also influence mammalian behavior (Norris et al. 2010). Comparing remnants in a forested landscape of the southern Brazilian Amazon, *D. leporina* had a more pronounced peak of activity earlier in the morning in larger fragments, while activity was distributed more evenly throughout the day in smaller fragments (Norris et al. 2010). The patterns we observed in both areas are similar to those reported in larger fragments (Norris et al. 2010), yet the peak before dusk was higher in our study. Also, the overall level of activity of *D. punctata* was inversely related to food availability in Panama (Suselbeek et al. 2014), which help to explain the fact that agoutis tend to be active for more time in forest fragments that have been isolated longer (Norris et al. 2010). The risk of predation is another biotic factor that affects the activity pattern, and *D. punctata* avoid periods of high exposure to predators (Lambert et al. 2009, Suselbeek et al. 2014). Despite the peculiarities among the studied reserves, the principal predators of agoutis, the ocelot *Leopardus pardalis* (Linnaeus, 1758) and puma *Puma concolor* (Linnaeus, 1771), are both present in EBSL and RNV. Therefore, we assume that the risk of predation may not be a factor that explains the differences in the activity pattern of the agouti between areas.

Red-rumped Agouti activity lasted more or less the duration of daylight (14–15 hours), similar to that of a previous study in the RNV (14 hours, Ferreguetti et al. 2018). The Coiban Agouti (*Dasyprocta coibae* Thomas, 1902) and *D. punctata* were active, respectively, 15 and 14 hours each day in Panama (Suselbeek et al. 2014, Duquette et al. 2017). The Black Agouti (*Dasyprocta fuliginosa* Wagler, 1832) was active for 13 h in Ecuador (Blake et al. 2012). In the Pantanal, *D. azarae* was active up to 17 hours each day (Cid et al. 2015). These data demonstrate that factors other than latitude (and hence photoperiod), as previously discussed, influence the agouti activity pattern also by considering broader geographic scales.

In a previous study in the RNV, *D. leporina* was not recorded during the hottest hours of the day, between 12:00 h and 13:30 h (more than 750 independent camera trap records; Ferreguetti et al. 2018). In this study we found less activity between 10:00 h and 13:00 h in RNV, but there was always some activity. This pattern has also been observed elsewhere in agoutis (Norris et al. 2010, Blake et al. 2012, Suselbeek et al. 2014, Cid et al. 2015, Duquette et al. 2017). No agouti activity was observed during the 09:00 h interval in the EBSL (between 08:55 h and 10:18 h), but this can be attributed to the lower number of records obtained in this area, associated with the reduction of the activities of the species at this time, similar to that observed in RNV.

We only had three nocturnal captures of *D. leporina*, all in the RNV. Occasional nocturnal activity of *D. punctata* occur in periods of fruit abundance, even though foraging at night may have greater predation risk (Lambert et al. 2009). In our study, the nocturnal captures were in February (n = 1) and October (2), which are rainy months with greater food availability.

Red-rumped Agouti was active throughout the day, with a bimodal pattern of activity (morning and afternoon), and nocturnal activity was rare. These activity characteristics are similar to that recorded for agoutis in one or more localities. The study areas had somewhat different activity patterns, demonstrating plasticity on the part of the agouti that is probably due to differences in ambient temperature, associated with local effects of altitude and topography of the terrain on the incidence of light in the interior of the forest in the two locations. This shows behavioral plasticity in the activity patter of *D. leporina* in the Atlantic Forest can be influenced by factors other than latitude. In addition to a better understanding of the temporal niche of the Red-rumped Agouti, our data can also be used to help to optimize strategies for *D. leporina* conservation in southeastern Brazil. We suggested the intensification of activities to combat agouti poaching at the times of the day when the species is most active and consequently more susceptible to hunting. This would be applied particularly to areas where agouti is one of the main hunting target species.

Acknowledgments

We would like to thank the staff of the Museu de Biologia Professor Mello Leitão and of the Reserva Natural Vale for the logistic support; and to the colleagues who kindly helped with data collection. We also acknowledge financial support from Vale S.A. – Instituto Ambiental Vale/Brazil, CAPES/Brazil and FAPES/Brazil. This project was funded by the CNPQ (grant # 469.321/2000-8) and Fundo de Incentivo à Pesquisa - PUC Minas (grant # FIP 2002/06-TLE).

Author Contributions

Ana Carolina Srbek-Araujo: Concept and design of the study, data collection, data analysis and interpretation, manuscript preparation.

Laura Martins Magalhães: data analysis and interpretation, manuscript preparation.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALVARES, C.A., STAPE, J.L., SENTELHAS, P.C., GONÇALVES, J.L. & SPAROVEK, G. 2013. Köppen's climate classification map for Brazil. Meteorol. Z. 22(6):711-728.

BELTRÁN, J.F. & DELIBES, M. 1994. Environmental determinants of circadian activity of free ranging Iberian lynxes. J. Mammal. 75(2):382-393.

BLAKE, J.G., MOSQUEIRA, D., LOISELLE, B.A., SWING, K., GUERRA, J. & ROMO, D. 2012. Temporal activity patterns of terrestral mammals in lowland rainforest of eastern Ecuador. Ecotropica. 18:137-146.

BRONSON, F.H. 1988. Mammalian reproductive strategies: genes, photoperiod and latitude. Reprod. Nutr. Develop. 28(2B):335-347.

CID, B., OLIVEIRA-SANTOS, L.G. & MOURÃO, G. 2015. The relationship between external temperature and daily activity in a large rodent (*Dasyprocta azarae*) in the Brazilian Pantanal. J. Trop. Ecol. 31:469-472.

CULLEN JR, L., BODMER, R.E. & PADUA, C.V. 2000. Effects of hunting in habitat fragments of the Atlantic Forests, Brazil. Biol. Cons. 95:49–56.

DUQUETTE, J.F., UREÑA, L., ORTEGA, J., CISNEROS, I., MORENO, R. & FLORES, E.E. 2017. Coiban agouti (*Dasyprocta coibae*) density and temporal activity on Coiba Island, Veraguas, Panama. Mammal Study. 42:153-160.

FERREGUETTI, A.C., TOMAS, W.M. & BERGALLO, H.G. 2018. Density, habitat use, and activity pattersns of the Red-rumped Agouti (*Dasyprocta leporina*) in the Atlantic Forest, Brazil. Stud Neotrop Fauna E. 53(2): 143-151.

FSOSMA & SOSMA & INPE. 2017. Atlas dos Remanescentes Florestais da Mata Atlântica: Período 2015-2016. Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais, São Paulo.

GALETTI, M., DONATTI, C.I., PIRES, A.S., GUIMARÃES JR, P.R. & JORDANO, P. 2006. Seed survival and dispersal of an endemic Atlantic Forest palm: the combined effects of defaunation and forest fragmentation. J. Proc. Linn. Soc., Bot. 151:141–149.

GOLDMAN, B.D. 1999. The circadian timing system and reproduction in mammals. Steroids 64:679-685.

HINZE, A. & PILLAY, N. 2006. Life in an African alpine habitat: diurnal activity patterns of the ice rat *Otomys aloggetti robertsi*. Arct. Antarct. Alp. Res. 38 (4):540–546.

JESUS, R.M. & ROLIM, S.G. 2005. Fitossociologia da Mata Atlântica de Tabuleiro. Bol. Téc. SIF. 9:1-149.

KENAGY, G.J., NESPOLO, R.F., VASQUEZ, R.A. & BOZINOVIC, F. 2002. Daily and seasonal limits of time and temperature to activity of degus. Rev. Chil. Hist. Nat. 75:567-581.

KIERULFF, M.C.M., AVELAR, L.H.S., FERREIRA, M.E.S., POVOA, K.F. & BÉRNILS, R.S. 2015. Reserva Natural Vale: história e aspectos físicos. Ciência & Ambiente 49:7-40.

KOVACH COMPUTING SERVICES. 2009. Oriana Users' Manual. Kovach Computing Services, Pentraeth, Wales, United Kingdon.

KRONFELD-SCHOR, N. & DAYAN, T. 2003. Partittioning of time as an ecological resource. Annu. Rev. Ecol. Evol. Syst. 34:81-153.

LAMBERT, T.D., KAYS, R.W., JANSEN, P.A., ALIAGA-ROSSEL, E.& WIKELSKI, M. 2009. Nocturnal activity by the primarily diurnal Central American agouti (*Dasyprocta punctata*) in relation to environmental conditions, resource abundance and predadion risk. J. Trop. Ecol. 25:211-215.

MENDES, S.L. & PADOVAN, M.P.A. 2000. Estação Biológica de Santa Lúcia, Santa Teresa, Espírito Santo. Bol. Mus. Biol. Mello Leitão 11/12:7-34.

NORRIS, D., MICHALSKI, F. & PERES, C.A. 2010. Habitat patch size modulates terrestrial mammal activity patterns in Amazonian forest fragments. J. Mammal. 91(3):551-560.

OLIVEIRA, J.A. & BONVICINO, C.R. 2006. In Mamíferos do Brasil. Capítulo 12: Ordem Rodentia. (Nelio R. dos Reis) Londrina, Paraná. Pp. 347-406.

PITA, R., MIRA, A. & BEJA, P. 2011. Circadian activity rhythms in relation to season, sex and interspecific interactions in two Mediterranean voles. Anim. Behav. 81:1023-1030.

ROWCLIFFE, J. M., KAYS, R., KRANSTAUBER, B., CARBONE, C. & JANSEN, P. A. 2014. Quantifying levels of animal activity using camera trap data. Methods Ecol. Evol. 5:1170–1179.

SASSI, P.L., TARABORELLI, P., ALBANESE, S. & GUTIERREZ, A. 2015. Effect of temperature on activity patterns in a small andean rodent: behavioral plasticity and intraspecific variation. Ethology 121:840-849.

SRBEK-ARAUJO, A.C. & CHIARELLO, A.G. 2005. Is camera-trapping an efficient method for surveying mammals in Neotropical forests? A case study in south-eastern Brazil. J. Trop. Ecol. 21:1–5.

SRBEK-ARAUJO, A.C. & CHIARELLO, A.G. 2007. Armadilhas fotográficas na amostragem de mamíferos: considerações metodológicas e comparação de equipamentos. Revista Brasileira de Zoologia, 24(3):647-656.

SRBEK-ARAUJO, A.C. & CHIARELLO, A.G. 2013. Influence of camera-trap sampling design on mammal species capture rates and community structures in southeastern Brazil, Campinas. Biota Neotrop. 13(2):51-62.

SRBEK-ARAUJO, A.C. & CHIARELLO, A.G. 2017. Population status of the jaguar *Panthera onca* in one of its last strongholds in the Atlantic Forest. Oryx 51(2):246-253.

SUSELBEEK, L., WILLEM-JAN, E. HIRSCH, B.T., KAYS, R., ROWCLIFFE, J.M., ZAMORA-GUTIERREZ, V. & JANSEN, P.A. 2014. Food acquisition and predador avoidance in a Neotropical rodent. Anim. Behav. 88:41-48.

THOMAZ, L.D. & MONTEIRO, R. 1997. Composição florística da Mata Atlântica de encosta da Estação Biológica de Santa Lúcia, município de Santa Teresa – ES. Bol. Mus. Biol. Mello Leitão 7:3–48.

WAGNER, G.C., JOHNSTON, J.D., CLARKE, I.J., LINCOLN, G.A. & HAZLERIGG, D.G. 2008. Redefining the limits of day length responsiveness in a seasonal Mammal. Endocrinology 149(1):32-39.

WALSBERG, G.E. 1988. Consequences of skin color and fur properties for solar heat gain and ultraviolet irradiance in two mammals. J. Comp. Physiol. B 158:213-221.

ZAR, J.H. 2010. Biostatistical analysis. Prentice Hall, Upper Saddle River.

Received: 30/07/2018
Revised: 12/11/2018
Accepted: 17/01/2019
Published online: 11/02/2019

Biota Neotropica 19(2): e20180620, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Ethnoecology and socioeconomic around an artificial reef: the case of artisanal fisheries from southeastern Brazil

Juliano Silva Lima[1,2], Camilah Antunes Zappes[3], Ana Paula Madeira Di Beneditto[1] &

Ilana Rosental Zalmon[1]*

[1]Universidade Estadual do Norte Fluminense, Av. Alberto Lamego, 2000, 28013-602, Rio de Janeiro, RJ, Brasil
[2]Instituto Federal de Educação, Ciência e Tecnologia de Sergipe, Av. Juscelino Kubitschek, 49680-000, Nossa Senhora da Glória, SE, Brasil
[3]Universidade Federal Fluminense, Rua José do Patrocínio, 71, 28010-385, Rio de Janeiro, RJ, Brasil
**Corresponding author: Ilana Rosental Zalmon, e-mail: ilana@uenf.br*

LIMA, J. S., ZAPPES, C. A., DI BENEDITTO, A. P. M., ZALMON, I. R. **Ethnoecology and socioeconomic around an artificial reef: the case of artisanal fisheries from southeastern Brazil**. Biota Neotropica. 19(2): e20180620. http://dx.doi.org/10.1590/1676-0611-BN-2018-0620

Abstract: The objective of this study is to describe the ethnoecological aspects, valorization, and commercialization of target species caught by artisanal fishers on the northern coast of Rio de Janeiro, southeastern Brazil. The data were obtained through semi-structured interviews conducted with 60 fishers from the fishing community of Guaxindiba (21°29'S, 41°00'W), which is associated with the Z-1 fishers' colony. Eighty-nine species were cited by the fishers and 44.1% are important commercial fish in the region. The fishers cited five distinct zones used to fish: border zone (68 species), estuary (41 spp.), artificial reef (27 spp.), "malacacheta" (24 spp.), and open ocean (10 spp.). The fishery resources were classified according to their gastronomic and economic characteristics: primary fish (35 spp.), secondary fish (32 spp.), mixed fish (10 spp.), discarded fish (7 spp.), and fish used as bait (5 spp.). The price of the target species increases along the production chain due to the greater number of people involved, processing costs, and improvement of the product. The data from this study can contribute to local fisheries management and point out to the use of artificial reefs in the maintenance of fishery resources in northern Rio de Janeiro.
Keywords: *artisanal fishing, traditional knowledge, fishery management.*

Etnoecologia e socioeconomia em torno de um recife artificial: o caso da pesca artesanal do sudeste do Brasil

Resumo: O objetivo desse estudo é descrever os aspectos etnoecológicos, a valoração e a forma de comercialização das espécies-alvo capturadas na pesca artesanal praticada na costa norte do Rio de Janeiro, sudeste do Brasil. Os dados foram obtidos a partir de entrevistas semiestruturadas realizadas com 60 pescadores da comunidade pesqueira Guaxindiba (21°29'S, 41°00'O), vinculados à colônia de pescadores Z-1. Oitenta e nove espécies foram citadas pelos pescadores e 44,1% desse total são importantes para o comércio da região. Os pescadores citaram cinco zonas distintas que são utilizadas para a pesca: zona da "borda" (68 espécies), estuário (41 spp.), recife artificial (27 spp.), "malacacheta" (24 spp.) e mar aberto (10 spp,). Os recursos pesqueiros foram classificados de acordo com suas características gastronômicas e econômicas: pescado-de-primeira (35 spp.), pescado-de-segunda (32 spp.), pescado-mistura (10 spp.), pescado-de-descarte (7 spp.) e pescado-isca (5 spp.). O preço das espécies-alvo aumenta ao longo da cadeia produtiva em decorrência do maior número de pessoas envolvidas, dos gastos com insumos e do processo de beneficiamento do pescado. Os dados desse estudo podem contribuir para o manejo da pesca local e apontam para o uso de recifes artificiais na manutenção dos recursos pesqueiros no norte do Rio de Janeiro.
Palavras-chave: *pesca artesanal, conhecimento tradicional, manejo pesqueiro.*

Introduction

Artisanal fishing is important to the economy in many fishing communities, since it provides food security, employment and income, and is part of the cultural identity of various social groups (FAO 2016). In these practices, the fishermen explore the coastline with small equipment that have low autonomy and capture species for subsistence or small-scale commercialization (Clauzet et al. 2005, Oliveira et al. 2016). Artisanal fishermen usually are organized in fishing colony or association with a local leader as representative (Brasil 2009, Brasil 2011). However, factors such as the use of active gears (*e.g.* trawls, dredges and seine nets), overexploitation of fisheries resources, degradation of coastal environments, and climate change have affected local fishing communities and intensified the economic crisis in this sector (Silvano & Valbo-Jorgensen 2008, Vasconcellos et al. 2011, Santos & Alves 2016). In this context, ethnoecological studies have provided relevant information about the environment and exploited species, because they consider the knowledge of subjects that are in direct and regular contact with natural resources (Johannes 2002, Silvano et al. 2009). Based on local ecological knowledge (LEK) it is possible to better understand, for example, aspects related to the behavior and occurrence of species, as well as environmental impacts that affect them, which often go unnoticed by researchers (Begossi et al. 2000, Silvano 2004, Hanazaki et al. 2013).

The LEK of artisanal fishers provides knowledge about ecological, taxonomic, and ethological aspects of aquatic communities that are important for inventories, especially in places where scientific studies are scarce (Mourão & Montenegro 2006, Silvano & Begossi 2012, Pinto et al. 2015, Pitcher & Lam 2015). Analyzing the interactions between fishers and fishery resources is important to understand distinct forms of use, capture, categorization, and commercialization of target species (Begossi 2006, Souza et al. 2007, Gerhardinger et al. 2009). Studies on fishing dynamics through LEK are related to developing management measures for the conservation of species that are the most vulnerable or threatened with extinction.

Rio de Janeiro State is ranked as seventh fish producer in Brazil, and the northern coast has six important artisanal fishing ports (Vianna 2009, ICMBIO 2011). Despite management strategies for fishing in Brazil (*e.g.*, regulation of fisheries and closed season, public benefits for fishers and input subsidies), public policies for artisanal fishing in northern Rio de Janeiro are still inefficient, due the impossibility of the State to ensure legal presuppositions (Pérez et al. 2001, Castello, 2007, Brasil, 2009, FIPERJ 2013, 2015). Co-shared management of fishing activities include information about fisheries resources and how they are exploited, besides the participation of fishers in the political and economic decision-making process (Berkes et al. 2006, Pomeroy et al. 2007).

Several studies have contributed on this subject, through daily observations of fishing activities, regular monitoring, and interviews with fishers (Ota & Just, 2008, Silvano & Begossi 2012, Pinto et al. 2015, Pitcher & Lam 2015). Studies along the coast of Rio de Janeiro (*e.g.*, Di Beneditto et al. 1998, Di Beneditto 2001, Fernandes et al. 2014, Oliveira et al. 2016, Zappes et al., 2016) have been conducted to analyze the activities of fishers and the management of fishery resources. These studies aimed to describe the main boats, fishing gear and target species. However, little is known about the catching process of target species in different fishing areas ("pesqueiros"), and the socioeconomics of fishing resources in the north coast of Rio de Janeiro.

In 1996, an artificial reef complex (ARs) composed of reef balls was installed on the north coast of Rio de Janeiro near the fishing community of Guaxindiba. These modules were installed to increase the abundance, biomass of fishing resources and improve the artisanal fishing rates (Santos et al. 2010, Santos & Zalmon 2015). Over the years, the local fishermen have used the area where the ARs were deployed as an ancillary fishing area to capture some target species (Lima et al. 2018). However, there is little information about the species caught in these artificial structures or in other fishing areas used for artisanal fishermen in the northern Rio de Janeiro.

Thus, the objective of this work is to describe the ethnoecological aspects and commercialization of target species caught by artisanal fishers in different fishing areas on the northern coast of Rio de Janeiro (especially in the ARs). The results will contribute to local fisheries management, besides the evaluation of ARs usage to co-management of species that are vulnerable and threatened.

Material and Methods

1. *Study area*

The northern coast of Rio de Janeiro is located in a transition zone between hot, oligotrophic waters and the cold, resurgent, nutrient-rich current from the South Atlantic (Valentin & Monteiro-Ribas 1993) (Figure 1). The continental platform in this region has a seabed that is composed of sand, mud, and biogenic sediment from Paraíba do Sul River (PSR) (Murilo et al. 2009). During rainy season (January to March), the estuarine plume of PSR reach up to 20 km offshore, which affects the ecological processes of marine and local fish communities (Ovalle et al. 2014).

Fishing community of Guaxindiba (21°29'S, 41°00'W) is characterized by capture of shrimp, weakfish, croakers, catfish and shark (Di Beneditto 2001, Vianna 2009). Fishing in this region is predominantly artisanal and the fishers are represented by the Z-1 Fishers' Colony, which has 328 associates (FIPERJ 2015). Most of these operate in coastal marine areas of Campos Basin between the municipalities of São Francisco de Itabapoana (21º18'S, 40º57'W) and Macaé (22º22'S, 41º47'W), from areas near the coast to 120 km offshore.

In 1996, ARs were installed 5 km from the community of Guaxindiba to increase coastal resources and improve artisanal fishing. These ARs are composed of 36 reef balls (1 m^3) covering an area of 60.000 m^2, each module weight 500 kg with holes 20 cm diameter. The reef balls are ideal for creating habitats for several marine species (fish, lobsters, oysters) and also to prevent industrial fishing (Sherman et al. 2002, Young et al. 2012). Lima et al. (2018) showed that fishermen on the northern coast of Rio de Janeiro have used the area where the ARs were deployed as an ancillary fishing area to capture some target species, using fishing gears that best suit the characteristics of that area as bottom lines and hook, longlines and surface gillnets.

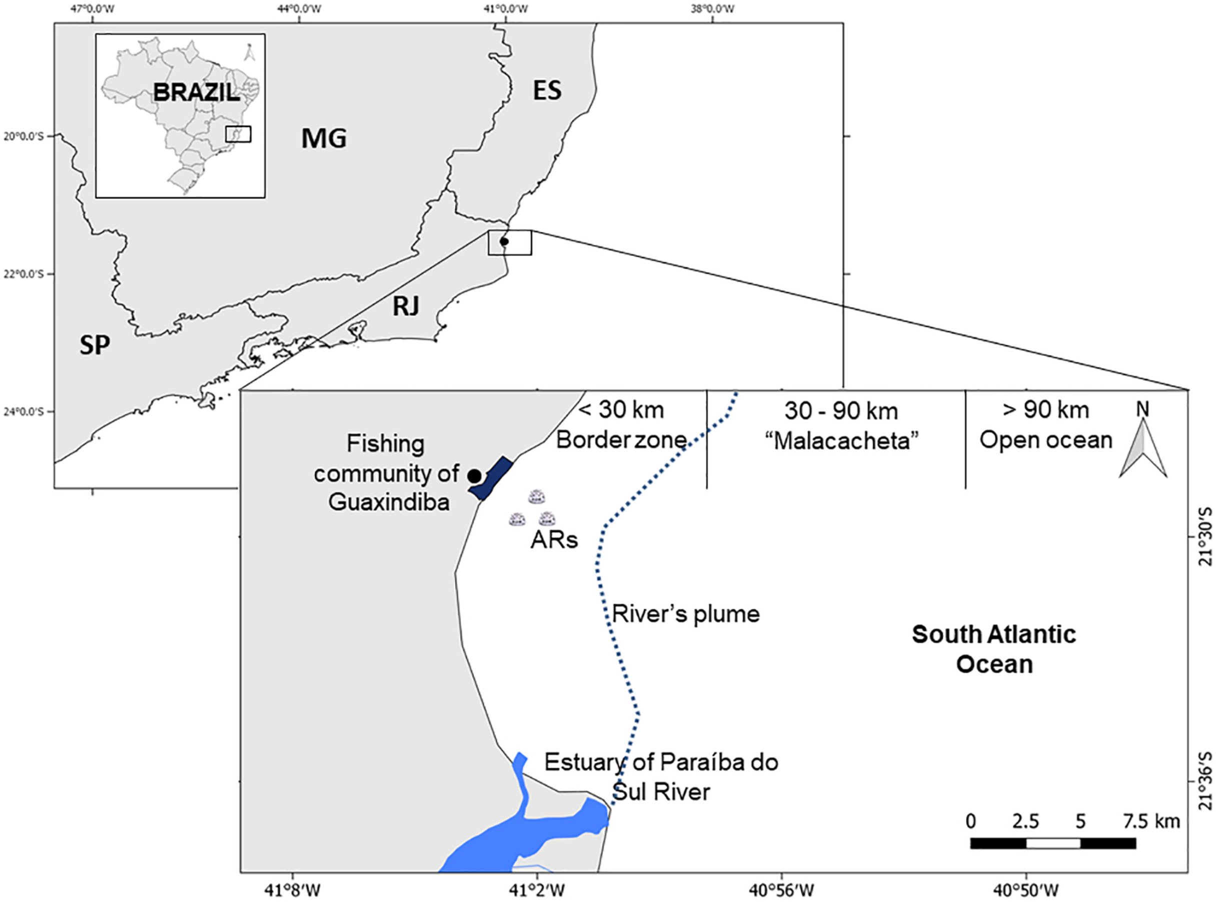


Figure 1. Location of the community of Guaxindiba and delineation of fishing zones according to the local fishermen. Southwestern states: MG - Minas Gerais, ES - Espírito Santo, SP - São Paulo and RJ - Rio de Janeiro.

2. *Data collection*

This study was approved by the Human Research Ethics Committee of the Federal Institute of Sergipe, Brazil (CAAE 64939116.1.0000.8042). The permission to perform this study was granted by the president of the Fishing Colony Z-1, an autocracy that represents the professional category studied. The data were collected from November 2016 to May 2017 through 60 semi-structured interviews with artisanal fishers from the fishing community of Guaxindiba. In the first phase of the study, the participant observation method was used through observations of the fishers routine (Malinowski 1984, Sieber et al. 2014).

The observations were recorded in a field diary to obtain data about the structural and cultural characteristics of the community (Clifford 1998). The interviews were conducted using a semi-structured questionnaire (Kendall 2008) with open and closed questions characterizing the local interviewees, description of artisanal fishing and commercialization of fish (Box 1).

The interviewee selection was based on the following mandatory aspects: i) to be an artisanal fisher based in northern Rio de Janeiro, and ii) having fishing as the main professional activity. The first interviewee involved the collaboration of a local guide (president of the Z-1 Fishers' Colony), and a snowball sampling was used with the second interview, where the interviewee indicate the next one (Lyra-Neves et al. 2015). At times the snowball sampling was interrupted, a fisherman was randomly chosen in order to minimize the possible personal tendencies of indicating someone (Silva et al. 2014).

To taxonomically identify the fishery resources mentioned by the interviewees, fish were caught with gill nets or obtained from local markets after verifying where they came from. Lobsters, oysters and shrimps were sampled at local markets, upon confirmation with the fisherman about the place of capture. The samples were preserved in a solution of 10% formaldehyde. Fish were identified using taxonomic keys (Figueiredo & Menezes 1977, 1978, 1980a, 1980b, 1985 and 2000, Carvalho Filho 1994, Eschmeyer & Fong 2018, Froese & Pauly 2018), and for mollusks and crustaceans, information was compared with literature (Costa et al. 2003, Jereb & Roper 2010, Tudesco et al. 2012, Amaral & Simone 2014, Giraldes & Smyth 2016). After the scientific names identification, the species common names were confirmed with the contribution of seven 'local knowledge experts'. These 'experts' were defined as major holders of fishing knowledge.

3. *Data analysis*

The responses were organized into categories according to the questionnaire (Ryan & Bernard 2000). This allowed the local knowledge of the artisanal fishers to be described and compared, as well as the ecological and economic characterization of the fishery resources in the

Box 1. Topics addressed in the semi-structured questionnaire addressed to fishers from the community of Guaxindiba, Brazil.

Topics	Questions
1. Characterization of the interviewees	Gender
	Age
	Education
2. Description of artisanal fishing	Boats
	Fishing gear
	Fishing areas
	Environmental interferences that influence fishing
3. Characterization of fishing	Marine species that occur in the region
	Habitat of target species
	Categorization of target species
4. Commercialization of fish	Main commercialized target species
	Fishing season of the target species
	Value of target species
	Production chain of target species

region. The comparisons were made based on the percentage frequencies of the responses to the questionnaire (Sieber et al. 2014). Topics that fishermen responded more than one option included the percentage frequency calculated by the number of total responses and not by the number of interviewed.

Based on the interviews and *in situ* observations, a fishing calendar was created including the months with the highest catch, average prices of the main commercial species, and conservation status of the target species according to the International Union for Conservation of Nature (IUCN 2017) and the Ministry of the Environment of Brazil (MMA 2014). Subsequently, the calendar was shown to the artisanal fishers to confirm the results. The LEK was analyzed through the triangulation method, which consists of crossing information collected through field diary, participant observation, and interview-questionnaire to compare similarities and discrepancies in the data (Yeasmin & Rahman 2012, Albuquerque et al. 2014).

Results

1. *Description of artisanal fishing and environmental interferences*

The interviewed fishers were mostly male (n = 59); only one professional fisher was female. Age varied from 40 to 78 years and the amount of time fishing ranged from 22 to 60 years. Considering the formal education, 61.7% (n = 37) of the fishers did not finish elementary school, 23.3% (n = 14) finished elementary school, and 15% (n = 9) never went to school.

The most common boats in are trawlers and canoes (paddled or motorized). Trawlers are used for short (up to 8 h) and long (up to 10 days) fishing trips. Motorized canoe is used for short fishing trips (up to 6 hours), and paddled canoe is used to transport cargo to trawlers, since there are no docks in the fishing community.

The most used fishing gear is the bottom trawl net (n = 41, 32.5%), followed by bottom line and hook (n = 21, 16.7%), longline (n = 19, 15.1%), bottom gillnet (n = 17, 13.5%), purse seine net (n = 12, 9.5%), trammel net (n= 9, 7.1%), and surface gillnet (n = 7, 5.6%). The same fisher sometimes uses more than one fishing gear, and consequently the number of responses is greater than the number of interviewees.

The fishers mentioned five distinct zones that are used for artisanal fishing in the region: border zone (0 to 30 km from the coast zone), ARs (zone with reef balls), estuary zone (PSR mouth), "malacacheta" (30 to 90 km), and open ocean (over 90 km) (Figure 1). According to the interviewees, the border zone has the greatest number of species that are caught (68 spp., 40%), followed by the PSR estuary (41 spp., 24.1%), ARs (27 spp., 15.9%), "malacacheta" (24 spp., 14.1%), and open ocean (10 spp., 5.9%). The same target species can be caught in more than one zone, and consequently the number of responses is greater than the species cited.

The fishers highlighted some environmental conditions (wind, tides, and moon phases) as determining factors in planning fishing trips. Wind direction is the main predictor used to conduct these activities in the region. Winds from the north (n = 26, 43.3%), northeast (n = 22, 36.7%), and southeast (n = 12, 20.0%) are considered the best for fishing. Winds from the north lead several target species to the coast and those from the northeast increase the turbidity of the water making it possible to catch shrimp and sharks. Winds from the southeast are less intense, which is good for fishing in areas farther from the coast.

However, southern (n = 31, 51.7%), southeastern (n = 18, 30.0%), and continental (n= 11, 18.3%) winds cause the worst fishing conditions. According to the fishers, wind from the south ("vento de viração") is very intense so they do not fish during this time (August and September). Wind from the southeast is related to an increase in the availability of target species; however, strong bottom currents hamper to handle the fishing gear. Continental winds are considered calm, but they direct the target species away from the coast, which increases the time and cost of fishing.

The interviewers considered high ("alive" or syzygy) tide (n = 32, 53.3%) as the best condition to fish compared to low ("dead" or quadrature) tide (n = 19, 31.6%). Full moon with a spring tide (n = 24, 40.0%) was reported as a favorable condition to fish for shrimp and when fish species move to regions closest to the coast. Neap tide is suitable for catching species such as mullet, snook, and sharks because the moonlight is less intense on the water's surface. Waxing moon (n = 14, 23.3%) is characterized by fishing in regions further than 30 km from the coast due to weaker sea currents. Fishers commonly associated waning moon (n = 13, 21.6%) with the fish spawning period in PSR estuary region.

2. *Characterization and commercialization of the fish*

Eighty-nine species belonging to four classes and 44 families were reported by the interviewees: Osteichthyes (69 species), Chondrichthyes (11), Malacostraca (7), Cephalopoda (1) and Bivalvia (1). The most representative families were Sciaenidae (14 species), Carangidae (7), Ariidae (6), Haemulidae (6) and Carcharhinidae (5).

The fishers differentiated the fish habit by their vertical distribution in the water column. The "peixes-boieiros" (surface fish) (12 spp., 13.5%) are represented by species that feed on the water surface, "peixes-meio-d'água" (midwater fish) (39 spp., 43.8%) include species that move a lot in the water column based on climatic conditions or to feed, and "peixes-de-fundo" (bottom fish) are divided into "peixes-de-lama" (mudfish) (23 spp., 25.8%) and "peixes-de-pedra" (rockfish) (15 spp., 16.9%), which inhabit and feed within soft and hard substrates, respectively.

The fishing resources in the region were classified into five categories by the interviewees: 1) Primary fish (35 spp., 39.3%), which are the most preferred species by the fishers due to their better flavor and high commercial value. These resources are exclusively for commercialization and are only eaten by the fishers and their families on special occasions. The sale price varies between US$ 2.90/kg and US$ 8.60/kg (Table 1). Secondary fish (32 spp., 36.0%), which are less preferred by the consumer market due the presence of many small bones ("espinhas"). In general, these fish are consumed by fishermen and their families. The sale price varies between US$ 0.60/kg and US$ 2.30/kg (Table 2).

Mixed fish (10 spp., 11.2%), which are less preferred by the consumer market due to their rancid flavor and/or presence of many small spines. The "mixed" category was designated by the fishers for by-catch from shrimping. The fish are sold together for a single price, which is US$ 0.20/kg. These species are also given to friends and family (Table 3). Discarded fish (7 spp., 7.9%) include species that lack commercial value and are discarded during fishing as the spotted moray (*Gymnothorax moringa* Cuvier 1829) and ocellated moray (*Gymnothorax ocellatus* Agassiz 1831), which the fishermen avoid due to the bite of these fishes. Some fishers (n = 6, 6.7%) wait for seabirds, such as the brown booby (*Sula leucogaster* Boddaert 1783) and seagulls (*Larus* sp.) to discard this type of fish, which keeps the birds from looking for fish on the boat that have a higher commercial value. Fish used as bait (5 spp., 5.6%), which are species of small size that are not consumed by humans in the region. This category includes some species that are used as bait in bottom line, hook and longline fisheries to catch commercially valuable fish species. The Atlantic thread herring (*Opisthonema oglinum* Lesueur 1818) is used as bait to catch the common dolphinfish (*Coryphaena hippurus* Linnaeus 1758) and the American coastal pellona (*Pellona harroweri* Fowler 1917) is used to catch the grey triggerfish (*Balistes capriscus* Gmelin 1789) and queen triggerfish (*Balistes vetula* Linnaeus 1758). Species of this category are not commercialized.

Among the 89 species mentioned by the fishers, only 39 (43.8%) are commercialized in the community of Guaxindiba (Table 4). Notable target species are shrimp (*Artemesia longinaris* Bate 1888, *Farfantepenaeus paulensis* Pérez Farfante 1967, *Litopenaeus schmitti* Burkenroad 1936 and *Xiphopenaeus kroyeri* Heller 1862), snook (*Centropomus parallelus* Poey 1860 and *Centropomus undecimalis* Bloch 1792), sharks (*Carcharhinus porosus* Ranzani 1839, *Rhizoprionodon porosus* Poey 1861 and *Rhizoprionodon lalandii* Valenciennes 1839), weakfish (*Cynoscion microlepidotus* Cuvier 1830, *Cynoscion virescens* Cuvier 1830, *Nebris microps* Cuvier 1830 and *Isopisthus parvipinnis* Cuvier 1830), mullet (*Mugil liza* Valenciennes 1836), "sargos" (*Anisotremus surinamensis* Bloch 1791 and *Archosargus probatocephalus* Walbaum 1792), and lobsters (*Panulirus argus* Latreille 1804 and *Panulirus* sp.).

The data indicate that ARs are important in the context of local fishing due to the ability to attract commercial fish to the fishing zone closer to the port of Guaxindiba. According to the fishermen bluefish (*Pomatomus saltatrix* Linnaeus 1766), lobsters (*Panulirus* sp. and *P. argus*), atlantic spanish mackerel (*Scomberomorus maculatus* Mitchill 1815) and "sargos" (*A. surinamensis* and *A. probatocephalus*) are captured in the coastal zone after the implantation of ARs in the north coast of Rio de Janeiro.

Based on the information provided by the interviewees about fishing periods, an ecological-economic calendar was created for the main target species caught along the northern coast of Rio de Janeiro (Table 4). The fishing season of commercialized target species is related to the rainy period and variation in the flow of PSR: rainy period and greatest flow (December to February); dry period and least flow (June to August). The rainy period is the best time to fish for lobsters, weakfish, "sargos" and mullet (except when the season is closed for fishing), and the dry period is the best time to fish for shark and snook. Shrimp are caught throughout year, except when the season is closed (March to May).

The market price for shrimp varies based on the time of year (season vs. off season). During the fishing seasons (June and July; October to January) shrimp are usually cheaper (-30%) compared to off seasons (February, August, and September). For the fish species, there was a relation between the average prices per kilo and the length of fishing season; the target species with the highest added value are those whose fishing season is the shortest.

Despite the lack of information on the conservation status of many target species that are commercially fished on the northern coast of Rio de Janeiro, some species are at risk (14 species) by the IUCN (2017) and the National List of Threatened (MMA 2014). The sharks are target species whose decrease in numbers have been most noticed by the fishers (n = 41). Replacing shark fillet with catfish fillet (*Bagre bagre* Linnaeus 1766 and *Bagre marinus* Mitchill 1815) is an example of a utilitarian substitution due to a stock decrease of sharks in the region. The similar flavor of these species favored an increase in the catfish fishery and a higher market value, resulting on a decrease on their stock population.

The production chain is a matrix of artisanal fishers that can comprise up to six steps (n = 47, 78.4%): 1) investment of goods and inputs, 2) catching target species, 3) bringing the catch to shore, 4) selling the species of commercial value (primary and secondary fish)

Table 1. Species related as primary fish by artisanal fishermen from the community of Guaxindiba, Brazil.

Common name	N	Scientific name	Fishery gear	Fishing zone
“Anchova”	15	*Pomatomus saltatrix*	L, SG	AR, M, OS
“Badejo”	9	*Mycteroperca microlepis*	B, SG	M, OS
“Bicuda”	7	*Sphyraena guachancho*	B, L	B, M, OS
“Bijupirá”	6	*Rachycentron canadum*	B, L	B, M
“Bonito”	8	*Euthynnus alletteratus*	B, SG	B, M
“Cação-anequim”	6	*Isurus oxyrinchus*	L, BL	B, M, OS
“Cação-areia”	12	*Carcharias taurus*	L, BL	B, M
“Cação-barriga-d'água”	6	*Carcharhinus* sp.	L, BL, SG	B, M, OS
“Cação-galha-preta”	27	*Carcharhinus porosus*	L, BL, SG	B, M
“Cação-olho-verde”	35	*Rhizoprionodon porosus*	L, BL, SG	AR, B
“Cação-panan”	6	*Sphyrna* sp.	BL, BT, BG	B
“Cação-tintureiro”	18	*Prionace glauca*	L, BL, SG	B, M
“Cação-torce-torce”	32	*Rhizoprionodon lalandii*	L, BL, SG	AR, B
“Cação-viola”	15	*Rhinobatos percellens*	BL, BT, BG	B
“Camarão-barba-russa”	37	*Artemesia longinaris*	BT	B, E
“Camarão-branco”	31	*Litopenaeus schmitti*	BT	B, E, M
“Camarão-rosa”	35	*Farfantepenaeus paulensis*	BT	B, M
“Camarão-sete-barbas”	52	*Xiphopenaeus kroyeri*	BT	B, E
“Cherne”	6	*Epinephelus niveatus*	B, SG	OS
“Dourado”	15	*Coryphaena hippurus*	B, L	OS
“Garoupa”	6	*Epinephelus marginatus*	B, SG	M, OS
“Lagosta-marrom”	20	*Panulirus* sp.	BT, BG	AR
“Lagosta-rainha”	20	*Panulirus argus*	BT, BG	AR
“Lula”	9	*Loligo* sp.	BT, PS	B, M
“Namorado”	14	*Pseudopercis numida*	BL, L	OS
“Pescada-banana”	16	*Nebris microps*	BL, BG, BT	B, E
“Pescada-branca”	25	*Cynoscion microlepidotus*	BL, BG, BT	B, E
“Pescada-selvagem”	45	*Cynoscion virescens*	BL, BG, BT	AR, B, E
“Prejereba”	10	*Lobotes surinamensis*	L, SG	B, M
“Robalo-fincudo”	42	*Centropomus undecimalis*	BL, BG	AR, E
“Robalo-peva”	48	*Centropomus parallelus*	BL, BG	AR, E
“Sarda”	21	*Scomberomorus maculatus*	L, SG	AR, M, OS
“Sargo-de-beiço”	32	*Anisotremus surinamensis*	BL, BG	AR
“Sargo-de-dente”	26	*Archosargus probatocephalus*	BL, BG	AR
“Tainha”	42	*Mugil liza*	RF, SG	B, E

N - Number of citations. Fishery gear: L - longline, BL - bottom line and hook, BT - bottom trawl net, TN - trammel net, SG - surface gillnet, BG - bottom gillnet and PS - purse seine net. Fishing zone: B - border area, E - estuary, AR - artificial reef, M - “Malacacheta” and OS - Open Sea.

to intermediaries or owners of local markets, 5) selling or donating mixed fish to family and friends, and 6) commercialization of fish in local markets. However, some fishers have tried to change the local production chain by selling fish they catch out of their home. In this new production chain model (n = 13, 21.6%) the fish is refrigerated, processed and sold directly to consumers, avoiding the intermediaries and owners of local markets.

Discussion

1. *Description of artisanal fishing and environmental interferences*

The artisanal fishers of Guaxindiba are mostly male, which is common in many Brazilian fishing communities (Alencar & Maia 2011, Côrtes et al. 2014, Fonseca et al. 2016). In general, fishers have a low

Table 2. Species related as secondary fish by artisanal fishermen from the community of Guaxindiba, Brazil.

Common name	N	Scientific name	Fishery gear	Fishing zone
"Bagre-amarelo"	39	*Aspistor luniscutis*	BT, BG	B, E
"Bagre-bandeira"	47	*Bagre bagre*	BL, BT, BG	B, E
		Bagre marinus	BL, BT, BG	B, E
"Bagre-branco"	45	*Genidens barbus*	BL, BT, BG	B, E
"Bagre-calafate"	21	*Notarius grandicassis*	BT, BG	B, E
"Bagre-urutu"	39	*Genidens genidens*	BT, BG	B, E
"Baiacú-ará"	24	*Lagocephalus laevigatus*	BL, L, BG	B, AR
"Barana"	9	*Elops saurus*	L, SG	B
"Carapeba"	15	*Diapterus rhombeus*	BL, L, BG	B
"Castanha"	6	*Umbrina canosai*	BT, BG, PS	B, E
"Cororoca"	7	*Orthopristis ruber*	BL, BG	B
"Corvina"	21	*Micropogonias furnieri*	BL,L,BT, BG	B, E
"Espada"	6	*Trichiurus lepturus*	BL, BG	B
"Goete"	34	*Cynoscion jamaicensis*	BL, BG, BT	B, E
"Goibira"	15	*Oligoplites saliens*	BL, BG	B, M
"Linguado"	7	*Syacium papillosum*	BT, BG	B, E
"Olhete"	8	*Seriola* sp.	L, SG	B, M
"Olho-de-cão"	6	*Priacanthus arenatus*	BL, L, BG	B
"Papa-terra"	16	*Menticirrhus americanus*	BT, BG, PS	B, E
"Pargo"	22	*Lutjanus analis*	BL, L	AR, M
"Parú"	15	*Chaetodipterus faber*	BL, BG, RF	AR
"Peroá-leste"	32	*Balistes vetula*	L, BL	M
"Peroá-preto"	45	*Balistes capriscus*	L, BL	M
"Pescadinha"	56	*Isopisthus parvipinnis*	BT, BG, PS	AR, B, E
		Macrodon ancylodon	BT, BG, PS	AR, B, E
"Raia-marrom"	15	*Hypanus americanus*	L, BT, BG	AR
"Raia-pintada"	9	*Aetobatus narinari*	L, BT, BG	B
"Sabãozinho"	11	*Peprilus paru*	BT, PS, BG	B, E
"Salema"	7	*Anisotremus virginicus*	BT, BG	B
"Xarelete"	11	*Caranx crysos*	L, SG	AR, B, M
"Xaréu"	11	*Caranx latus*	L, SG	AR, B, M
"Xixarro"	8	*Trachurus lathami*	L, SG	B, M

N - Number of citations. Fishery gear: L - longline, BL - bottom line and hook, BT - bottom trawl net, TN - trammel net, SG - surface gillnet, BG - bottom gillnet and PS - purse seine net. Fishing zone: B - border area, E - estuary, AR - artificial reef and M - "Malacacheta".

level of education and are highly dependent on fishing. Low education level affects the fishers socioeconomic situation and can interfere in the process of changing professions (Oliveira et al. 2016, Zappes et al. 2016). Artisanal fisheries involve fishers and their families as the main labor force related to catching and processing the fish. Socioeconomic data related to these fishers are similar to those recorded in other coastal areas of Brazil (Diegues 2008, Cunha 2009, Colaço 2012).

Fishing out of Guaxindiba port involves different gear related to the target species. Surface gillnets, bottom gillnets, and trammel nets are important to local fishing and are used to catch most of the commercially important species. Bottom trawl net is the most used fishing gear in the region to catch shrimp (*X. kroyeri* and *A. longinaris)* (Semensato & Di Beneditto 2008, Fernandes et al. 2014). The boats have low or medium autonomy, which limits the fishers to a few hours or days of consecutive fishing. In addition, fishing gear is limited to the type of boat. Trawling and purse seine nets, for example, are associated with larger boats with more powerful engines due to the mechanical traction associated with launching and pulling in the fishing nets. Many authors have noted that use and type of fishing gear are associated with the boats used by the fishing community (Diegues 2004, Begossi 2006, Oliveira et al. 2016).

Fishing effort in the region is concentrated along the coastal zone (border, ARs and PSR estuary). Only the larger boats can reach the farthest fishing grounds ("malacacheta" and open water) due to their greater autonomy (high speed, capacity of displacement and space to

Table 3. Species related as mixed, discarded and bait by artisanal fishermen from the community of Guaxindiba, Brazil.

Common name	N	Scientific name	Fishery gear	Fishing zone	C
"Boca-de-velho"	6	*Anchovia* sp.	BT, SG	AR, B, E	B
"Cabeça-dura"	10	*Stellifer brasiliensis*	BT, BG, PS	AR, B, E	M
		Stellifer rastrifer	BT, BG, PS	AR, B, E	M
		Stellifer stellifer	BT, BG, PS	AR, B, E	M
"Cangoá-relógio"	16	*Larimus breviceps*	BT, BG, PS	B, E	M
"Cobra-caramuru"	3	*Gymnothorax ocellatus*	BT, PS, BG	B, E	D
"Cobra-preta"	3	*Gymnothorax moringa*	BT, PS, BG	B, E	D
"Folha-de-mangue"	6	*Chloroscombrus chrysurus*	BT, OS	B, E	D
"Gurundeia"	5	*Haemulon aurolineatum*	BT, BG	B	M
"Língua-de-mulata"	5	*Trinectes paulistanus*	BT, BG, PS	B, E	D
"Manjuba"	5	*Anchoviella* sp.	BT, SG	B, E	B
"Maria-luiza"	12	*Paralonchurus brasiliensis*	BT, BG, PS	AR, E	M
"Maria-sapeba"	5	*Symphurus plagusia*	BT, BG, PS	B, E	D
"Ostra"	3	*Crassostrea* sp.	BG	AR	D
"Pargo-vermelho"	6	*Haemulon steindachneri*	BT, BG	B	M
"Peixe-galo"	6	*Selene setapinnis*	BT, BG	AR, E	M
"Peixe-voador"	6	*Dactylopterus volitans*	BT, PS, BG	B	M
"Piaba-olhuda"	5	*Pellona harroweri*	BT, PS	B, E	B
"Roncador"	6	*Conodon nobilis*	BT, BG	AR, B, E	M
"Sardinha-de-vidro"	5	*Odontognathus mucronatus*	BT, BG, PS	B, E	B
"Sardinha-laje"	8	*Opisthonema oglinum*	BT, OS	B, E	B
"Siri"	9	*Callinectes* sp.	BT	B, E	D

N - Number of citations. Fishery gear: BT - bottom trawl net, SG - surface gillnet, BG - bottom gillnet and PS - purse seine net Fishing zone: B - border area, E - estuary and AR - artificial reef. C - Category: M - mixed fish, D - discarded and B - bait.

store the fish). By living in this marine environment, the fishers can recognize and identify the best fishing grounds and environmental conditions to catch various target species. The empirical knowledge about the dynamics of environmental factors (winds, tides, currents and moon phases) and the ethology of the target species allow the fishers to understand the marine environment and consequently, increase their chances of catching the fishery resources (Diegues 2004, Begossi 2006, Berkes & Turner 2006).

2. *Characterization and commercialization of fish*

The fishers of Guaxindiba have vast LEK about fishing resources on the northern coast of Rio de Janeiro, which is demonstrated by their recognition of target species and their occurrence characteristics. In this study, 39 species were recorded as having commercial value in the region. This number is slightly greater than those recorded in previous studies conducted for fishing ports near our local study (Di Beneditto 2001: 23 species of commercial importance, Oliveira et al. 2016: 17 species, Zappes et al. 2016: 29 species).

The spatial distribution of the fish in the water column allowed some habitat categories to be assigned by the artisanal fishers. Studies indicate that spatial location of fish in a marine environment influences the definition of fishing strategies and fishing gear used by Brazilian fishers (*e.g.*, Diegues 1998, Silvano et al. 2006, Silvano & Begossi 2010, Bezerra et al. 2012). Most of the target species caught is concentrated near the coast (border, ARs and estuary). Some species are caught 30 km from the coast and have a high commercial value (some primary fish) due to their quality and size, besides the higher fishing cost in remote regions. In this context, the implementation of the ARs resulted in benefit to the fishers and these structures emerge as an ancillary fishing area to capture some commercial species as *C. parallelus*, *C. undecimalis, R. porosus* and *R. lalandii* (Lima et al. 2018). After the implantation of ARs the local fishermen captured some target species closer the coastal zone with fewer inputs. The role of ARs in the enrichment of fish stocks is demonstrated in others regions of the world (e.g., Portugal: Ramos et al. 2011, India: Kassim et al. 2013; France: Tessier et al. 2015, Philippines: Macusi et al. 2017, Scotland: Rouse et al. 2018).

Another important question to consider is that categorizing the target species is mainly based on their economic value, which is influenced by gastronomic characteristics and the amount of each available species. In Brazil, artisanal fishers use various emic systems to classify target species, which is dependent on use and cultural significance; however the fishermen preference may increase the pressure on certain target species (Berry 1999, Berkes et al. 2006, Silvano & Begossi 2012, Santos & Alves 2016).

Shrimp, especially the species *X. kroyeri*, is the main fishing resource in Guaxindiba. However, shrimp fishing with bottom trawl net capture a large number of young fish species and also some of lower commercial value (Eayrs 2007). It is estimated that for each kilo

Table 4. Annual calendar of the fish marketed by fishermen from the community of Guaxindiba, Brazil. Gray-light frames represent the months of capture of a target species and dark-gray frames represent the closed seasons established by Brazilian legislation (IBAMA 189/2008(1) and MPA/MMA 04/2015(2)).

Common name	Scientific name	J	F	M	A	M	J	J	A	S	O	N	D	US$	RS
"Anchova"	*Pomatomus saltatrix*								░	░	░	░	░	2.9	VU
"Bagre-amarelo"	*Aspistor luniscutis*	░	░	░	░						░	░	░	0.8	ND
"Bagre-bandeira"	*Bagre bagre*	░	░	░								░	░	2.0	NT
	Bagre marinus	░	░	░								░	░		
"Bagre-branco"	*Genidens barbus*					░	░	░	░					1.7	EN
"Bagre-calafate"	*Notarius grandicassis*	░	░	░	░						░	░	░	1.4	LC
"Bagre-urutu"	*Genidens genidens*					░	░	░	░					1.4	LC
"Baiacu-ará"	*Lagocephalus laevigatus*						░	░	░	░				2.3	LC
"Cação-galha-preta"	*Carcharhinus porosus*						░	░	░	░				4.2	CR
"Cação-olho-verde"	*Rhizoprionodon porosus*						░	░	░	░			░	4.2	VU
"Cação-torce-torce"	*Rhizoprionodon lalandii*						░	░	░	░			░	3.7	NT
"Cação-viola"	*Rhinobatos percellens*	░	░	░								░	░	2.9	NT
"Camarão-barba-russa"	*Artemesia longinaris*(1)	░	░	▓	▓	░	░	░	░	░	░	░	░	3.0	ND
"Camarão-rosa"	*Farfantepenaeus paulensis*(1)	░	░	▓	▓	░	░	░	░	░	░	░	░	4.2	ND
"Camarão-sete-barbas"	*Xiphopenaeus kroyeri*(1)	░	░	▓	▓	░	░	░	░	░	░	░	░	3.4	ND
"Camarão-branco"	*Litopenaeus schmitti*(1)	░	░	▓	▓	░	░	░	░	░	░	░	░	8.6	ND
"Corvina"	*Micropogonias furnieri*	░	░	░	░	░	░	░	░	░	░	░	░	2.3	LC
"Goete"	*Cynoscion jamaicensis*	░	░	░										1.7	LC
"Goibira"	*Oligoplites saliens*					░	░	░	░	░				4.0	LC
"Lagosta-comum"	*Panulirus* spp	░	░										░	8.6	ND
"Lagosta-rainha"	*Panulirus argus*	░	░										░	8.6	ND
"Papa-terra"	*Menticirrhus americanus*	░	░	░	░	░	░	░	░	░	░	░	░	1.7	LC
"Pargo"	*Lutjanus analis*	░	░	░	░	░	░	░	░	░	░	░	░	1.4	NT
"Parú"	*Chaetodipterus faber*	░	░	░	░								░	1.7	LC
"Pescada-banana"	*Nebris micros*	░	░	░										2.9	LC
"Pescada-branca"	*Cynoscion microlepidotus*	░	░	░									░	2.9	NT
"Pescada-selvagem"	*Cynoscion virescens*	░	░	░										5.2	NT
"Pescadinha"	*Isopisthus parvipinnis*	░	░	░	░								░	1.2	LC
	Macrodon ancylodon	░	░	░	░								░		
"Raia-marrom"	*Hypanus americanus*	░	░	░									░	0.6	VU
"Robalo-fincudo"	*Centropomus undecimalis*						░	░	░	░				5.7	NT
"Robalo-peva"	*Centropomus parallelus*						░	░	░	░				5.7	NT
"Sabãozinho"	*Peprilus paru*	░	░	░	░	░	░	░	░	░	░	░	░	0.6	LC
"Sarda"	*Scomberomorus maculatus*	░	░	░									░	2.9	LC
"Sargo-de-beiço"	*Anisotremus surinamensis*	░	░	░									░	2.9	NT
"Sargo-de-dente"	*Archosargus probatocephalus*	░	░										░	2.9	LC
"Tainha"	*Mugil liza*(2)	░	░	▓	▓	▓	▓	▓	▓	▓	░	░	░	2.9	LC
"Xarelete"	*Caranx crysos*	░	░	░	░						░	░	░	1.7	LC
"Xaréu"	*Caranx latus*	░	░	░	░						░	░	░	1.7	LC

US$ - Average value of the species (one pound) in dollars. RS - Risk situation of target species established by IUCN (2017) and (MMA, 2014): LC - least concern, NT - near threatened, VU - vulnerable, EN - endangered, CR - criticaly endangered and ND - no data.

of shrimp there is about four to six kilos of by-catch (Beckman 2013, Fernandes et al. 2014). In Guaxindiba, part of the by-catch (mixed fish) is sold at a low price and the other part (bait fish) is used to fish for species of higher commercial value. The shark species of the family Carcharhinidae, for example, deserve the greatest attention because they are particularly vulnerable to the effects of overfishing and also to bottom gears (Lack & Sant 2011). Studies conducted in the Southeast Region of Brazil have demonstrated that low-selectivity fishing gear is considered one of the factors responsible for the decrease in fishing stocks and degradation of the seabed (São Paulo: Souza et al. 2007, Rio de Janeiro: Vianna 2009, Fernandes et al. 2014, and Espírito Santo: Musiello-Fernandes et al. 2017).

Despite the frequent use of bottom trawling, this equipment is not used in the vicinity of the ARs. The ARs have provide refuge and feeding areas for local species, since these artificial structures prevent the use of bottom gears and their negative impact on seabed health (Seaman & Jensen 2000, Conceição & Nascimento 2009). Practices that control the use of active gears should be encouraged for the Guaxindiba coast. Thus, this study and several researches along 20 years suggest that the deployment of ARs can contribute to the sustainable management of fishery resources in northern Rio de Janeiro (Zalmon et al. 2002, Brotto et al. 2006, Santos et al. 2008, Santos et al. 2010, Rocha et al. 2014, Santos & Zalmon, 2015).

Defining fishing seasons is one of the rules adopted to limit the exploitation of fishery resources. In northern Rio de Janeiro, the Normative Instruction nº 195/2008 by IBAMA defines the fishing seasons and protection of reproductive periods of the ichthyofauna (IBAMA 195/2008). Some species, such as mullet (MPA/MMA 04/2015) and shrimp (IBAMA 189/2008) have their own rules that define off seasons when commercial fishing is prohibited. However, most species caught in northern Rio de Janeiro do not have fishing seasons defined and the seasons are determined by the fishers. In Guaxindiba, fishing season of target species is defined according to rainfall and outflow rates of Paraiba do Sul River. However, local fishers recognize that fishing period can change due to environmental variations that influence the life cycle (reproduction period) and ontogenetic cycle (differences in habitat between juveniles and adults) of some target species. Previous studies have also found an association between fishing period and greater precipitation or drought period in many communities along the Brazilian coast (*e.g.*, southeastern Brazil: Begossi 1996 and Silvano et al. 2010, northeastern Brazil: Costa-Neto 2001 and Pinto et al. 2015).

In general, the fish price in Guaxindiba has been influenced by the availability of target species and also by the marketing chain of fish. Intermediaries and owners of local markets that make up the end of the production chain of fish in the region are responsible for increasing the prices of target species caught by the artisanal fishers, which reduces the profit made by the fishers (producer). Vianna (2009), through diagnosing the production chain of fish in Rio de Janeiro, found that seasonality of target species supply is one of the factors that influence the final price of a fishing resource. In addition, the costs related to fishing include inputs (fuel, fishing gear, ice, meals on the boat) and fish processing (salting, filleting, deboning), which also influence the final price. Other studies in Brazil point that the length of the production chain is included in the final cost of the fish; the longer the production chain, the higher the final consumer price (Santos 2005, Rapozo 2011, Musiello-Fernandes et al. 2017). These studies demonstrated that higher price of commercialized fish species is due to intermediaries and owners of local markets, as occurs in Guaxindiba. Despite this usual way of commercializing fish, there have been initiatives to shorten the production chain and increase the profits of the producer (fisher). Thus, the creation of local cooperatives should be encouraged in order to make artisanal fishing socially just and environmentally less destructive. Long-term actions that consider such recommendations could contribute to co-managing fishery resources and maintaining artisanal fishing in northern Rio de Janeiro.

Our results provide different contributions to the fishery management on the northern coast of Rio de Janeiro related to the main target species. In addition, we show some data about the vulnerability of several local species. The use of these information's and the annual calendar of commercial species provide important data that can support new proposals for fishing regulations, especially fishing species not yet regulated by law. Our findings also provide new data about species associated to artificial structures, mainly of commercial interest. These results are important because it complements past data related to fish assemblages associated with ARs on the northern coast of Rio de Janeiro and reinforces the importance of these structures to local fishing.

Acknowledgments

The authors thank the artisanal fishers of Guaxindiba for their collaboration in the study and to Diviane Santos da Chagas Barreto, president of the Fishing Colony Z-1. JL Lima is grateful to the team from the Environmental Sciences Laboratory for their logistical support and to the Foundation for Research Support of the State of RJ (FAPERJ) for the doctoral fellowship. This research received financial resources from Research Foundation of Rio de Janeiro - FAPERJ (E-26/203.202/2016; E-26/202.770/2017) and the Brazilian Agency for Research Development - CNPq (301084/2016-5; 400053/2016-0; 301.259/2017-8). This study was also partially financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Author Contributions

Juliano Silva Lima: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Camilah Antunes Zappes: Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Ana Paula Madeira Di Beneditto: Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Ilana Rosental Zalmon: Contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALENCAR, C.A.G. & MAIA, L.P. 2011. Perfil Socioeconômico dos pescadores brasileiros. Arq. Cienc. Mar., 44: 12-19.

AMARAL, V.S., SIMONE, L.R.L. 2014. Revision of genus Crassostrea (Bivalvia: Ostreidae) of Brazil. J. Mar. Biol. Assoc. U. K., 94(4), 811–836.

BECKMAN, D. 2013. Marine Environmental Biology and Conservation. 1 ed. Jones & Bartlett Learning, Burlington. p. 1-452.

BEGOSSI, A. 1996. Fishing Activities and Strategies at Búzios Island. Fish. Res. Util. and Policy, 2: 125-141.

BEGOSSI, A. 2006. Temporal stability in fishing spots: conservation and co-management in Brazilian artisanal coastal fisheries. Ecol. Soc., 11: 1-25.

BERKES, F. & TURNER, N.J. 2006. Knowledge, learning and the evolution of conservation practice for social-ecological system resilience. Hum. Eco., 34: 479-494.

BERKES, F., MAHON, R., MCCONNEY, P., POLLNAC, R., POMEROY, R. 2006. Gestão da pesca de pequena escala: diretrizes e métodos alternativos. Ed. FURG, Rio Grande do Sul. p.1-360.

BERRY, J.W. 1999. Emics and etics: a symbiotic conception. Cul. Psy., 5: 165-171.

BEZERRA, D.M.S.Q., NASCIMENTO, D.M., FERREIRA, E.N., ROCHA, P.D., MOURÃO, J.S. 2012. Influence of tides and winds on fishing techniques and strategies in the Mamanguape river estuary, Paraíba State, NE Brazil. An. Acad. Bras. Cienc. (Impresso), 84: 775-787.

BRASIL. 2009. Decreto nº. 11.959, de 29 de junho de 2009. Dispõe sobre a Política Nacional de Desenvolvimento Sustentável da Aquicultura e da Pesca, regula as atividades pesqueiras, revoga a Lei no 7.679, de 23 de novembro de 1988, e dispositivos do Decreto-Lei no 221, de 28 de fevereiro de 1967, e dá outras providências. Diário Oficial da União, Brasília, 30 de junho de 2009, nº. 122, Seção 1: 1-3.

BRASIL. 2011. Instrução Normativa nº. 02, de 25 de janeiro de 2011. Dispõe sobre os procedimentos administrativos para a inscrição de pessoas físicas no Registro Geral da Atividade Pesqueira nas categorias de Pescador Profissional e de Aprendiz de Pesca no âmbito do MPA. Diário Oficial da União, 26 de janeiro de2011, nº. 18, Seção 1, p. 34-36.

BROTTO, D.S., KROHLING, W., ZALMON, I.R. 2006. Fish community modeling agents on an artificial reef on the Northern Coast of Rio de Janeiro – Brazil. Braz. J. Oceanogr., 54(4): 205-212.

CARVALHO FILHO, A. 1994. Peixes da Costa Brasileira. Marca D'água Ltda, São Paulo. p.1-304.

CASTELLO, J.P. 2007. Gestão sustentável dos recursos pesqueiros, isto é realmente possível? Pan-American J. Aquat. Sci. 2(1): 47-52.

CLIFFORD, J. 1998. Sobre a autoridade etnográfica. In: Gonçalves, J.R.S. (org.) A experiência etnográfica: antropologia e literatura do século XX. Ed. UFRJ, Rio de Janeiro. p.17-62.

COLAÇO, J. 2012 Segredos, Pescadores e Etnógrafos. Vivência: Rev. Antr., 40: 121-130.

CÔRTES, L.H.O, ZAPPES, C.A., DI BENEDITTO, A.P.M. 2014. Ethnoecology, gathering techniques and traditional management of the crab Ucides cordatus Linnaeus, 1763 in a mangrove forest in south-eastern Brazil. Ocean Coast. Manage. 93: 129-138.

COSTA, R.C., FRANSOZO, A., MELO, A.S., FREIRE, F.A.M. 2003. Chave Ilustrada Para Identificação Dos Camarões Dendrobranchiata Do Litoral Norte Do Estado De São Paulo, Brasil. Biota Neotrop. 3(1): 1-12.

COSTA-NETO, E.M. 2001. A Cultura Pesqueira do Litoral Norte da Bahia. Etnoictiologia, Desenvolvimento e Sustentabilidade. EDUFBA, Salvador, EDUFAL, Maceió. p.1-159.

CUNHA, L.H.O. 2009. O mundo costeiro: temporalidades, territorialidades, saberes e alternativas. Desenvolvimento e Meio Ambiente. 20: 59-67.

DI BENEDITTO, A.P.M. 2001. A pesca artesanal na costa norte do Rio de Janeiro. Bioikos, 15: 103-107.

DI BENEDITTO, A.P.M., RAMOS, R.M.A., LIMA, N.R.W. 1998. Fishing on Northern Rio de Janeiro States (Brazil) and relation with small cetaceans. Braz. Arch. Biol. Technol., 41(3): 296-302.

DIEGUES A.C.S. 2008. Marine protected areas and artisanal fisheries in Brazil. In: Samudra monograph. International Collective in Support of Fishworkers. p.1-54.

DIEGUES, A.C.S. 1998. Environmental impact assessment: the point of view of artisanal fishermen communities in Brazil. Ocean Coast. Manage., 39: 119-133.

DIEGUES, A.C.S. 2004. A pesca construindo sociedades. NUPAUB, São Paulo. p.1-315.

EAYRS, S. 2007. A guide to bycatch reduction in tropical shrimp-trawl fisheries. FAO - Food and Agricultural Organization of the United Nations, Rome. p.1-124.

ESCHMEYER, W. N. & FONG, J. D. Catalog of Fishes. https://www.calacademy.org/scientists/projects/catalog-of-fishes (last accessed 20/07/2018).

FAO - Food and Agriculture Organization of the United Nations. 2016. The state of world fisheries and aquaculture. Contributing To Food Security And Nutrition For All. FAO, Rome. p.1-204.

FERNANDES, L.P., KEUNECKE, K.A., DI BENEDITTO, A.P.M. 2014. Produção e Socioeconomia da Pesca do Camarão sete-barbas no norte do estado do Rio de Janeiro. Bol. Inst. Pesca, 40(4): 541-555.

FIGUEIREDO, J.L. & MENEZES, N.A. 1977. Manual de peixes marinhos do sudeste do Brasil. II. Introdução, Cação, Raias e Quimeras. Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-104.

FIGUEIREDO, J.L. & MENEZES, N.A. 1978. Manual de peixes marinhos do sudeste do Brasil. II. Teleostei (1). Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-110.

FIGUEIREDO, J.L. & MENEZES, N.A. 1980a. Manual de peixes marinhos do sudeste do Brasil. III. Teleostei (2). Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-93.

FIGUEIREDO, J.L. & MENEZES, N.A. 1980b. Manual de peixes marinhos do sudeste do Brasil. IV. Teleostei (3). Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-98.

FIGUEIREDO, J.L. & MENEZES, N.A. 1985. Manual de peixes marinhos do sudeste do Brasil. V. Teleostei (4). Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-107.

FIGUEIREDO, J.L. & MENEZES, N.A. 2000. Manual de peixes marinhos do sudeste do Brasil. VI. Teleostei (5). Museu de Zoologia da Universidade de São Paulo, São Paulo. p.1-116.

FIPERJ - Fundação Instituto de Pesca do Estado do Rio de Janeiro. 2015. Relatório, Rio de Janeiro. p.1-174.

FIPERJ - Fundação Instituto de Pesca do Estado do Rio de Janeiro. 2013. Diagnóstico da Pesca do Estado do Rio de Janeiro, Rio de Janeiro. p.1-101.

FONSECA, M., ALVES, F., MACEDO, M.C., AZEITEIRO, U.M. 2016. O papel das mulheres na pesca artesanal marinha: estudo de uma comunidade pesqueira no município de Rio das Ostras, RJ, Brasil. J. Integ. Coast. Zone Manage., 16(2): 231-241.

FROESE, R. & PAULY, D. 2017. FishBase. World Wide Web electronic publication. http://www.fishbase.org/search.php (last accessed 20/07/2018).

GIRALDES, B.W. & SMYTH, D.M. 2016. Recognizing Panulirus meripurpuratus sp. nov. (Decapoda: Palinuridae) in Brazil - Systematic and biogeographic overview of Panulirus species in the Atlantic Ocean. Zootaxa 4107(3): 353-366.

HANAZAKI, N., BERKES, F., SEIXAS, C., PERONI, N. 2013. Livelihood diversity, food security and resilience among the Caiçara of Coastal Brazil. Hum. Ecol. Rev., 41: 153–164.

IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 2008a. Instrução Normativa Nº 189, de 23 de Setembro de 2008. IBAMA, Brasília.

IBAMA - Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis. 2008b. Instrução normativa n° 195, de 2 de outubro de 2008. IBAMA, Brasília.

ICMBIO - Instituto Chico Mendes de Conservação da Biodiversidade. 2011. Boletim Estatístico da Pesca e Aquicultura. ICMBIO, Brasília. p.1-60.

IUCN - International Union for Conservation of Nature. 2017. UCN red list of threatened species. http://www.iucnredlist.org (last accessed 20/07/2018).

JEREB, P. & ROPER, C.F.E. 2010. Cephalopods Of The World: An Annotated And Illustrated Catalogue Of Cephalopod Species Known To Date. Vol. 2. Myopsid and Oegopsid Squids. FAO Species Catalogue for Fishery Purposes. Food and Agriculture Organization of the United Nations, Rome. p.1-605.

JOHANNES, R.E. 2002. The Renaissance of Community Based Marine Resource Management in Oceania. Annu. Rev. Ecol. Syst. 33:317-340.

KENDALL, L. 2008. The conduct of qualitative interview: Research questions, methodological issues, and researching online. In Handbook of research on new literacies (J. Coiro, M. Knobel, C. Lankshea, D.J. Leu, eds). Lawrence Erlbaum Associates, Nova York. p. 133-149.

LACK, M. & SANT, G. 2011. The Future of Sharks: A Review of Action and Inaction. TRAFFIC International and the Pew Environment Group. p.1-44.

LIMA, J.S, ZAPPES, C.A.DI BENEDITTO, A.P.M., ZALMON, I.R. 2018. Artisanal fisheries and artificial reefs on the southeast coast of Brazil: Contributions to research and management. Ocean Coast. Manage. 163: 372-382.

LYRA-NEVES, R.M., SANTOS, E.M., MEDEIROS, P M., ALVES, R.R.N., ALBUQUERQUE, U.P. 2015. Ethnozoology in Brazil: analysis of the methodological risks in published studies. Braz. J. Biol., 75: 184-191.

MALINOWSKI, B.K. 1984. Os pensadores: Argonautas do Pacífico Ocidental: Um relato do empreendimento e da aventura dos nativos nos Arquipelagos da Nova Guine Melanesia, second ed. Abril Cultural, São Paulo. p.1-424.

MMA – Ministério do Meio Ambiente. 2014. Instrução Normativa nº 14, de 14 de Outubro de 2004. MMA, Brasília.

MPA/MMA – Ministério da Pesca e Aquicultura e Meio Ambiente. 2015. Portaria nº 04, de 14 de Maio de 2015. MPA & MMA, Brasília.

MURILO, V., SILVA, C.G., FERNANDES, G.B. 2009. Nearshore Sediments and Coastal Evolution of Paraíba do Sul River Delta, Rio de Janeiro, Brazil. J. Coastal Res. 56(1): 650-654.

MUSIELLO-FERNANDES, J., ZAPPES, C.A., HOSTIM-SILVA, M. 2017. Small-scale shrimp fisheries on the Brazilian coast: Stakeholders perceptions of the closed season and integrated management. Coast. Zone Manage., 148: 89-96.

OLIVEIRA, P.C., DI BENEDITTO, A.P.M., BULHÕES, E.M.R., ZAPPES, C.A. 2016. Artisanal fishery versus port activity in southern Brazil. Coast. Zone Manage., 129: 49-57.

OTA, Y. & JUST, R. 2008. Fleet sizes, fishing effort and the “hidden” factors behind statistics: Na anthropological study of small-scale fisheries in UK. Mar. Policy, 32(3): 301-308.

PÉREZ, J.A.A., PEZZUTO, P.R., RODRIGUES, L.F., VALENTINI, H., VOOREN, C.M. 2001. Relatório da reunião técnica de ordenamento da pesca de arrasto nas regiões Sudeste e Sul do Brasil. Facimar, 5: 1-34.

PINTO, M.F., ALVES, R.R.N., MOURÃO, J.S. 2015. Use of ichthyofauna by artisanal fishermen at two protected areas along the coast of Northeast Brazil, Journal of Ethnobiology and Ethnomedicine, 11(20): 1-32.

POMEROY, R., PARKS, J., POLLNAC, R., CAMPSON, T., GENIO, E., MARLESSY, C., HOLLE, E., PIDO, M., NISSAPA, A., BOROMTHANARAT, S., NGUYEN, T.H. 2007. Fish Wars: Conflict and Collaboration in Fisheries Management in Southeast Asia. Mar. Policy, 31(6): 645-656.

RAPOZO, P. 2011. A renda da água: Trabalhadores da pesca e as redes de comercialização na Amazônia brasileira. REDD, 4(1): 1-22.

ROCHA, D.F., FRANCO, M.A.L., GATTS, P.V., ZALMON, I.R., 2014. The effect of an artificial reef system on the transient fish assemblages – south-eastern coast of Brazil. J. Mar. Bio. Assoc. U.K., 95(3): 635–646.

RYAN, G. & BERNARD, H.R. 2000. Data management and analysis methods. In Handbook of Qualitative Research (N.K. Denzin & Y.S. Lincoln, eds). Sage, Lon- don. p. 769-802.

SANTOS, C.A.B. & ALVES, R.R.N. 2016. Ethnoichthyology of the indigenous Truká people, Northeast Brazil. J. Ethnobiol. Ethnomed., 12: 1-10.

SANTOS, L.N, ZALMON, I.R. 2015. Long-term changes of fish assemblages associated with artificial reefs off the northern coast of Rio de Janeiro, Brazil. J. Appl. Ichthyol.. 31: 15-23.

SANTOS, L.N., BROTTO, D.S., ARAÚJO. F.G. 2008. Artificial structures as tools for fish habitat rehabilitation in a Neotropical reservoir. Aquat. Conserv., 18: 896-908.

SANTOS, L.N., BROTTO, D.S., ZALMON, I.R., 2010. Fish responses to increasing distance from artificial reefs on the Southeastern Brazilian coast. J. Exp. Mar. Biol. Ecol., 386: 54-60.

SANTOS, M.A.S.D. 2005. A cadeia produtiva da pesca artesanal no Estado do Pará: Estudo de Caso no nordeste paraense. Amazônia Cienc. Des., 1(1): 61- 81.

SIEBER S.S., SILVA T.C., CAMPOS, L.Z.O., ZANK, S., ALBUQUERQUE, U.P. 2014. Participatory Methods in Ethnobiological and Ethnoecological Research. In Methods and Techniques in Ethnobiology and Ethnoecology (U.P. Albuquerque, L.V.F.C. Cunha, R.F.P. Lucena, R.R.N. Alves, eds). Springer, New York Heidelberg Dordrecht, London, p. 39-58.

SILVA, V.A, NASCIMENTO, V.T., SOLDATI, G.T., MEDEIROS, M.F.T.; ALBUQUERQUE, U.P. 2014. Techniques for Analysis of Quantitative Ethnobiological Data: Use of Indices. In Methods and Techniques in Ethnobiology and Ethnoecology (U.P. Albuquerque, L.V.F.C. Cunha, R.F.P. Lucena, R.R.N. Alves, eds). Springer, New York Heidelberg Dordrecht, London, p. 379-395.

SILVANO, R. A. M. & VALBO-JORGENSEN. 2008. Beyond fishermen’s tales: contributions of fisher’s local ecological knowledge to fish ecology and fisheries management. Environ. Dev. Sustain., 10: 657-675.

SILVANO, R.A.M. & BEGOSSI, A. 2010. What can be learned from fishers? An integrated survey of fishers’ local ecological knowledge and bluefish (Pomatomus saltatrix) biology on the Brazilian coast. Hydrobiologia, 637: 3-18.

SILVANO, R.A.M. & BEGOSSI, A. 2012. Fishermen’s local ecological knowledge on Southeastern Brazilian coastal fishes: contributions to research, conservation, and management. Neotrop. Ichthyol. (Imp), 10: 133-147.

SILVANO, R.A.M., GASALLA, M.A., SOUZA, S.P., 2009. Applications of Fishers’ Local Ecological Knowledge to Better Understand and Manage Tropical Fisheries. In Current Trends in Human Ecology (P. Lopes & A. Begossi, eds). Newcastle upon Tyne, Cambridge Scholars Publishing, p. 76-100.

SILVANO, R.A.M., MACCORD, P.F.L., LIMA, R.V., BEGOSSI, A. 2006. When does this fish spawn? Fishermen’s local knowledge of migration and reproduction of Brazilian coastal fishes. Environ. Biol. Fish., 76: 371–86.

SOUZA, M.R., CARNEIRO, M.H., QUIRINO-DUARTE, G., SERVO, G.J.M. 2007. Caracterização da Mistura na pesca de arrasto de parelha desembarca em Santos e Guarujá, São Paulo, Brasil. Bol. Inst. Pesca, 33(1): 43-51.

TUDESCO, C.C., FERNANDES, L.P., DI BENEDITTO, A.P.M. 2012. Population structure of the crab *Callinectes ornatus* Ordway, 1863 (Brachyura: Portunidae) bycatch in shrimp Bol. fishery in northern Rio de Janeiro State, Brazil. Biota Neotrop., 12(1): 93-98.

VALENTIN, J.L. & MONTEIRO-RIBAS, W.M. 1993. Zooplankton community structure on the east-southeast Brazilian continental shelf (18-23ºS latitude). Cont. Shelf Res., 13(4): 407-424.

VASCONCELLOS, M., DIEGUES, A.C., KALIKOSKI, D.C. 2011. Coastal fisheries of Brazil. In Coastal fisheries of Latin America and the Caribbean 544 (S. Salas, R. Chuenpagdee, A. Charles, J.C. Seijo, eds), Rome: FAO - Food and Agriculture Organization of the United Nations, 430p.

VIANNA, M. 2009. Diagnóstico da cadeia produtiva de pesca marítima no Estado do Rio de Janeiro: Relatório de pesquisa. FAERJ/SEBRAE-RJ, Rio de Janeiro p.1-200.

ZALMON, I.R, NOVELLI, R.N., GOMES, M.P., FARIA, V.V. 2002. Experimental results of an artificial reef program on the Brazilian coast north of Rio de Janeiro. J. Mar. Sci., 59: 83–87.

ZAPPES, C.A.; OLIVEIRA, P. C.; DI BENEDITTO, A.P.M. 2016. Percepção de pescadores do norte fluminense sobre a viabilidade da pesca artesanal com a implantação de megaempreendimento portuário. Bol. Inst. Pesca, 42(1): 73-88.

Received: 24/07/2018
Revised: 09/01/2019
Accepted: 26/02/2019

Biota Neotropica 19(2): e20180697, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Reproduction and growth in a Neotropical arboreal mouse: *Oecomys rutilus* (Sigmodontinae: Cricetidae) in French Guiana

François Catzeflis[1]*

[1]*Université de Montpellier, Institut des Sciences de l'Evolution, Montpellier, France*
*Corresponding author: François Catzeflis, e-mail: *francois.catzeflis@umontpellier.fr*

CATZEFLIS F. **Reproduction and growth in a Neotropical arboreal mouse: *Oecomys rutilus* (Sigmodontinae: Cricetidae) in French Guiana**. Biota Neotropica. 19(2): e20180697. http://dx.doi.org/10.1590/1676-0611-BN-2018-0697

Abstract: Postnatal growth and development of the small Neotropical mouse *Oecomys rutilus* (Sigmodontinae: Cricetidae) were investigated from birth to day 143, in the laboratory. Morphometric measurements at age of 3 days, of both sexes combined, revealed body weight to be 3.4 ± 0.3 g, mean tail length as 27.4 ± 1.1 mm, and mean hind foot length as 9.3 ± 0.7 mm. Body weight was found to increase steadily until at least 69 days, whereas the instantaneous growth rates of other measurements declined earlier: the daily growth of hind foot length declined to a minimum at age of 24 days, and the growth of tail and of ear declined by the age of 33 days. Average litter size for 12 captive births was 2.5, ranging from 2 to 3. The preserved eye crystalline lens was weighted in 23 captive-born animals of known age, allowing a rough estimate of the age of reproduction in wild-caught animals. Based on the inferred relation between eye-lens weight and age, the youngest reproductive (pregnant) wild-caught females had an estimated age of 90 and 95 days.

Keywords: *allometry, Gompertz model, rodents, eye-lens weight, life history.*

Reprodução e crescimento de um rato arbóreo neotropical: *Oecomys rutilus* (Sigmodontinae: Cricetidae) na Guiana Francesa

Resumo: O crescimento pós-natal e o desenvolvimento do pequeno roedor neotropical Oecomys rutilus (Sigmodontinae: Cricetidae) foram investigados desde o nascimento até o dia 143, em laboratório. Mensurações morfométricas na idade de 3 dias, de ambos os sexos combinados, revelaram a massa corporal de 3,4 ± 0,3 g, comprimento médio da cauda de 27,4 ± 1,1 mm, e comprimento médio do pé de 9,3 ± 0,7 mm. A massa do corpo aumentou rapidamente até o dia 69, enquanto as taxas de crescimento instantâneo de outras medidas diminuíram mais cedo: o crescimento diário do pé diminuiu para o mínimo na idade de 24 dias, e os crescimentos da cauda e da orelha diminuíram na idade de 33 dias. O tamanho de prole médio para 12 nascimentos em cativeiro foi de 2,5, variando de 2 a 3. As lentes preservadas do cristalino do olho foram pesadas em 23 animais nascidos em cativeiro de idades conhecidas, permitindo fazer uma estimativa da idade de reprodução em animais capturados na natureza. Baseado na relação inferida entre peso da lente do cristalino e idade, as mais jovens fêmeas (prenhes) capturadas na natureza tiveram uma estimativa de 90 a 95 dias de idade.

Palavras-chave: *alometria, modelo de Gompertz, roedores, peso do cristalino do olho, história de vida.*

Introduction

Being able to ascertain age is important in studies of mammals, as the knowledge of age structures of populations is necessary for understanding the life history strategies (Martin et al., 2011). When samples of field-caught mammals are taken from a population, it is usually impossible to assign a known age to any specimen unless birth was observed and the individual was uniquely marked for later identification. The procedures for establishing absolute and relative ages can be standardized by study of individuals of known age (Morris 1972), and this is were captive-born animals can bring much useful data. Increases in linear dimensions and weight may be useful indicators of age during the earliest portions of a mammal's life but rapidly lose their usefulness once adult dimensions are reached (Hoffmeister and Zimmerman 1967; Morris, 1972).

The weight of the eye crystalline lens increases with age and thus many studies of age structures of populations have relied on weighing the lenses removed from mammal specimens (see Lord 1959 and review in Friend 1967; Morris 1972). Several investigators (Dapson and Irland 1972; Myers et al. 1977) found eye lens weight increasing linearly during approximately the first year of life in several species of myomorph rodents. For example, Birney et al. (1975) have shown that, in the cotton rat *Sigmodon hispidus*, both body weight and eye lens weight were regularly growing with age during the first weeks of life, inasmuch that a strong (i.e. within 95% confidence intervals) correlation between age and weight lasted much less for body (until to ca. 70 days) than for the eye lens (until to ca. 150 days). In this study, eye lens weights of 22 captive-born specimens with known age will provide the first data linking age and eye lens growth in a small arboreal Neotropical rodent.

Here we are interested in the reproduction and in ontogenetic allometry, ie the description of differential growth in individual ontogeny (Gould, 1966; D'Andrea et al., 1996) of a neotropical Cricetidae. By rearing small arboreal mice in captivity, we aim at addressing changes of one part to another during growth, through measurements taken in six metric features at different ages after birth.

In early life, growth in most kinds of mammals is continuous, but some structures cease growth sooner than others, hence not all morphological features are useful for aging individuals in a population. Growth rates of mammals are most commonly described by the sigmoidal function of the Gompertz model, although other models sometimes perform equally well (Zullinger et al., 1984).

It is a matter of common observation that not all parts of the body grow at a uniform rate (D'Andrea et al., 1996). A newborn mouse, for example, is obviously short-tailed, relative to body length, as contrasted with a mouse that has attained adult proportions (Green and Fekete, 1933).

The Neotropical *Oecomys rutilus* Anthony, 1921 (Sigmodontinae: Cricetidae) is a small-sized oryzomyine (average weight of 18 - 22 g), externally characterized by soft fur, short and broad hindfeet with relatively long toes, and a tail moderately longer than the head and body (tail length about 115 % of head and body length). The fur is soft, and moderately long in length. Dorsal coloration of adults varies from tawny to reddish brown, bright in tone; ventral pelage color is pure white. Tail hairs relatively long, typically concealing distal scale rows and forming conspicuous terminal tuft (5– 11 mm per Voss et al. 2001). Females possess eight mammae (Carleton and Musser, 2015).

In French Guiana (Voss et al. 2001; Catzeflis et al., 2014), nearly all examples of O. *rutilus* were captured in primary forest, with few animals in secondary growth. At Trois-Sauts Amerindian villages, the arboreal *O. rutilus* has also been caught in traditional houses, together with the more common and slightly larger *O. bicolor* (Catzeflis, 2012). As so far documented, the geographic range of O. *rutilus* adheres to the Guiana subregion of Amazonia, along with those of *O. auyantepui* and *O. rex* (Carleton and Musser, 2015).

This study brings an additional taxon to the data base of growth patterns in New World cricetid rodents examined by Creighton and Strauss (1986), and it is the first case for an arboreal sigmodontine from the Neotropical rainforests.

Materials and Methods

Wild-caught *Oecomys rutilus* come from the primary rainforest (upland, terra firme) from Les Nouragues scientific station (municipality of Regina, French Guiana), and were obtained in July 1998 and in May 1999, with due permission from the acting director of the campsite.

Two males (N-330; N-251) and one female (N-217) were caught via canopy traps in large trees at heights 32, 14, and 15 m, respectively; one female (N-351) was caught at 3 m height when visiting the food stores of a wooden lodge of the camp. The four wild-caught breeders passed a routine health examination at the French Veterinary Services (Department of Agriculture and Forests) at Cayenne in September 1998 and June 1999, respectively.

Animals were kept in a large terrarium (in cm: 400 length x 60 breadth x 80 height) filled by 5 to 7 cm of earth mixed with vegetable-mould, and containing a few dry natural branches. Artificial wooden nest-boxes (12 x 12 x 12 cm) with a single circular opening (diameter 30 mm) were randomly scattered on the ground, with always at least two more nest-boxes than pairs of adult animals.

Food (various seeds, grains and fruits) and water were given ad libitum.

We checked the content of the nest-boxes every 3 to 5 days. When discovering a new litter, we refrained from handling the mother and youngs until the third day of age. Then, at each control, we gently seized each juvenile for taking the following measurements in six metric features: weight (in grams), tail (without terminal hair-tuft; in mm); hind foot (without claws), vibrissae from the muzzle (mystacial vibrissae), vibrissae from above eye (superciliary vibrissae), and ear (from inner notch).

Obviously many factors, such as litter size, age and condition of the mother, differential mortality of smaller young, and laboratory conditions, influence the growth rates of individual young (Myers and Master, 1983). Yet, the mean values approximate the general pattern of growth in these features for a typical individual (Creighton and Strauss, 1986). Data were also recorded on the mean length of the gestation period.

Upon termination of the breeding experiments (27 August 2000), animals were sacrificed and preserved as vouchers deposited in the collections of the Museum National d'Histoire Naturelle (MNHN, Paris). For 23 specimens (see vouchers numbers in Appendix 1) with known age, the eye-lens (eye crystalline) could be preserved, cleaned, dried, and weighed with a dedicated analytical balance (model AE-240 of Mettler-Toledo company, Bekasi -Indonesia) to the nearest tenth of mg. Among various "saturation" models available for linking eye-lens weight and age, we selected the Gompertz equation as implemented in the software PAST (Hammer et al., 2011). Other saturation models do exist, such as Bertalanffy, or Michaelis-Menten models, or else polynomial, but we used Gompertz' for the sake of comparison with published studies (see discussions in Creighton and Strauss, 1986; Zullinger et al., 1984).

The parameters obtained for each metric feature were the asymptotic length (mm) or mass (g) A; the constant growth rate (b) and the age at the first inflexion point of the growth curve (c) in the Gompertz equation. Estimates of adult size were based either on measurements of wild-caught specimens or on the asymptotic size calculated from the growth curves using the Gompertz model.

Choosing 90% of the asymptotic size provides a landmark from within the range of observed data that indicates the age at which a constant proportion of total exponential growth has occurred (further details in Creighton and Strauss, 1986). For assessing the role of sexual size dimorphism, parametric (paired t-tests) and non-parametric tests (Mann-Whitney) were done with the software PAST (Hammer et al., 2011).

For comparing the estimated asymptotic growth values of the captive-bred animals, we used biometric data for 40 wild-caught *Oecomys rutilus* (20 females, 20 males) originating from different localities in French Guiana (see Appendix 2 for details). Our data set includes 3 juveniles (with only two erupted molars), 4 sub-adults (with three molars but no dental wear), and 33 adults. Adult wild-caught females include 7 pregnant and 4 lactating individuals.

All procedures with animals (captures in the wild, rearing in captivity, handling, euthanasy) followed the ethical guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes and Gannon 2011).

Results

From the captive breeding colony, we obtained a total of 12 litters (on average 2.5 young per litter, from 2 to 3) between 25 August 1998 and 13 June 2000. Five consecutive litters are separed by intervals of 26 to 31 days (average 28.8 days), which suggests a gestation time of about four weeks. Reproduction in captivity occurred in May (2 births of litters), June (2), July (2), August (2), September (1), and October (3).

In French Guianan wild-caught animals, seven females were found pregnant, with a mean of 2.3 embryos (range from 1 to 3); pregnant and/or lactating females were caught in June (3 individuals), July (1), September (1), October (4), and November (2).

As previously observed in other sigmodontine rodents (see Voss, 1991, for *Zygodontomys*; or Percequillo et al., 2008, for *Cerradomys*), there is no sexual size dimorphism in wild-caught adult animals of *O. rutilus* (17 females versus 16 males: t-tests and/or Mann-Whitney tests: data not shown), thus growth measurements did not consider each sex separately.

The growth pattern was examined in six external variables, for which a minimum of 3 young animals could be measured at each of 25 different ages ranging from 3 to 143 days after birth, and the mean values are detailed in Table 1. (For the body weight, data span a much longer period up to 567 days, with 42 additional ages, each with at least 3 measures).

Plotting the absolute values (in g for the body weight, otherwise in mm for other variables) for the 25 age classes (Figure 1) indicates that maximum size is essentially reached by the age of 55 days.

The Gompertz model provides the following asymptotic values: Tail 92.5 mm (correlation $r = 0.739$); Hindfoot 18.3 mm ($r = 0.635$), Ear 11.7 mm ($r = 0.734$), Mystacial vibrissae 36.3 mm ($r = 0.680$), Supraciliary vibrissae 23.3 mm ($r = 0.672$), and Body weight 17.9 g ($r = 0.862$).

Relative growth values are plotted on Figure 2, which shows that the first variable to reach 90% of its final (asymptotic) size is the Ear (at age 22 days), followed by the Hindfoot (at 24 days), the Supraciliary vibrissae (25 days), the Mystacial vibrissae and the Tail (30 days each). Finally, the body weight continues to grow for a longer span, reaching 90% of asymptotic completion by the age of 50 days.

The weight of the eye lens in captive-born animals (Figure 3) has a very steep growth during the first two months - from 0.9 mg at age of 11 days to 8.1 mg at age of 67 days -, and then the slope of the curve diminishes but the growth continues during the next 12 months, at least. The oldest animals (aged 405 and 430 days) have an eye lens weighting 14.4 and 15.4 mg, respectively. The examination of the growth of the eye-lens in 23 captive *Oecomys rutilus* of known ages ($N = 17$ ages ranging from 11 to 430 days) indicates a regular increase for that organ, and there is a rather good fit of the data to the Gompertz model (Akaike IC = 27.0; $r = 0.903$). Wild-caught animals (sample size of 40 individuals) have eye lens weights ranging from 3.0 mg (a juvenile with only two erupted molars) to 14.1 mg (an adult male with worn molars). After excluding 7 pregnant females from the data set of wild-caught animals, there is a good linear correlation ($r = 0.839$, $p < 0.0001$, $N = 52$) between eye-lens weight and body-weight in the complete data set (30 wild-caught and 22 captive-born: see Figure 4).

Discussion

In *Oecomys rutilus*, the average litter size measured in captivity (mean of 2.5 youngs for 12 litters) is not different from the average number of embryos in wild-caught females (mean of 2.3 for 7 pregnant individuals; see also Catzeflis et al., 2018). And the values of this study are comparable to those found in an other small species of the genus - *O. bicolor* -, with mean values ranging from 2.1 to 2.6 (Patton et al., 2000; Hice and Velazco, 2012; Catzeflis et al., 2018).

Interbirth intervals range from 26 to 31 days (average 29 days), but we caution that we have only five data; in a breeding colony of *Zygodontomys brevicauda*, interbirth intervals ranged from 24 to 28 days, with a mode at 25 days for 53 out of 71 observations (Voss et al., 1992).

In *O. rutilus*, the growth of newborn animals is very intense during the 30 first days, and then stabilizes around 52 to 55 days of age; a similar pattern is illustrated for a few taxa of Sigmodontinae by Creighton and Strauss (1986), as well as in various species of the Old-World murine genus *Apodemus* (Pelz et al., 1996). Among the five metric variables of this study, the length of the hind foot grows the fastest, as 90% of its asymptotic value is reached by the age of 24 days, with an average length of 16.6 mm to be compared with a mean of 18.5 mm in 33 adult wild-caught animals. The slower growing measures, as illustrated on Figure 2, are the Tail and the Ear length which reach 90% of their asymptotic value by the age of 33 days.

Asymptotic values (A) derived from Gompertz model are in adequacy with mean values for 33 adult wild-caught animals (W), although three variables are clearly smaller in captive bred animals: Tail length ($A = 92.5$; $W = 98.6 \pm 5.5$), Ear ($A = 11.7$; $W = 14.2 \pm 0.8$), and Weight ($A = 18.0$, $W = 21.1 \pm 3.3$). The other three measurements are similar between captive-bred and wild-caught animals: Hindfoot ($A = 18.3$; $W = 18.5 \pm 0.8$); Mystacial vibrissae ($A = 36.6$; $W = 37.3 \pm 2.9$); and Supraciliary vibrissae ($A = 24.3$; $W = 25.2 \pm 3.1$).

During the younger ages of *O. rutilus*, no potential effect of sexual differences could be evidenced, as shown by paired t-tests ($p = 0.160$ for mean; $p = 0.199$ for median) or Mann-Whitney unpaired tests ($p = 0.809$; U = 10’480) for comparing 26 males and 26 females aged 3 to 55 days after birth. This is similar to some other sigmodontine rodents, like *Akodon lindberghi* (De Conto and Cerqueira, 2007), for which no significant sexual size dimorphism is found during growth of the young in captivity.

We interprete the gestation length as ranging from 26 to 31 days (average of 29 days for 5 values), but this is only true if fecundation was occurring just after parturition. A value of 29 days for gestation in this arboreal oryzomyine compares well with other sigmodontine data presented by Moscarella and Aguilera (1999) such as 30 days for *Sigmodon hispidus* or *Nectomys rattus*, and 29 days for *Holochilus brasiliensis* and *H. sciureus*.

Table 1. Average values (in mm. except for weight in g) for six metric features in captive *Oecomys rutilus* aged from 3 to 143 days after birth.

Age (days)	Tail	Hind foot	Ear	Mystacial vibrissae	Superciliary vibrissae	Weight	Sample size
3	27.4	9.3	3.8	6.7	4.3	3.4	5
5	34.6	11.0	5.8	9.5	6.0	3.7	4
6	36.5	11.3	6.0	9.8	6.5	4.8	3
7	38.8	12.6	6.1	12.0	7.8	4.5	4
11	55.0	14.7	7.8	16.2	11.4	6.5	5
13	63.3	15.3	9.0	19.0	13.0	5.9	4
18	68.2	16.3	9.6	24.8	16.8	8.5	5
24	79.7	16.6	11.0	30.3	21.5	9.2	5
31	83.9	17.4	10.8	33.8	22.8	12.6	4
33	86.7	18.2	11.7	36.0	23.7	13.7	3
38	86.3	17.1	11.2	33.8	21.5	14.6	6
44	88.0	17.0	11.6	33.0	21.3	15.0	6
45	90.7	17.8	11.2	36.0	22.3	15.7	3
52	93.3	18.3	11.6	36.0	23.3	16.5	7
55	93.3	17.8	11.8	35.4	22.8	15.4	3
59	91.5	17.3	11.8	34.8	22.3	15.9	6
65	93.3	17.9	11.7	34.0	24.0	16.2	7
66	94.0	17.8	11.7	34.0	23.7	16.1	3
69	93.0	17.8	12.3	33.0	24.3	18.4	3
72	90.8	17.5	11.6	34.5	23.3	16.5	5
75	94.0	17.8	11.7	32.0	24.0	17.5	3
85	91.7	17.7	11.7	34.0	22.7	16.5	3
99	93.0	17.5	11.8	36.3	24.0	16.8	3
120	92.7	17.3	12.0	33.7	21.7	18.1	3
143	93.3	17.5	12.5	33.3	21.3	19.5	4

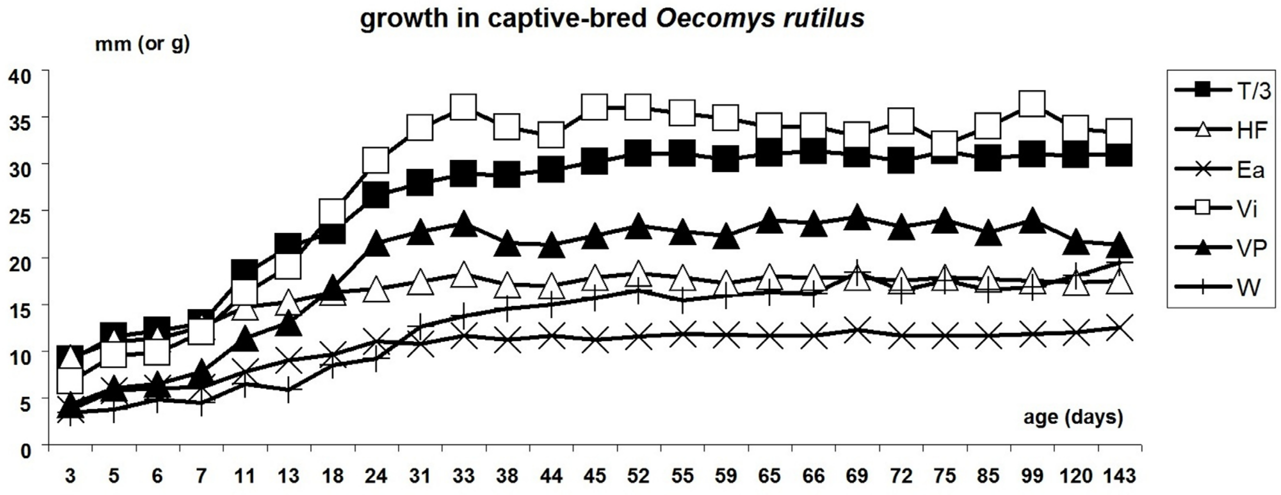


Figure 1. Growth values in captive bred *Oecomys rutilus*. Ages cover 25 classes ranging from 3 days after birth until 143 days, and each age class has at least 3 individuals measured. For the sake of clarity along the vertical axis, the Tail values have been divided by 3.

Abbreviations: T = tail; HF = hind foot; Ea = ear; Vi = mystacial vibrissae; VP =superciliary vibrissae; W = weight. A mere visual inspection suggests that maximum size is reached by the age of ca. 55 days.

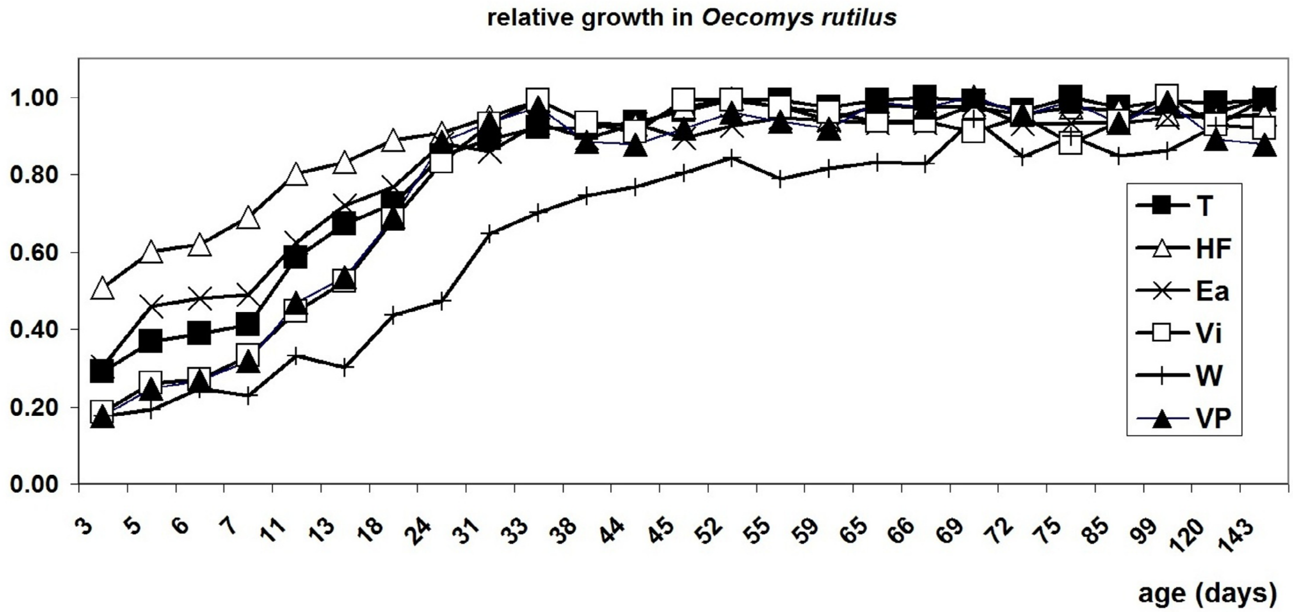


Figure 2. Relative growth values in captive bred *Oecomys rutilus*. Values expressed as percentage of maximum size reached after 55 days of age. Abbreviations as in Figure 1.

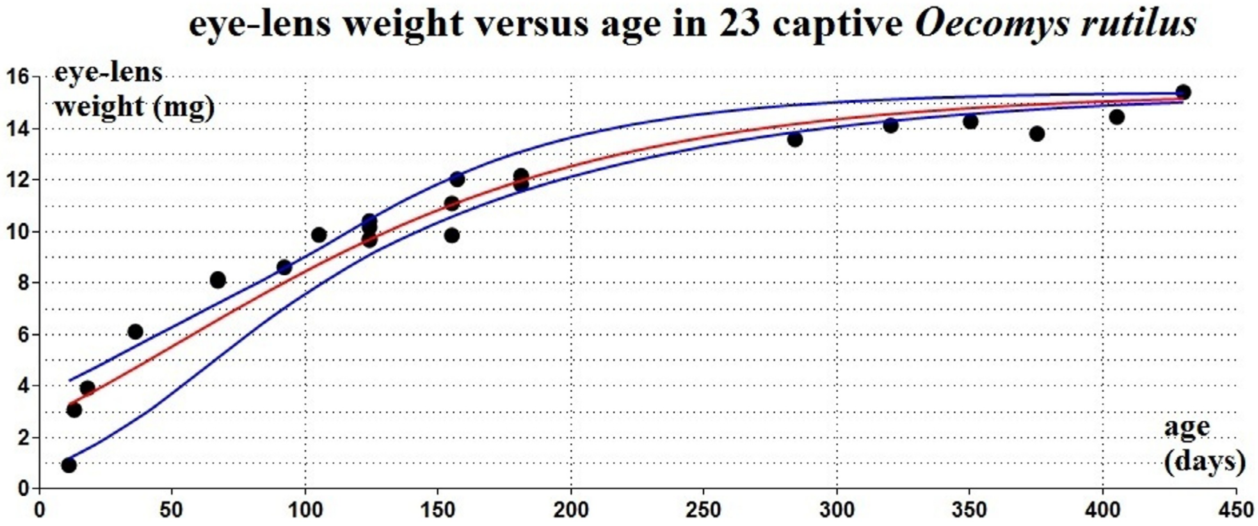


Figure 3. Eye lens weight in 23 captive bred *Oecomys rutilus* of known age. The curve (red) is a fit through the Gompertz model, bordered by 95% confidence intervals (blue).

Among the various "saturation" models available for linking eye-lens weight and age, the Michaelis-Menten formula had the lowest Akaike IC (10.67) as compared to other models (see under Methods): its parameters ($y = ax/(b+x)$) were a = 17.4 (95% confidence interval 16.3 to 18.5) and b = 81.5 (60.5 to 97.4). Since the Gompertz model seems to be the one that best fits most mammalian growth curves (D'Andrea et al., 1996; Pillay, 2001; Zullinger et al., 1984), and for the sake of comparison with published studies, we applied this model to our data which resulted in a slightly higher Akaike IC (24.58) and in the following values for the main parameter: a = 15.42 (95% confidence interval 15.40 to 15.44); b = - 1.82 (-2.17 to -0.62), and c = -0.01 (-0.011 to -0.006).

Thus, it appears that the two wild-caught pregnant females with the smallest eye-lens (7.7 and 8.2 mg) have an age of about 90 and 95 days, respectively; our data would suggest that breeding can occur at an age as early as three months in females of *Oecomys rutilus*.

In studies of relative age, the technique of regression analysis is often used (Sokal and Rohlf 1995). With known-age individuals, age is a non-random variable and thus the quantity measured - such as eye lens weight - must be regressed on age. With samples of unknown age, age can be estimated by the method of inverse prediction (Dapson and Irland 1972; Sokal and Rohlf 1995) using values obtained from the measured variable (e.g., eye-lens or body weight), and Rowe et al.

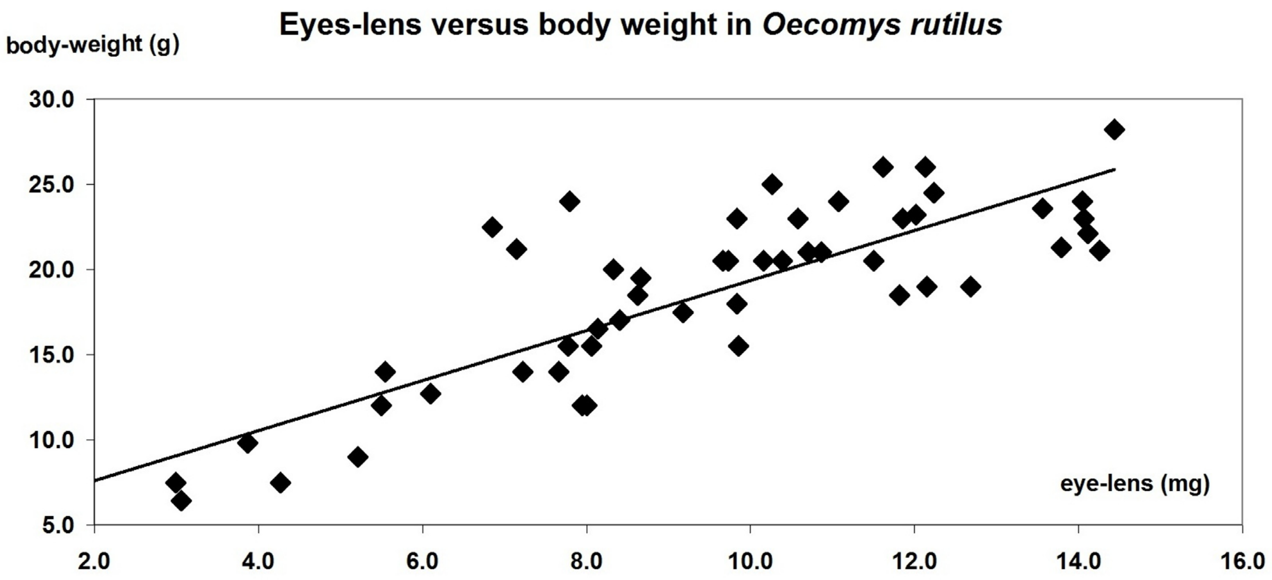


Figure 4. Eye-lens weight versus body weight in 52 *Oecomys rutilus* (30 captive-born and 22 wild-caught), with a good linear correlation (r = 0.839; p <0.0001) between these two variables.

(1985) have shown that, in house mice (*Mus musculus*), eye-lens weight-age relationship were comparable in captive and wild-caught animals.

Thus, this study provides a first clue to assess the age in wild-caught specimens of the arboreal *Oecomys rutilus*, in the case preserved specimen have their eye-lens kept, cleaned, and dried for a precise weight. When animals can not be sacrificed in the field, a careful measure of their body weight can provide an indirect estimate of their age, via the rather good correlation between body weight and eye-lens weight.

Supplementary material

The following online material is available for this article:

Appendix 1

Appendix 2

Acknowledgements

The friendly hospitality of Pierre Charles-Dominique, director of the Nouragues field station, has been much appreciated, as was the technical help of Jean-François Mauffrey in the field. At Montpellier University, Delphine Bosch generously offered the use of a dedicated analytical balance for weighing eye-lenses at the Earth Sciences Department, and Elisabeth Cabirou executed the cleaning of the eye crystalline lenses with much care. With his usual kindness, Nilton Caceres translated the summary into Brazilian Portuguese, and Philip Dunn helped improve my awful English language - many thanks! This work has benefited from an "Investissements d'Avenir" grant managed by Agence Nationale de la Recherche (CEBA, ref. ANR-10-LABX-25-01).

Authors' Contributions

François Catzeflis is accountable for the whole of that article: Data acquisition, analysis, interpretation, and overall writing.

Conflicts of interest

The author declare that they have no conflict of interest related to the publication of this manuscript.

References

BIRNEY, E. C., R. JENNESS, and D. D. BAIRD. 1975. Eye lens proteins as criteria of age in cotton rats. J. Wildlife Manag. 39:718-728.

CARLETON, M. D., and G. G. MUSSER. 2015. Genus *Oecomys* Thomas 1906, p. 393 - 416. *In:* Mammals of South America, Volume 2. Rodents. J. L. Patton, U. F. J. Pardinas, and G. D'Elia (eds.). The University of Chicago Press, Chicago.

CATZEFLIS, F. 2012. A survey of small non-volant mammals inhabiting Wayampi Amerindian houses in French Guiana. Mammalia. 76:327-330.

CATZEFLIS, F., S. BARRIOZ, J.-F. SZPIGEL, and B. DE THOISY. 2014. Marsupiaux et Rongeurs de Guyane. Institut Pasteur de la Guyane, Cayenne, Guyane française.

CATZEFLIS, F. M., B. K. LIM, and C. R. DA SILVA. 2018. Litter size and seasonality in reproduction for Guianan rodents and opossums, Studies on Neotropical Fauna and Environment. https://doi.org/10.1080/01650521.2018.1528655

CREIGHTON, G. K., and R. E. STRAUSS. 1986. Comparative patterns of growth and development in cricetine rodents and the evolution of ontogeny. Evolution. 40:94-106.

D'ANDREA, P. S., A. C. HORTA, R. CERQUEIRA, and L. REY. 1996. Breeding of the water rat (*Nectomys squamipes*) in the laboratory. Lab. Anim. 30:369-376.

DAPSON, R. W., and J. M. IRLAND. 1972. An accurate method of determining age in small mammals. J. Mammal. 53:100-106.

DE CONTO, V., and R. CERQUEIRA. 2007. Reproduction, development and growth of *Akodon lindberghi* (Hershkovitz, 1990) (Rodentia, Muridae, Sigmodontinae) raised in captivity. Braz. J. Biol., 67:707-713.

FRIEND, M. 1967. A review of research concerning eye lens weight as a criterion of age in animals. New York Fish and Game Journal. 14:152-165.

GOULD, S. J. 1966. Allometry and size in ontogeny and phylogeny. Biol. Rev. 41:587-640.

GREEN, C. V., and E. FEKETE. 1933. Differential growth in the mouse. Journal of Experimental Zoology. 66:351-370.

HAMMER, Ø., D. A. T. HARPER, and P. D. RYAN. 2011. PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica. 4:1-9.

HICE, C. L., and P. M. VELAZCO. 2012. The non-volant mammals of the Reserva Nacional Allpahuayo-Mishana, Loreto, Peru. Special Publications of the Museum of Texas Tech University. 60:1-135.

HOFFMEISTER, D. F., and E. G. ZIMMERMAN. 1967. Growth of the skull in the Cottontail (*Sylvilagus floridanus*) and its application to age-determination. The American Midland Naturalist. 78:198-206.

LORD, R. D. 1959. The lens as an indicator of age in cottontail rabbits. The Journal of Wildlife Management. 23:358-360.

MARTIN, R. E., R. H. PINE, and A. F. DEBLASE. 2011. A Manual of Mammalogy: with Keys to Families of the World. Waveland Press, Long Grove, Illinois, USA.

MORRIS, P. 1972. A review of mammalian age determination methods. Mammal Review. 2:69-104.

MOSCARELLA, R. A., and M. M. AGUILERA. 1999. Growth and reproduction of *Oryzomys albigularis* (Rodentia: Sigmodontinae) under laboratory conditions. Mammalia. 63:349-362.

MYERS, K., J. CARSTAIRS, and N. GILBERT. 1977. Determination of age of indigenous rats in Australia. The Journal of Wildlife Management 41:322-326.

PATTON, J. L., M. N. F. DA SILVA, and J. R. MALCOLM. 2000. Mammals of the Rio Jurua and the evolutionary and ecological diversification of Amazonia. Bull. Am. Mus. Nat. Hist. 244:1-306.

PELZ, H.-J., H. GEMMEKE, R. HUTTERER, and U. JÜDES. 1996. Jugendentwicklung der Brandmaus *Apodemus agrarius* (Mammalia: Muridae) im Vergleich zu anderen Arten der Gattung. Bonner zoologische Beiträge. 46:233-247.

PERCEQUILLO, A. R., E. HINGST-ZAHER, and C. R. BONVICINO. 2008. Systematic review of genus *Cerradomys* Weksler, Percequillo and Voss, 2006 (Rodentia, Cricetidae, Sigmodontinae, Oryzomyini), with description of two new species from eastern Brazil. American Museum Novitates 3622:1-46.

PILLAY, N. 2001. Reproduction and postnatal developmental in the bush Karoo rat *Otomys unisulcatus* (Muridae, Otomyinae). J. Zool., Lond. 254:515-520.

ROWE, F. P., A. BRADFIELD, R. J. QUY, and T. SWINNEY. 1985. Relationship between eye lens weight and age in the wild House Mouse (*Mus musculus*). Journal of Applied Ecology. 22:55-61.

SIKES, R. S., and W. L. GANNON. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy. 92:235-253.

SOKAL, R. R., and F. J. ROHLF. 1995. Biometry: the principles and practice of statistics in biological research. W. H. Freeman & Co, New York.

VOSS, R. S. 1991. An introduction to the Neotropical muroid rodent genus *Zygodontomys*. Bull. Amer. Mus. Nat. Hist. 210:1-113.

VOSS, R. S., P. D. HEIDEMAN, V. L. MAYER, and T. M. DONNELLY. 1992. Husbandry, reproduction and postnatal development of the Neotropical muroid rodent *Zygodontomys brevicauda*. Laboratory animals. 26:38-46.

VOSS, R. S., D. P. LUNDE, and N. B. SIMMONS. 2001. The mammals of Paracou, French Guiana: A Neotropical lowland rainforest fauna. Part 2: Nonvolant species. Bull. Amer. Mus. Nat. Hist. 263:1-236.

ZULLINGER, E. M., R. E. RICKLEFS, K. H. REDFORD, and G. M. MACE. 1984. Fitting sigmoidal equations to mammalian growth curves. J. Mamm. 65:607-636.

Received: 20/11/2018
Revised: 14/02/2019
Accepted: 19/02/2019

Biota Neotropica 19(2): e20170333, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Diversity of Meliponini and anothers Apiformes (Apidae *sensu lato*) in a Cerrado fragment and its surrounding, Campo Grande, MS

Antonia Railda Roel[1], Ricardo Dias Peruca[1,2]*, Felipe Varussa de Oliveira Lima[3], Kwok Chiu Cheung[1], Armando Araujo Neto[1], Leticia Vieira Da Silva[1] & Suellen Soares[1]

[1]Universidade Católica Dom Bosco, Campo Grande, MS, Brasil.
[2]Agência de Desenvolvimento Agrário e Extensão Rural - Gerência Desenvolvimento Agrário, Campo Grande, MS, Brasil.
[3]Universidade Federal da Grande Dourados, Dourados, MS, Brasil.
**Corresponding author: Ricardo Dias Peruca, e-mail: ricardoperuca@yahoo.com.br*

ROEL, A. R., PERUCA, R. D., LIMA, F. V. O., CHEUNG, C., ARAUJO NETO, A., SILVA, L. V., SOARES, S. **Diversity of Meliponini and anothers Apiformes (Apidae *sensu lato*) in a Cerrado fragment and its surrounding, Campo Grande, MS**. Biota Neotropica. 19(2): e20170333. http://dx.doi.org/10.1590/1676-0611-BN-2017-0333

Abstract: The aim of this study was to evaluate the diversity of Meliponini and anothers Apiformes (Apidae *sensu lato*) from a Cerrado fragment and its surrounding. Malaise traps were installed in a pasture near a fragment of Cerrado and its surrounding, from October to December 2010. Samples were collected at seven-day intervals and preserved in Dietrich solution. The apiforms specimens from each sample were separated in morphospecies and sent to identification. The tribe Meliponini was the most abundant in open areas of pasture than in the Cerrado fragment. The Meliponini species present were *Trigona* gr. *fulviventris*, *Paratrigona lineata*, *Trigona recursa*, *Partamona* sp, *Trigona spinipes*, *Trigona* gr. *hyalinata*, *Trigona* gr. *fulviventris*, *Tetragonisca fiebrigi*, *Tetragona clavipes*, *Frieseomelitta* cf. *flavicornis*, *Lestrimelitta rufipes*. The dominant species was *Paratrigona lineata*, comprising 82.47% of all species collected.

Keywords: *native bees, pollinators, stingless bees.*

Diversidade de Meliponini e demais Apiformes (Apidae *sensu lato*) em fragmento de Cerrado e entorno, Campo Grande, MS

Resumo: O objetivo deste estudo foi avaliar a diversidade de Meliponini e demais apiformes (Apidae *sensu lato*) de um fragmento de Cerrado e seus arredores. Armadilhas de Malaise foram instaladas em área de pastagem próximas a fragmento de Cerrado, de outubro a dezembro de 2010. As amostras foram coletadas em intervalos de sete dias e conservadas em solução de Dietrich. Das amostras foram separados os espécimes apiformes em morfoespécies e enviadas para identificação. A tribo Meliponini foi a mais abundante em áreas abertas de pastagem que em áreas de fragmento de Cerrado. As espécies de Meliponini presentes foram de Meliponini *Trigona* gr. *fulviventris*, *Paratrigona lineata*, *Trigona recursa*, *Partamona* sp, *Trigona spinipes*, *Trigona* gr. *hyalinata*, *Trigona* gr. *fulviventris*, *Tetragonisca fiebrigi*, *Tetragona clavipes*, *Frieseomelitta* cf. *flavicornis*, *Lestrimelitta rufipes*. A espécie dominante com 82,47% de indivíduos coletados foi *Paratrigona lineata*.

Palavras-chave: *abelhas nativas, polinizadores, abelhas sem ferrão.*

Introduction

Bees are responsible for the pollination of plants in general, ensuring the fruit formation and the perpetuation of plant species. On the other hand, bees are rewarded with nectar supply and pollen from the plant flowers visited. According to Kerr et al. (1996), these insects are responsible for 40 to 90% of the pollination of native plants in a tropical environment. They are the best pollinator in Cerrado Biome, where they pollinate 60 to 75% of all plants species (Silberbauer-Gottsberger & Gottsberger 1988).

In Brazil there are about 3,000 bee (Apidae) species described, from a total of 17,533 bee species described in the world, with recent estimations of 20,000 (Michener 2007). Many species of native bees are in danger of extinction for several reasons. Among the main factors responsible for the extinction of bees are the fragmentation of original vegetation and the use of non-selective insecticides (Turner 1996, Kerr et al. 1996). Bees of the Tribe Meliponini, otherwise known as native stingless bees, are important pollinators for plants like strawberry, guarana, açai, coffee, and other cultivated species (Malagodi-Braga & Kleinert 2004).

The maintenance of honeybees in the field is very important for crop plants, but this practice is not very common in developing countries. In the United States the annual value of pollination by bees has been estimated at US$ 14,6 billion, thus justifying its importance for agriculture (Morse & Calderone 2000). The levels of plant pollination in Brazil are usually unsatisfactory, which cause reduction of crop yields, particularly those that depend on pollinators such as bees (Imperatriz-Fonseca 2010).

Currently, public policies are being discussed in order to assess the necessity of maintain native vegetation areas, their efficiency in maintaining beneficial organisms in adjacent areas, and how to measure this efficiency or interference in the altered environment. According to Freitas et al. (2009), the causes of pollinators' population declining in agricultural areas may be the deforestation of native vegetation, inadequate use of agricultural practices, such as the use of pesticides and monoculture. Native vegetation areas have nectar and pollen source plants for pollinators, and continuously provide flowers throughout the year for nesting and breeding (Larsen et al. 2005). However, little attention has been given to the impact of agricultural products on pollinators, especially in cultivated areas (Freitas & Pinheiro 2010). Identifying the diversity of bees present in cultivation sites close to native vegetation areas can become a tool in the evaluation of the effect of agricultural practices.

Therefore, the aim of this work was to estimate the diversity of Apidae family (*sensu lato*) in a Cerrado fragment and its surrounding in Campo Grande, MS, Brazil.

Material and Methods

The experiment was performed at an experimental area on São José farm, Campo Grande, MS, in October, 2010. The experimental area was divided into three sub areas in two different environments, inside and outside the of the Cerrado fragment. Six Malaise traps (Townes 1972) were installed, three in the outside fragment area (A) and three inside the Cerrado fragment area (B). A distance of 100 meters separated each Malaise trap installed. The geographical coordinates per site of collection were: In Pasture area: 1) Latitude 20°24'13.16"S and Longitude 54°36'45.69"W; 2) Latitude 20°24'09.60"S and Longitude 54°36'47.32"W; 3) Latitude 20°24'03.62"S and Longitude 54°36'44.93"W. In the Cerrado fragment: 1) Latitude 20°24'11.87"S and Longitude 54°36'42.20"W; 2) Latitude 20°24'08.35"S and Longitude 54°36'43.83"W; 3) Latitude 20°24'06.22"S and Longitude 54°36'42.63"W.

The samples were collected once a week, during a period of four weeks. The collected material was packed in glass vials containing 70% alcohol. In the laboratory the Apidae family (*sensu lato*) (apiforms) were removed from the total samples and were separated in Tribe Apini, Meliponini, Bombini and Euglossini. Dr. Gabriel Mello from Universidade Federal do Paraná, PR, carried out the Identification.

The type of soil is neossolo and the climate is humid tropical, characterized by a pronounced dry season in the winter and rainy season in summer, annual average temperature is 23.0 °C. At the time the experiments were carried out the temperature was high and there was a lot of rain. The seasonal climate date was obtained from a center installed at the University Farm (Table 1).

The area has three remaining fragments of the original vegetation of Cerrado Biome and constitute the Legal Reserve of the Property according to the current Forest Code. These three areas have 9.9 ha, 5.2 ha and 0.6 ha respectively, comprising a total of 15.6 ha of native vegetation. The most common trees in the area are Pau-terra-folha-grande (*Qualea grandiflora* Mart.), pau-terra-folha-pequena (*Qualea multiflora* Mart.), pau-pombo (*Matayba ghuianensis* Aubl.), Capororoca (*Myrsine ferrugínea* Spreng.), pimenta-de-macaco (*Xylopia aromatica* (Lam.) Mart.) and lixeira (*Curatella americana* L). These species together make up more than 50% of the wealth of the three fragments of tree presents. Some species of figueira (Moraceae) and many individuals of *Alibertia sessilis* Vell. K. Schum. (marmelinho do campo) are common, and occurs in high frequency and have wide distribution. In open areas (pasture), the lighting changes the composition edge, favoring some individuals like *Dipteryx alata* Vogel (Cumbaru), *Cecropia pachystachya* Trécul. (Embankment) and *Annona crassiflora* Mart. (Marolo).

Results and Discussion

There were 994 bees of the Apinae subfamily (Corbiculate bees), collected from two areas (Pasture and Cerrado) during four weeks. In total, 726 individuals were sampled from pasture sites and 268 individuals were sampled from Cerrado fragment sites. The most abundant tribe was Meliponini (n=672; 72% in Pasture and n=258; 26% in Cerrado), followed by Bombini (n=14; 12 in Pasture and 2 in Cerrado), Apini (n=2, 2 in pasture and 0 in Cerrado), Euglossini (n=4; 2 in Pasture and 2 in Cerrado) and other Apiforms (n=44; 40 in pasture and 4 in Cerrado) (Table 2).

In this study, nine species from Meliponini were found: *Frieseomelitta* cf. *flavicornis* (Fabricius 1798), *Lestrimelitta rufipes* (Friese 1903), *Paratrigona lineata* (Lepeletier 1836), *Partamona* sp., *Tetragona clavipes* (Fabricius 1804), *Tetragonisca fiebrigi* (Schwarz 1938), *Trigona* gr. *fulviventris* (Guérin 1844), *Trigona* gr. *hyalinata* (Lepeletier 1836), *Trigona recursa* (Smith 1863), *Trigona spinipes* (Fabricius 1793), one morphospecies from Bombini (*Bombus* sp. 1); one specie from Apini (*Apis mellifera* Linnaeus 1758); two morphospecies from Euglossini and seven from other Apidae s. lato, *Tropidopedia*

Table 1. Weekly climate data, average temperature, total precipitation and average Wind velocity, in the experimental area, latitude 20°26'50"S and longitude 54°43'21"W, Campo Grande, MS, October, 2010.

Sampling date	Average Temperature (ºC) (max-min)	Total Precipitation (mm) (max-min)	Average Wind (km/h) (max-min)
04/10/2010	21.98(34.2-13.9)	12.4(2.2-0.0)	7.01(33.8-22.5)
11/10/2010	23.41 (32.8-25.3)	3.2(2.0-0.0)	8.34(11.6-3.1)
18/10/2010	24.40(34.8-17.2)	51.6(54.4-0.0)	7.0(12.9- 2.3)
25/10/2010	21.83(34.3-13.4)	43.40(26.4-0.0)	7.72(13.4-3.3)

Table 2. Numbers of Apidae and other apiforms specimens, collected during four weeks, in Malaise traps, in pasture areas, around Cerrado fragment, Campo Grande, MS, October, 2010.

Family/Tribe	week 1		week 2		week 3		week 4	
	Pastage	Cerrado	Pastage	Cerrado	Pastage	Cerrado	Pastage	Cerrado
Meliponini	129	38	158	98	209	76	176	46
Bombini	2	0	3	2	7	0	0	0
Apini	0	2	0	0	0	0	0	0
Euglossini	0	0	0	0	0	0	2	2
Others apiforms	2	0	4	0	22	0	12	4
Total	133	40	165	100	238	76	190	52
Meliponini (%)	96.99	95.00	95.76	98.00	87.82	100	92.63	88.46

flavolineata (Aguiar & Melo 2007) (Tapinotaspidini)*, Exomalopsis* sp. 1. (Exomalopsini), Halictinae – seven morphospecies. Meliponini was the predominant tribe in the two environments, the Cerrado fragment and pasture around.

In this area few individuals (2) of *Apis mellifera* (Apinae) were found. Krug & Alves-dos-Santos (2008) reported from surveys in Santa Catarina that the dominant bee was *Peponapis fervens* (Eurecini) showing 51% of all visits to flowers, followed by *A. mellifera* with 46% and the other three species of native bees represented 3% of all visits to the pumpkin flowers. D'Avila & Marchini (2008), working in a Cerrado area in São Paulo State, observed greater abundance of the subfamily Apidae, or *A. mellifera* (55.8%), followed by *Trigona spinipes* (14.4%) and *Exomalopsis* sp. (8.3%). The authors concluded that the low diversity of bees might be due to fragmentation and the plantation of exotic species (*Pinus* and *Eucalyptus*) in the surroundings areas of this fragment. In a survey developed at the Private Reserve of Natural Heritage Eliezer Batista, State of Mato Grosso do Sul, Aoki et al. (2012) reported bees belonging to *A. mellifera* as the most abundant species (50% of total).

It was observed a variation according to the collection area (pasture and cerrado fragment) and sampled week (Figure 1). This could be explained by the fact that these insects are known as good flyers and live in social colonies. As a consequence, it could increase the probability of occasional catches in certain areas, with varying resource availability. The local climate, temperature, rain and wind may also help to understand the changes between different weeks of collection. The predominance of stingless bees (Meliponini) in the pasture area (Figure 1) may be due to a large capacity of flight to catch pollen from the flowers, which is more accessible in open areas.

In the pasture area it was observed an increase in the abundance of Meliponini at the three first weeks, with 129, 158, and 209 individuals captured. But in the fourth week a reduction was observed (176) (Figure 1). When fewer individuals were collected, it was observed a decrease in temperature (Table 1).

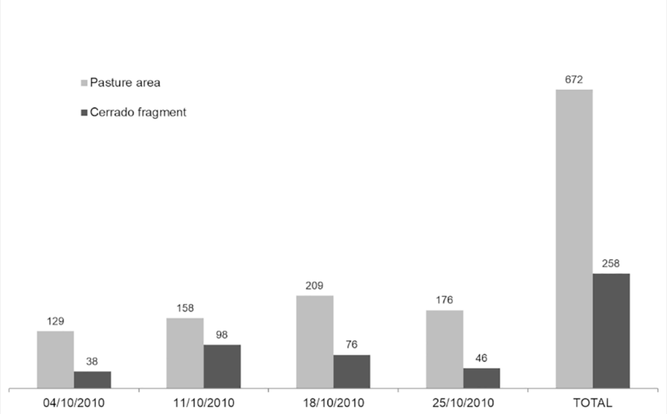


Figure 1. Abundance flutuation of Meliponini, collected during four weeks in Malaise traps in Pasture area around a Cerrado fragment. Campo Grande, MS, October, 2010.

The average temperature during the experiment ranged from 19 to 24°C, indicating excellent conditions for foraging. However, in the third and fourth week, heavy rains occurred with a total of 51.6 and 43.40 mm (Table 1). This cannot be the cause of a reduction in the number of collected individuals observed in all experimental areas.

Considering only Meliponini in the Cerrado fragment, it was observed a small number of stingless bees compared to the pasture area, for all traps, totalizing 258 individuals, whereas in the pasture area, 672 individuals of stingless bees were collected. However, a reduction was observed in the first and fourth week of collection (pasture and Cerrado fragment). In addition, an increase in the third week was observed (Figure 1).

The population dynamics of insects in the field is directly related to weather conditions, rain, wind and especially temperature. According to Silveira Neto et al. (1995) temperature is an important factor in the activity of the bees, which prefer a time of the day when the temperature is around 25°C for foraging. Thomazini & Thomazini (2002) reported that an increase in temperature and less rain, increased bee numbers, at Rio Branco, Acre.

According to Kerr et al. (1996) around 400 species of Meliponini were described and at least 100 are in danger of extinction due to human activities, and to the loss of natural habitats. According to Imperatriz-Fonseca (2010) it is necessary the conservation of the forest for the maintenance of bee populations and pollination services on agricultural landscapes.

The colonies of stingless bees, unlike Apini, have multiple queens or bee masters, but only one queen is fertilized. In order to breed, the worker bee stay in the alveoli, which is full of honey, pollen and glandular secretions, where the larvae feed alone. The honey is deposited in pots of wax nests of hollow trees, wood and abandoned termite mounds. However, the species *Trigona spinipes* (Fabr.), bee Arapuá, is a citrus pest, which destroys leaves and flowers. In the case of native bees, which are social insects, pollen is used to feed the larvae, after collected from nearby plants. Therefore, the availability of flowers also influences the dynamics of the population. Social bees have no food preference, like *Apis mellifera* (Apinae) and *T. spinipes* (Meliponini), which explains the abundance of different species of plants (D'Avila & Marchini 2008).

In this study the most frequent species was *Paratrigona lineata* (Lepeletier 1836) with 82% (n=767) of all Meliponini captured, 72% in pasture and 28% in Cerrado area. A lower proportion was *Trigona* gr. *fulviventris* with 15% (137) of individuals captured, 41% in pasture and 59% in Cerrado area. The other species identified were only occasionally collected and in fewer numbers. Most individuals were captured in traps in the pasture area, 100 meters from the Cerrado fragment, with a total of 672 individuals. For the traps in the forest, a total of 258 individuals were captured, however, the same proportion of was maintained (Figure 2).

According to Caceres & Legendre (2009) 'indicator species" are recognized by ecologists when they are restricted to one or a few habitat types. In this study all species of Meliponini occurred in both sites. Therefore we were not able to point out an indicator species to monitor environmental changes.

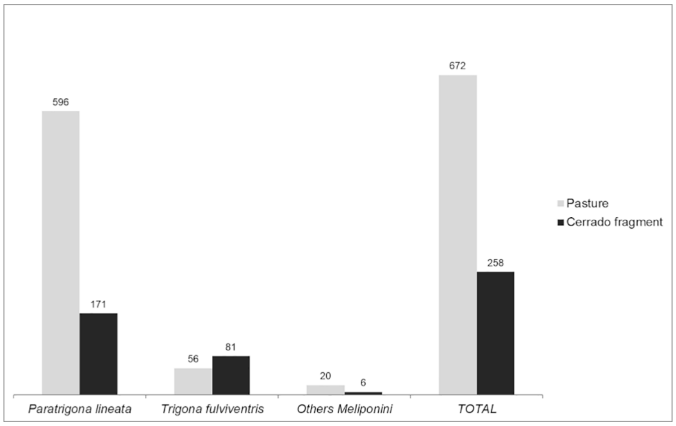


Figure 2. ***Paratrigona lineata***, *Trigona fulviventris* and another Meliponini in two environments (Pasture and Cerrado fragment), October, 2010, Campo Grande, MS.

Lima & Silvestre (2017) cataloged 386 species of bees (Apidae *s. lato*) in Mato Grosso do Sul, with citation of some species of Meliponini also recorded in the present study, such as *Paratrigona lineata*, *Tetragonia clavipes*, *Tetragonisca fiebrigi, Trigona hyalinata*. In this context, this study presents four new occurrence records for the state of Mato Grosso do Sul, *Frieseomelitta* cf. *flavicornis* (Fabricius 1798), *Lestrimelitta rufipes* (Friese 1903), *Trigona gr. fulviventris* (Guérin 1844), and *Trigona recursa* (Smith 1863).

Conclusions

The Meliponini was the most abundant Tribe in the area of study, most of them were captured in open areas around the Cerrado fragment. The species present in this area were *Trigona* gr. *fulviventris*, *Paratrigona lineata*, *Trigona recursa*, *Partamona* sp, *Trigona spinipes*, *Trigona* gr. *hyalinata*, *Trigona* gr. *fulviventris*, *Tetragonisca fiebrigi*, *Tetragona clavipes*, *Frieseomelitta* cf. *flavicornis*, *Lestrimelitta rufipes.* The first most abundant species was *Paratrigona lineata* and the second one *Trigona fulviventris.* Moreover, this study present new data on the species occurrence for Mato Grosso do Sul state.

Acknowledgments

To Centro de Pesquisas do Pantanal (CPP), Instituto Nacional de Ciência e Tecnologia em Áreas Úmidas (INAU) - Programa Institutos Nacionais de Ciência e Tecnologia - CNPq/MCT, Fundação de Suporte para o Ensino, Ciência e Tecnologia do estado do Mato Grosso do Sul (FUNDECT).

Author Contributions

Antonia Railda Roel: Substantial contribution to the design and design of the work; Contribution to data acquisition: Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in critical review adding intellectual content.

Armando Araujo Neto: Contribution to data acquisition.

Kwok Chiu Cheung: Substantial contribution to the design and design of the work; Contribution to data acquisition.

Felipe Varussa de Oliveira Lima: Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in critical review adding intellectual content.

Ricardo Dias Peruca: Substantial contribution to the design and design of the work; Contribution to data acquisition; Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in critical review adding intellectual content.

Leticia Vieira da Silva: Contribution to data acquisition.

Suellen Soares: Contribution to data acquisition.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AOKI, C., TEIXEIRA-GAMARRA, M.C., BARÔNIO, G.J., SIGRIST, M.R. & LAROCA, S. 2012. Diversidade de abelhas (Hymenoptera: Apoidea) e Recursos Florais na RPPN Engenheiro Eliezer Batista, Pantanal de Mato Grosso do Sul. p. 82-111. In: Rabelo, A.P.C.; Moreira, V.F.; Bertassoni, A.; Aoki, C., eds. Descobrindo o Paraíso Aspectos Biológicos da Reserva Particular do Patrimônio Natural. Instituto Homem Pantaneiro, Rio de Janeiro, RJ, Brazil.

CÁCERES, M.D. & LEGENDRE, P. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology, v. 90, n. 12, p. 3566-3574.

D'AVILA, M. & MARCHINI, L. C. 2008. Análise faunística de himenópteros visitantes florais em fragmento de cerradão em Itirapina, SP. For. Sci., v. 18, n.2, p. 271-279.

IMPERATRIZ-FONSECA, V.L. 2010. Conservação de polinizadores no ano internacional da biodiversidade. Oecolog. Australis, v. 14, p. 14-15.

FREITAS, B.M., IMPERATRIZ-FONSECA, V.L., MEDINA, L.M., KLEINERT, A.M.P., GALLETO, L., NATES-PARRA, G. & QUEZADA-EUÁN, J.J.G. 2009. Diversity, threats and conservation of native bees in the Neotropics. Apidologie, v.40, p. 332-346.

FREITAS, B.M. & PINHEIRO, J. N. 2010. Efeitos sub-letais dos pesticidas agrícolas e seus impactos no manejo de polinizadores dos agroecossistemas brasileiros. Oecol. Aust., v. 14, p. 282-298.

KERR, W.E., CARVALHO, G.A. & NASCIMENTO, V.A. 1996. Abelha uruçu: biologia, manejo e conservação. Acangaú, Belo Horizonte, MG, Brasil.

KRUG, C. & ALVES-DOS-SANTOS, I. 2008. Use of different methods to sample the bee fauna (Hymenoptera: Apoidea): a study in mixed temperate rainforest in Santa Catarina state. Neotrop. Entomol., v. 37, n. 3, p. 265-278.

LARSEN, T.H., WILLIAMS, N.W. & KREMEN, C. 2005. Extinction order and altered community structure rapidly disrupt ecosystem functioning. Ecol. Lett., v. 8, p. 538-547.

LIMA, F.V.O. & SILVESTRE, R. 2017. A fauna de abelhas (Hymenoptera, Apidae sensu lato) de Mato Grosso do Sul, Brasil. Iheringia. Sér. Zoo., v.107, Suppl.: e2017123.

MALAGODI-BRAGA, K.S. & KLEINERT, A.D.P. 2004. Could *Tetragonisca angustula* Latreille (Apinae, Meliponini) be effective as strawberry pollinator in greenhouses? Aust. J. Agric. Res., v. 55, p. 771-773.

MICHENER, C.D. 2007. The bees of the world. 2ed. Johns Hopkins University, Baltimore, MD, USA, 992pp.

MORSE, R.A. & CALDERONE, N.W. 2000. The value of honey bees as pollinators of U.S. crops in 2000. Bee Culture v. 128, p. 1-15.

SILBERBAUER-GOTTSBERGER, I. & GOTTSBERGER, G. 1988. The pollinization of Cerrado plants. Rev. Bras. de Biol, v. 48, p. 651-663.

SILVEIRA NETO, S., MONTEIRO, R.C., ZUCCHI, R.A. & DE MORAES, R.C.B. 1995. Faunistic insect analysis for environmental change evaluation. Sci. Agric., v. 52, n. 1, p. 9-15.

THOMAZINI, M.J. & THOMAZINI, A.P.B.W. 2002. Bee diversity (Hymenoptera: Apoidea) in inflorescences of *Piper hispidinervum* (C.DC.). Neotrop. Entomol., v. 31, n. 1, p. 27-34.

TOWNES, H.A. 1972. A light-weight malaise trap. Entomol. News, v. 83, p. 239-247.

TURNER, I.M. 1996. Species loss in fragments of tropical rain forest: a review of the evidence. J. Appl. Ecol., v. 33, p. 200-209.

Received: 07/02/2017
Revised: 11/06/2018
Accepted: 19/02/2019

Biota Neotropica 19(2): e20190727, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Amphibians of the Parque Nacional da Restinga de Jurubatiba, a sandy coastal environment in southeastern Brazil

Luiz Fernando Carmo[1,2], Ingrid Ribeiro Miguel[1,3], Pedro H. Pinna[1,2], Daniel Silva Fernandes[1,2] &*

Manoela Woitovicz-Cardoso[1]

[1]Universidade Federal do Rio de Janeiro, Museu Nacional, Departamento de Vertebrados, Setor de Herpetologia, Quinta da Boa Vista, 20940-040, Rio de Janeiro, RJ, Brasil
[2]Universidade Federal do Rio de Janeiro, Instituto de Biologia, Departamento de Zoologia, Ilha do Fundão, 21941-902, Rio de Janeiro, RJ, Brasil
[3]Universidade Federal do Rio de Janeiro, Campus Macaé, Núcleo em Ecologia e Desenvolvimento Socioambiental de Macaé, 27965-045, Barreto, Macaé, RJ, Brasil
**Corresponding author: Luiz Fernando Carmo, e-mail: luiz.carmo@ufrj.br*

CARMO L. F., MIGUEL I. R., PINNA P. H., FERNANDES D. S., WOITOVICZ-CARDOSO M. **Amphibians of the Parque Nacional da Restinga de Jurubatiba, a sandy coastal environment in southeastern Brazil**. Biota Neotropica. 19(2): e20190727. http://dx.doi.org/10.1590/1676-0611-BN-2019-0727

Abstract: We provide an inventory checklist of the amphibians from Parque Nacional da Restinga de Jurubatiba (PNRJ), a sandy coastal environment in southeastern Brazil. As a result of three years of surveys, from August 2013 to June 2016, a total of 36 amphibians species were recorded – one Gymnophiona (Typhlonectidae) and 35 species of five anuran families: Bufonidae (2 species), Craugastoridae (1), Hylidae (21), Leptodactylidae (8), and Microhylidae (3). One specie is reported for the first time for the restinga ecosystem (*Chthonerpeton braestrupi*) and 24 species were recorded for the first time to the PNRJ. This result reinforces the importance of long-term studies for accurate knowledge of the biodiversity. Considering that the biodiversity of the fragments of the sandy coastal environments in the state of Rio de Janeiro is poorly known, the present study provided a more detailed knowledge about different aspects of the biodiversity in the PNRJ, contributing to the preservation of this threatened ecosystem.
Keywords: *Herpetology, Anura, Inventory, Species Richness, Rio de Janeiro, Conservation.*

Anfíbios do Parque Nacional da Restinga de Jurubatiba, um ambiente costeiro no sudeste do Brasil

Resumo: Nós fornecemos um inventário dos anfíbios do Parque Nacional da Restinga de Jurubatiba (PNRJ), uma zona costeira de ambiente arenoso no sudeste do Brasil. Como resultado de três anos de pesquisas, no período de Agosto de 2013 a Junho de 2016, foram registradas um total de 36 espécies de anfíbios – uma Gymnophiona (Typhlonectidae) e 35 espécies de cinco famílias de anuros: Bufonidae (2 espécies), Craugastoridae (1), Hylidae (21), Leptodactylidae (8) e Microhylidae (3). Uma espécie é registrada pela primeira vez para o ecossistema de restinga (*Chthonerpeton braestrupi*) e 24 espécies foram registradas pela primeira vez para o PNRJ. Este resultado reforça a importância de estudos de longo prazo para um conhecimento preciso da biodiversidade. Considerando que a biodiversidade dos fragmentos dos ambientes costeiros no estado do Rio de Janeiro é pouco conhecida, o presente estudo proporcionou um conhecimento mais detalhado sobre os diferentes aspectos da biodiversidade no PNRJ, contribuindo para a preservação desse ecossistema ameaçado.
Palavras-chave: *Herpetologia, Anura, Inventário, Riqueza de Espécies, Rio de Janeiro, Conservação.*

Introduction

Brazil has the greatest amphibian richness in the world, with about 1080 species distributed in 25 families (Segalla et al. 2016; Frost, 2018). The morphoclimatic domain of the Atlantic Forest is known as a conservation hotspot due to its high concentration and maintenance of biodiversity (Myers et al. 2000), comprising about 540 species, of which 88% are endemic to this ecosystem (Haddad et al. 2013). This remarkable richness is related to the diversity of microenvironments found in the biome, which possibly contributes to the evolution of various reproductive modes and speciation events (Haddad & Prado 2005; Vasconcelos et al. 2010).

Originally, the Atlantic Forest extended over nearly the entire coastline of Brazil, displaying different vegetation landscapes throughout its distribution (Falkenberg 1999). One of the habitats observed in this biome are the restingas, dating from the Quaternary, and whose seasonal dynamic and morpho-physiological composition is mostly shared with other ecosystems, such as Tabuleiros and Caatingas (Freire 1990). Restingas extend over about 79% of the coast (Lacerda et al. 1993), being one of the most extensive ecosystems in Brazil and having multiple fragmented areas along its distribution (Teixeira et al. 2002). The flora is adapted to withstand extreme environmental conditions, such as sandy substrate with high salt concentration, high exposure to sunlight and low water potential (Henriques et al. 1986; Assumpção & Nascimento 2000; Scarano 2002). Bromeliads are very common and widely distributed in this environment (Cogliatti-Carvalho et al. 2001), being an essential element especially for amphibians (Teixeira et al. 2002). They provide a significant amount of rainwater in their central tube, serving as sites for foraging, breeding and protection against possible predators (Peixoto 1995; Schineider & Teixeira 2001).

Despite the great extension of the Brazilian coastline, the coastal ecosystems are the most anthropically impacted (directly and indirectly), mostly due to the unequal and unrestrained occupation of the country initiated in the 16th century, from the sea towards inner lands (Dean 1997; Tabarelli et al. 2010). In addition to the loss of natural habitats, another issue concerning the conservation of amphibians in the Atlantic Forest is that recent studies estimate the decline of populations in the coming years, largely due to global climatic changes and the contamination by the fungus *Batrachochytrium dendrobatidis* (Fisher et al. 2012; Loyola et al. 2014; Jenkinson et al. 2016). Some studies related to amphibians assemblage in restingas have been carried out in the last years in order to provide a more detailed inventory of their composition (e.g. Bastazini et al. 2007; Rocha et al. 2008; Narvaes et al. 2009, Wachlevski & Rocha 2010; Vilela et al. 2011; Telles et al. 2012; Oliveira & Rocha 2015; Gondim-Silva et al. 2016; Oliveira et al. 2017).

The Parque Nacional da Restinga de Jurubatiba (PNRJ) was created on April 29, 1998 with the main purpose of protecting and preserving one of the most threatened ecosystems of the country since the colonial period (Esteves & Lacerda 2000). Currently, despite being a federal conservation unit, the PNRJ is still under strong anthropic pressure (Rocha et al. 2007). The taxocenosis of amphibians from the PNRJ was previously studied by Van Sluys et al. (2004). Herein we provide an updated checklist of amphibians for the PNRJ.

Material and Methods

1. *Study site*

The present study was carried out in the Parque Nacional da Restinga de Jurubatiba (22° 16' S, 41° 39' W) located on northern state of Rio de Janeiro, southeastern Brazil (Fig. 1). It is one of the largest remnants of restinga in Brazil with 14,992 hectares, extending through the municipalities of Macaé, Carapebus, and Quissamã. It is the only fragment of restinga in the country to be titled as national park (Brasil 2016). As in most of the restingas found in Brazil, the vegetation is composed of a mosaic of forested and open areas.

The study was carried out in the municipality of Carapebus, in three distinct zones of the PNRJ. These zones were selected and classified based on phytophysiognomic characteristics as follow: *Clusia* spp. open formation; dry forest of restinga; and an intermediate zone between these two formations (Fig. 2). The *Clusia* spp. open formation is closer to seashore and was defined by Araújo et al. (1998) as "shrub vegetation of *Clusia*" (SVC). It was characterized as having more sparse vegetation, separated by strips of bare sand, with high solar incidence and low potential of fresh water. The zone here classified as an intermediate area also had its physiognomy defined by Araújo et al. (1998), who called it "open shrub of Ericaceae" (OSE). This region shows fragments of irregular vegetation varying from shrubs to trees. It is located between the *Clusia* spp. open formation and the dry forest of restinga. The third zone, dry forest of restinga (DFR), corresponds to an area with vegetation more densely distributed. In this area, one can find large trees and a large ammount of decomposing plant material, forming a dense litter. The availability of water is larger compared to the other zones, with temporary swamps and periodically flooded areas (pers. obs).

2. *Field methods and data analyses*

Fieldwork consisted of quarterly surveys over three years, from August 2013 to June 2016, in a total of 12 surveys lasting seven days each. We applied two methods for sampling amphibians: pitfall traps (according to the standard procedures of Cechin & Martins 2000) and Complete Species Inventory (*sensu* Scott Jr. 2001). These methods were chosen as they are the most effective for accessing anuran taxocenosis in restingas (Rocha et al. 2004a). Twenty one pitfall traps arrays were used, each one with four 60 L buckets. Seven arrays were placed in each of the three zones previously determined (Fig. 1). Nineteen pitfall arrays were Y-shaped (one central bucket linked to other three peripherals forming angles of approximately 120º), and two pitfall arrays were linked in one straight line with eight buckets. Buckets of each pitfall array were connected by 4-5 m long and 0.5-1m high drift fences. Each pitfall array was sampled once along the consecutive days of each survey, corresponding to a total sample effort of 7,056 bucket nights. Regarding the Complete Species Inventory, eight sampling areas were selected and sampled in all surveys (Periodically sampled areas). Two of these correspond to areas of "dry forest of restinga", four are located in "*Clusia* spp. open formations", and two in areas of "open shrub of Ericaceae" (Fig. 1). We occasionally performed Complete Species Inventory on another twenty-six areas (extra sample areas) in order to maximize sampling (Fig. 1). Everyday nocturnal Complete Species Inventory was performed by three to five researchers, during two

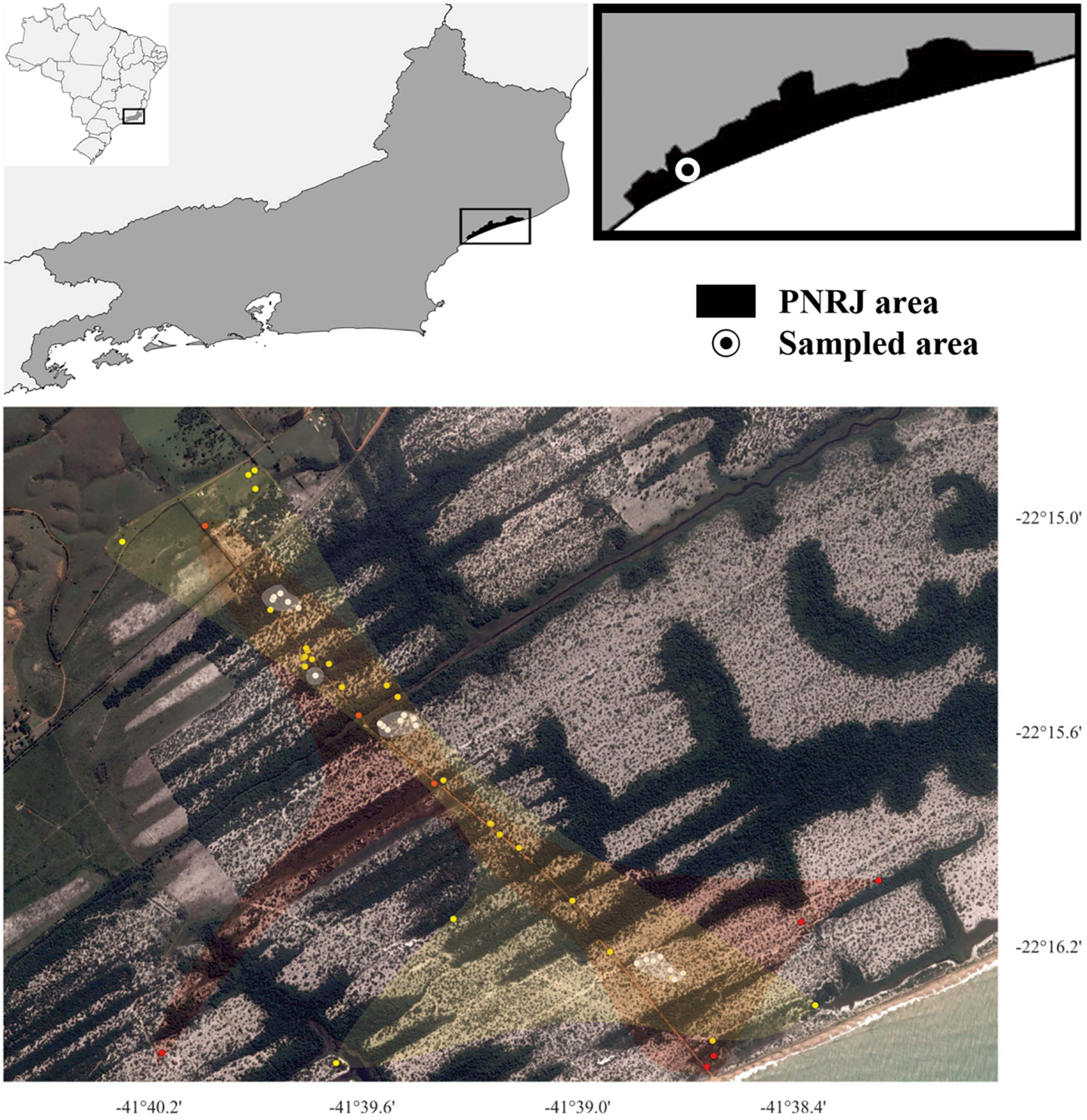


Figure 1. Localization of Parque Nacional da Restinga de Jurubatiba (PNRJ) in the state of Rio de Janeiro, southeastern Brazil. Sampling areas are indicated by: White circles = Pitfall traps; Red circles = Periodically sampled areas of Complete Species Inventory; Yellow circles = Extra sample areas, occasionaly sampled by Complete Species Inventory.

hours per night, resulting in a total effort of 722 hours person. Voucher specimens were killed and fixed according to the usual techniques described by McDiarmid (1994) and deposited in the amphibians collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). We also gathered data from specimens previously collected within the PNRJ and deposited in the herpetological collection of MNRJ. All voucher specimens (collected by us or already deposited at MNRJ) are listed in Appendix.

Species richness was estimated by the extrapolation of the accumulation curve through the Jackknife 1 estimator (Burnham & Overton, 1979) for incidence data, in the software EstimateS Win 9.1.0 (Colwell 2013). Only species recorded during the surveys of the present study were included in the Jackknife 1. Third-party records (*Itapotihyla langsdorffii* [Duméril & Bibron, 1841]), species collected close to the limits of the park (*Physalaemus marmoratus* [Reinhardt & Lutken, 1862]) or data from herpetological collections (*Chthonerpeton*

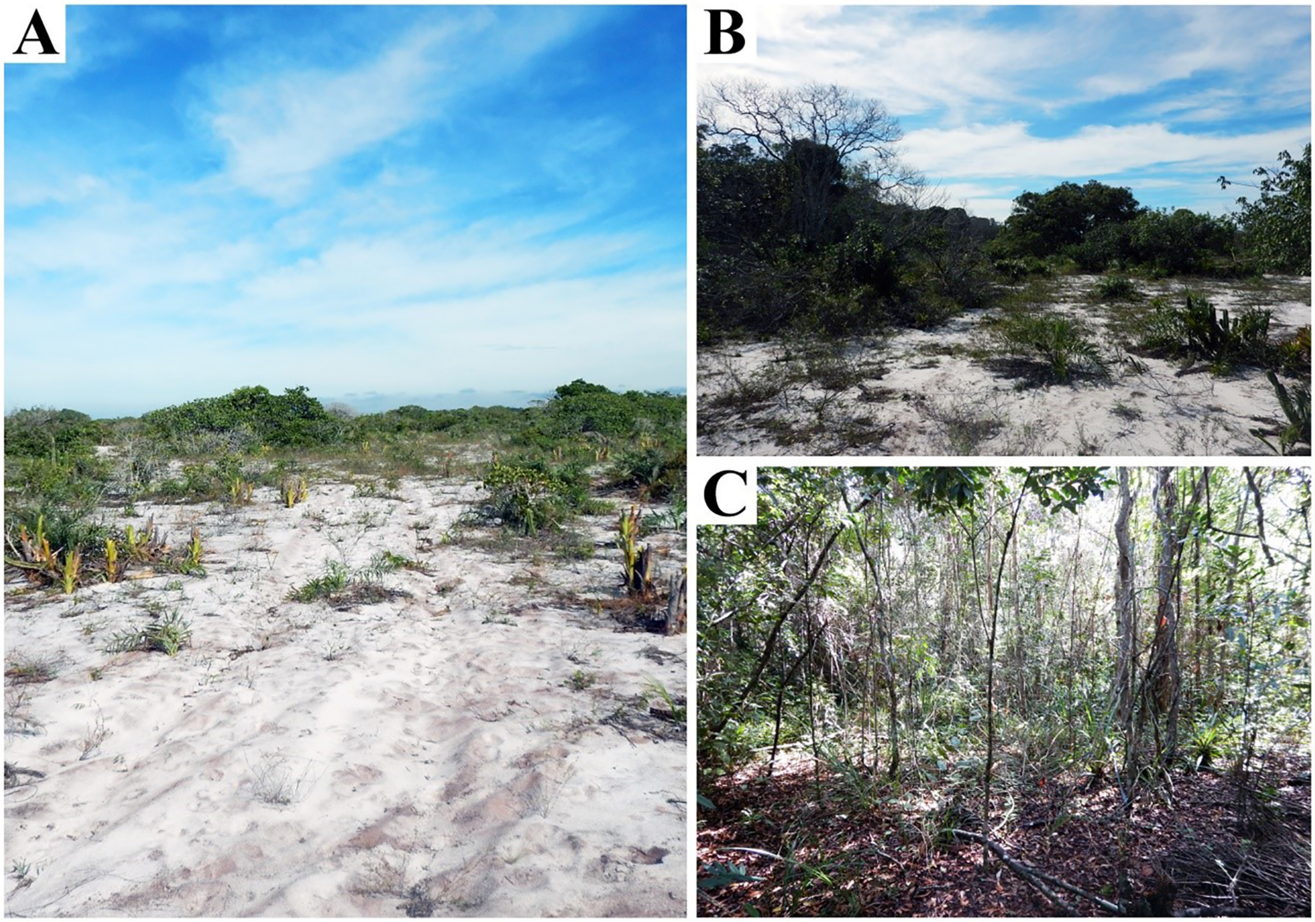


Figure 2. Sampled areas in the Parque Nacional da Restinga de Jurubatiba, state of Rio de Janeiro, southeastern Brazil: A = Shrub Vegetation of *Clusia* (SVC); B = Open Shrub of Ericaceae (OSE); C = Dry forest of resting (DFR).

braestrupi Taylor, 1968; *Dendropsophus meridianus* [Lutz, 1954]) were included in the species list, but were not included in the accumulation curve. Jackknife 1 was used since this analysis takes into account the number of *uniques* (samples with the only record of a species in the area). The estimated species richness was compared to the total recorded species richness, in order to check the accuracy of the richness estimator and to predict the effort necessary to achieve a richness value much closer to the "real" species richness.

Results

We recorded a total of 36 amphibian species occurring in the PNRJ area (Table 1): one Gymnophiona (Typhlonectidae) and 35 species of five anuran families: Bufonidae (2 species), Craugastoridae (1), Hylidae (21), Leptodactylidae (8) and Microhylidae (3). The Complete Species Inventory was the most effective survey method, recording 29 from the 36 species found in PNRJ, 21 of them recorded only by this method (Table 1).

One taxa is reported for the first time in the restinga ecosystem: *Chthonerpeton braestrupi* Taylor, 1968. Moreover, 24 species were recorded for the first time to the PNRJ area. Some of the recorded species present a well-defined distribution pattern within the decribed sampling areas: 25 species were recorded in the phytophysiognomic zone characterized as Shrub Vegetation of *Clusia* (SVC); 21 were recorded in the Open Shrub of Ericaceae (OSE); and 28 in the Dry Forest Restinga (DFR). Two species (*Scinax alter* [Lutz, 1973] and *S. tymbamirim* Nunes, Kwet & Pombal, 2012) were recorded in all the study sites. However, species as *Dendropsophus berthalutzae* (Bokermann, 1962)*, Dendropsophus* cf. *haddadi, Stereocyclops parkeri* (Wettstein, 1934)*, Adenomera thomei* (Almeida & Angulo, 2006), and *Chiasmocleis lacrimae* Peloso, Sturaro, Forlani, Gaucher, Motta & Wheeler, 2014 were found only in phytophysiognomic zone characterized as Dry Forest Restinga. *C. lacrimae*, *S. parkeri* and *Physalaemus signifer* (Girard, 1853) were recorded only by pitfall traps.

The steep slope in the rarefaction curve occurred until the seventeenth day of sampling, when 29 species were recorded in the PNRJ area. From this day onwards, the rarefaction curve indicated a high tendency to stabilize, with only five new records until the end of the sampling. The species richness was estimated at 34.97 (Jacknife 1), a value quite close to the actual number of species registered to PNRJ (Fig. 3).

Discussion

Previous studies on amphibian composition in restinga habitats were carried out by Silva et al. (1988) in the Restinga da Barra de Maricá, state of Rio de Janeiro. Since then, the number of studies concerning restinga habitats increased significantly. Oliveira & Rocha (2015)

Table 1. Amphibian species found in the Parque Nacional da Restinga de Jurubatiba; Carapebus; state of Rio de Janeiro; southeastern Brazil. IUCN (International Union for Conservation of Nature and Natural Resources): DD = Data Deficient; EN = Endangered; LC = Least Concern; NE = Not Evaluated. Sampling Methods: Pt = Pitfall traps; CSI = Complete Species Inventory. * = Species recorded only in literature; ** = Species recorded only in herpetological collection; *** = Third-party records; **** = Species collected close to the limits of the park; + = Species recorded for the first time to the PNRJ area. Study Sites: SVC = Shrub Vegetation of *Clusia*; OSE = Open Shrub of Ericaceae; DFR = Dry Forest Restinga. Habitat: Lt = Leaf litter; Ss = Sandy substrate; Br = Bromeliads; TrB = Trees and Buchers; Av = Aquatic Vegetation; Pn = Ponds.

TAXA			**SVC**						**OSE**						**DFR**					
Family/Species	**IUCN**	**Sampling Methods**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**
Bufonidae																				
Rhinella crucifer (Wied-Neuwied, 1821)$^{+}$	LC	CSI; Pt	X						X	X					X	X				
Rhinella pygmaea (Myers and Carvalho, 1952)	LC	CSI; Pt	X	X				X	X	X					X	X				
Craugastoridae																				
Haddadus binotatus (Spix, 1824)	LC	CSI; Pt							X	X	X				X	X				
Hylidae																				
Aparasphenodon brunoi Miranda-Ribeiro, 1920	LC	CSI			X	X					X	X					X	X	X	X
Boana albomarginata (Spix, 1824)	LC	CSI					X	X					X	X					X	X
Boana semilineata (Spix, 1824)	LC	CSI					X	X					X	X					X	X
Dendropsophus berthalutzae (Bokermann, 1962)$^{+}$	LC	CSI																	X	
Dendropsophus bipunctatus (Spix, 1824)$^{+}$	LC	CSI											X	X					X	X
Dendropsophus decipiens (Lutz, 1925)$^{+}$	LC	CSI					X	X					X	X					X	X
Dendropsophus elegans (Wied-Neuwied, 1824)$^{+}$	LC	CSI					X	X					X	X					X	X
Dendropsophus cf. *haddadi*$^{+}$	LC	CSI																	X	
Dendropsophus meridianus (Lutz, 1954)*	LC																			
Dendropsophus aff. *oliveirai*$^{+}$	LC	CSI					X	X					X	X					X	X
Dendropsophus pseudomeridianus (Cruz, Caramaschi & Dias, 2000)$^{+}$	LC	CSI											X					X	X	X
Dendropsophus seniculus (Cope, 1868)$^{+}$	LC	CSI				X	X	X				X	X	X				X	X	X
Itapotihyla langsdorffii (Duméril & Bibron, 1841)***$^{+}$	LC																			
Ololygon argyreornatus (Miranda-Ribeiro, 1926)$^{+}$	LC	CSI									X						X	X	X	X
Scinax alter (Lutz, 1973)	LC	CSI			X	X	X	X			X	X	X	X			X	X	X	X
Scinax cuspidatus (Lutz, 1925)	LC	CSI			X	X	X	X			X	X	X	X			X		X	X
Scinax eurydice (Bokermann, 1968)$^{+}$	LC	CSI				X												X	X	X
Scinax similis (Cochran, 1952)	LC	CSI; Pt				X	X	X				X	X				X	X	X	X
Scinax tymbamirim Nunes, Kwet & Pombal, 2012^{+}	LC	CSI			X	X	X	X			X	X	X	X			X	X	X	X
Sphaenorhynchus planicola (Lutz & Lutz, 1938)	LC	CSI											X						X	
Trachycephalus nigromaculatus Tschudi, 1838^{+}	LC	CSI		X	X	X					X	X								

Continuation Table 1.

TAXA			SVC						OSE						DFR					
Family/Species	**IUCN**	**Sampling Methods**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**	**Lt**	**Ss**	**Br**	**TrB**	**Av**	**Pn**
Leptodactylidae																				
Adenomera thomei (Almeida & Angulo, 2006)$^{+}$	LC	Pt														X				
Leptodactylus fuscus (Schneider, 1799)$^{+}$	LC	CSI	X					X		X										
Leptodactylus latrans (Steffen, 1815)	LC	CSI; Pt	X	X					X	X			X	X	X	X			X	X
Leptodactylus mystacinus (Burmeister, 1861)$^{+}$	LC	CSI; Pt		X						X						X				
Leptodactylus natalensis Lutz, 1930^{+}	LC	CSI; Pt		X					X						X	X				
Physalaemus marmoratus (Reinhardt & Lütken, 1862)****	LC	CSI																		
Physalaemus signifer (Girard, 1853)$^{+}$	LC	Pt																		
Pseudopaludicola restinga Cardozo, Baldo, Pupin, Gasparini & Haddad, 2018^{+}	NE	CSI						X		X				X	X					
Microhylidae																				
Chiasmocleis lacrimae Peloso, Sturaro, Forlani, Gaucher, Motta & Wheeler, 2014^{+}	EN	Pt													X					
Elachistocleis cesarii (Miranda-Ribeiro, 1920)$^{+}$	LC	CSI; Pt						X			X									
Stereocyclops parkeri (Wettstein, 1934)$^{+}$	LC	Pt														X				
Typhlonectidae																				
Chthonerpeton braestrupi Taylor, 1968**$^{+}$	DD																			

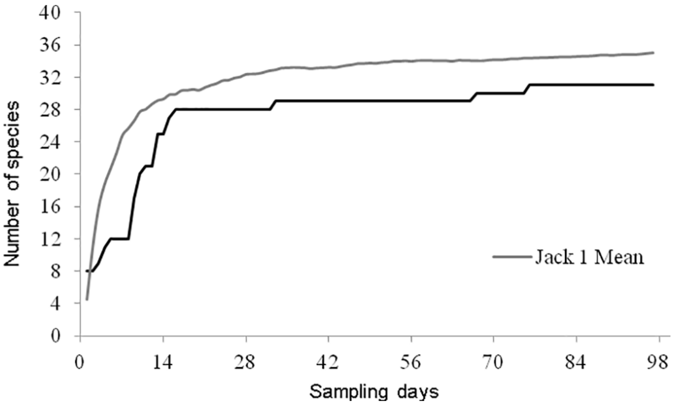


Figure 3. Extrapolation of the accumulation curve through the Jackknife 1 from 1000 randomizations on the order of samplings (grey line) and accumulation curve (black line) for amphibiam species at the Parque Nacional da Restinga de Jurubatiba, state of Rio de Janeiro, southeastern Brazil.

published a review on the anurofauna of the Brazilian sandy coastal plains, recording the occurrence of 145 species at this ecosystem. The 36 species registered for the PNRJ in the present study correspond to 24.8% of all the amphibian richness found in the ecosystem comprising restingas (Oliveira & Rocha 2015), standing out as the restinga environment with the highest amphibian species richness recorded. Some other areas of restinga with studied amphibian communities are: Mata de São João, in the state of Bahia, with 34 recorded species (Bastazini et al. 2007); restinga of the municipality of Conde, in the state of Bahia, with 33 species (Gondim-Silva et al. 2016); Parque Natural Municipal de Grumari, in the state of Rio de Janeiro, with 22 species (Telles et al. 2012); Estação Ecológica Juréia-Itatins, in the state of São Paulo, with 20 species (Narvaes et al. 2009); Parque Estadual da Ilha do Cardoso, in the state of São Paulo, with 17 species (Vilela et al. 2011); Baixada do Maciambu, in the state of Santa Catarina, with 15 species (Wachlevski & Rocha 2010); Restinga da Barra de Maricá, state of Rio de Janeiro, with 14 recorded species (Silva et al. 1988); Praia das Neves, in the state of Espírito Santo, with 13 species (Rocha et al. 2008), and Marambaia, in the state of Rio de Janeiro, with 12 species (Silva et al. 2008).

Although PNRJ is the Restinga with the second most studied anuran community (Oliveira & Rocha, 2015), the present study registered 24 species not recorded to the region (Table 1). This result reinforces the importance of long-term studies for accurate knowledge of the biodiversity. The family Hylidae is the most representative, corresponding to 60% of the total anuran species recorded for the PNRJ (21 of 35). The greater representativeness of this family reinforces the pattern found in all ecosystems studied within the Atlantic Forest Biome (e.g., Heyer et al. 1990; Duellman 1999; Canelas & Bertoluci 2007; Dias et al. 2014; Garey et al. 2014).

Some of the reported species (Fig. 4-5), such as *Scinax tymbamirim* and *S. alter*, show a vast spatial distribution within of the PNRJ and were not recorded for only two of the sampled microhabitats: leaf litter (Lt) and sandy substrate (Ss) (Table 1). These species present plasticity in relation to environmental changes, being able to inhabit different ecosystems, a favorable aspect for species that live in degraded and unstable environments (Vasconcelos et al. 2018). Others species such as *Dendropsophus berthalutzae*, *D*. cf. *haddadi*, *Stereocyclops parkeri, Adenomera thomei*, and *Chiasmocleis lacrimae* were found in only one microhabitat each, all in the dry forest restinga study site (Table 1).

One of the 36 species registered, *Chiasmocleis lacrimae*, is categorized as "Endangered" (EN) by the IUCN Red List (Pimenta & Peixoto 2004), reinforcing the importance of the restingas as conservation priority areas. One of the anurans registered in PNRJ was recently described: *Pseudopaludicola restinga* Cardozo, Baldo, Pupin, Gasparini & Haddad, 2018, compromising adequate avaliation of its conservational status. In the present study, we report the first record of this taxon outside the state of Espírito Santo, Brazil. This record represents an increase of its known geographic distribution of approximately 140 km to south from its nearest locality (Restinga de Praia das Neves, municipality of Presidente Kennedy [21°14'41.53"S; 40°58'45.80"W; 6 m asl], state of Espírito Santo, Brazil; according to Cardozo et al. 2018). The only species representative of the order Gymnophiona, *Chthonerpeton braestrupi*, is categorized in the IUCN Red Book as "Data Deficient" (DD) concerning its distribution, as well as taxonomy and ecological parameters (Wilkinson et al. 2004). The similarity with *Chthonerpeton indistinctum* (Reinhardt & Lütken, 1862), a relatively close and better known species (Maneyro & Langone 2001; Canavero et al. 2010), which is affected by urban occupation in coastal and agricultural areas, corroborates that these anthropic impacts may also affect populations of *C. braestrupi* in the Brazilian coastal zone. The other 33 species are listed and considered as "Least Concern" (LC) according to the IUCN Red Book (IUCN 2018) (Table 1).

None of the species recorded to the PNRJ is endemic to the park, reinforcing the pattern already described for the other restingas. Only five anuran species are known as endemic to this environment (Rocha et al. 2005; Peloso et al. 2012): *Ololygon agilis* (Cruz & Peixoto, 1983), *Ololygon littoreus* Peixoto, 1988*, Xenohyla truncata* (Izecksohn, 1959), *Leptodactylus marambaiae* Izecksohn, 1976, and *Melanophryniscus setiba* Peloso, Faivovich, Grant, Gasparini, & Haddad, 2012. Until recently, *Rhinella pygmaea* (Myers & Carvalho, 1952) was considered endemic to the restinga habitats. However, Silveira et al. (2009) recorded the species at semideciduous seasonal forest formations in the states of Rio de Janeiro and Espírito Santo. Considering that the biodiversity of the fragments of the sandy coastal environments in the state of Rio de Janeiro is poorly known, the present study provided a more detailed knowledge about different aspects of the biodiversity in the PNRJ, contributing to the preservation of this threatened ecosystem.

Currently, the PNRJ is the only area within the 72 Brazilian national parks distributed throughout the country to enclose this ecosystem (Brasil 2016). In addition, few restinga areas correspond to other categories of conservation units and only a few receive integral protection (Rocha et al. 2004b). Therefore, more studies are needed to subsidize the maintenance and implementation of conservation policies in other areas of restinga in Brazil. The PNRJ has a long tradition regarding conservation initiatives, including protection of threatened species and the socio-environmental development of sustainable alternatives for those who live from the resources offered by this ecosystem (Rocha et al. 2004b; Esteves 2011; Silva et al. 2012). Many of these measures were supported by science-based conservation planning (see Esteves 2011). According to Margules & Pressey (2000),

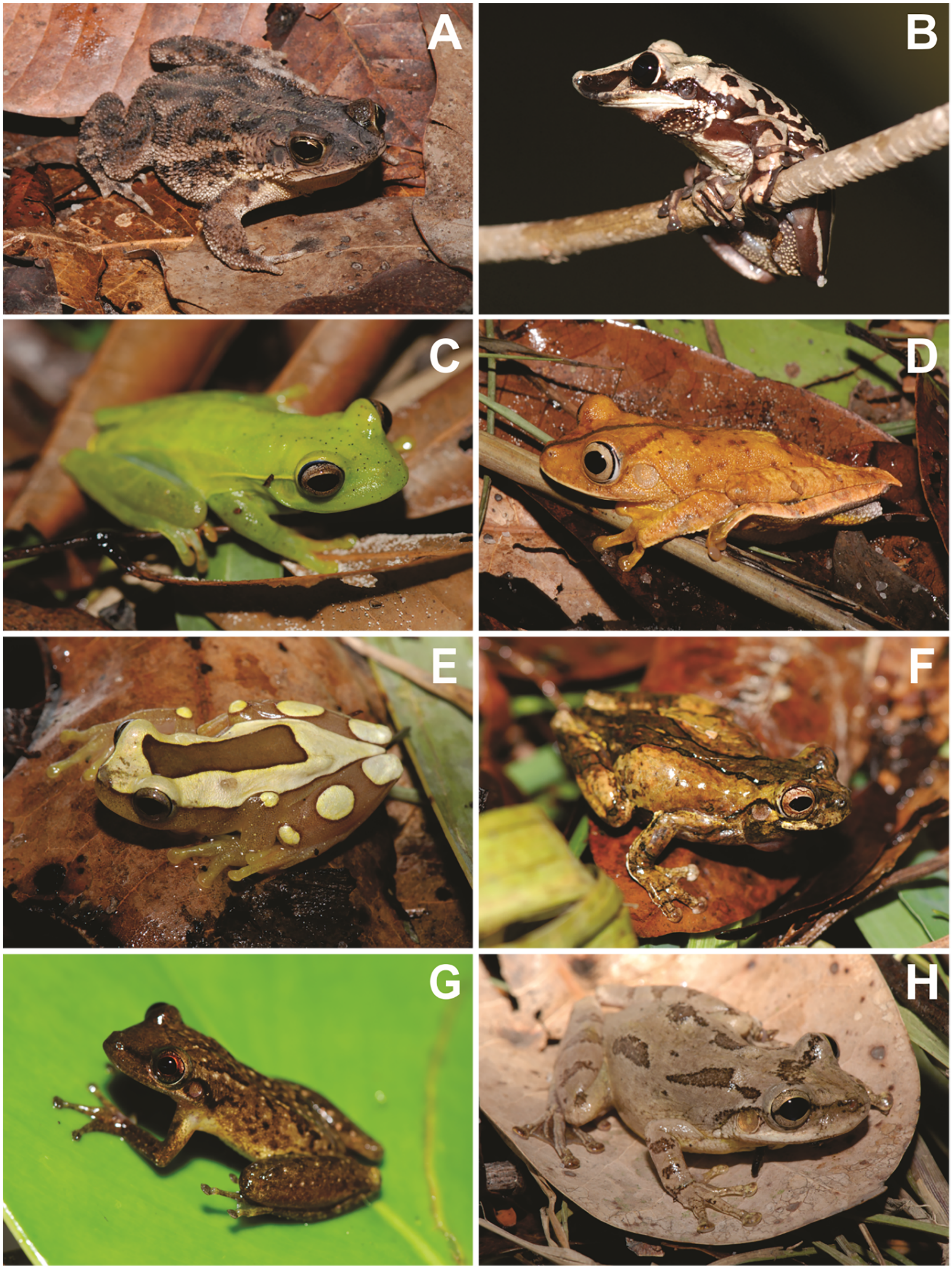


Figure 4. Some anurans from Parque Nacional da Restinga de Jurubatiba, state of Rio de Janeiro, southeastern Brazil: A) *Rhinella pygmaea*; B) *Aparasphenodon brunoi*; C) *Boana albomarginata*; D) *Boana semilineata*; E) *Dendropsophus elegans*; F) *Dendropsophus marmoratus*; G) *Scinax eurydice*; H) *Scinax cuspidatus*. Photos from MNFoto/Setor de Herpetologia, Museu Nacional, UFRJ.

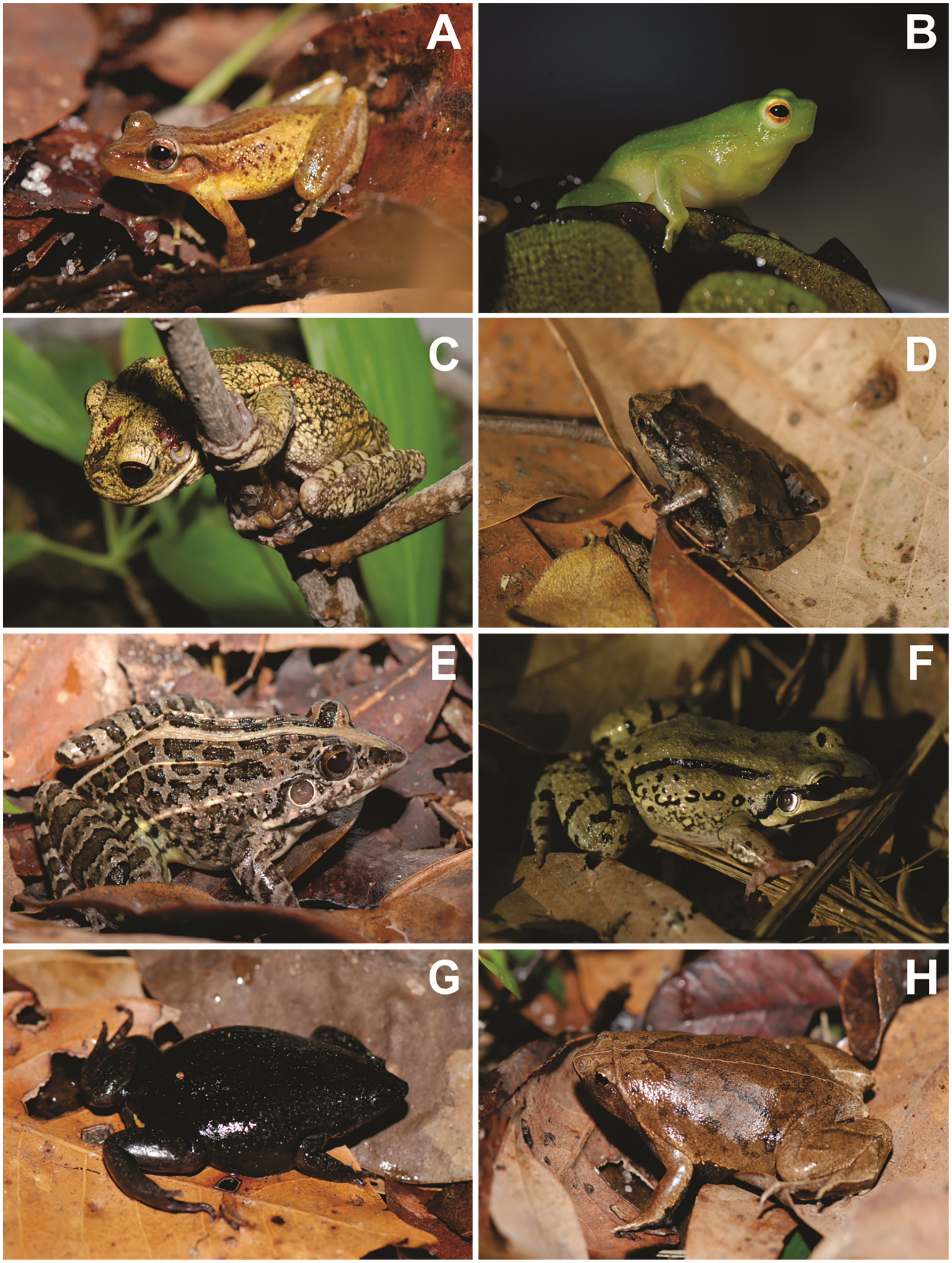


Figure 5. Some anurans from Parque Nacional da Restinga de Jurubatiba, state of Rio de Janeiro, southeastern Brazil: A) *Scinax tymbamirim*; B) *Sphaenorhynchus planicola*; C) *Trachycephalus nigromaculatus*; D) *Physalaemus signifer*; E) *Leptodactylus fuscus*; F) *Leptodactylus mystacinus*; G) *Elachistocleis cesarii*; H) *Stereocyclops parkeri*. Photos from MNFoto/Setor de Herpetologia, Museu Nacional, UFRJ.

one can only preserve what is known, and the first stage to conserve biodiversity is to describe, to map and to measure it. In the last years, several studies pointed to the occurrence of population declines in different parts of the world (Stuart et al. 2004), mostly caused by the destruction of natural areas. The PNRJ is a region of special relevance for the protection of the biodiversity from the coastal region of state of Rio de Janeiro. According to Rocha et al. (2005), the areas between the restingas of Maricá and Jurubatiba are of great importance for the maintenance of the biodiversity of local terrestrial vertebrates. Rocha et al. (2007) identified 14 sites of degradation in restinga areas and estimate that 1,257 hectares of the PNRJ are degraded, mostly due to the removal of native flora communities for the establishment of grazing areas for livestock and urban occupation. All these factors tend to cause significant impacts on the faunal communities present in the park. It is well known that amphibians comprise the most threatened vertebrate group and this, coupled with the current dynamics of changes in this ecosystem, will lead to the extinction of many species, some of them even before being described (Howard & Bickford 2014).

In addition, the PNRJ is an important area for the protection of different taxa, harboring some of the species that are endemic to the restingas ecosystem, such as the lizard *Glaucomastix littoralis* (Rocha, Bamberg Araújo & Vrcibradic, 2000) (Squamata: Teiidae) (Cosendey et al. 2016), the rodent *Cerradomys goytaca* Tavares, Pessôa & Gonçalves, 2011 (Rodentia: Cricetidae) (Lemos & Gonçalves 2015), and the fish *Atlantirivulus jurubatibensis* (Costa, 2008) (Cyprinodontiformes: Rivulidae) (Costa, 2008).

In conclusion, the detailed knowledge about the diverse aspects of the biodiversity existing in the PNRJ, provide means to subsidize the maintenance and implementation of conservation policies to other areas of restinga in Brazil. Ecosystems with high biodiversity, such as the Atlantic Forest, demand a continuous expansion of protected areas in the most diverse categories of Conservation Units according to the federal legislation. The Atlantic Forest suffered severe deforestation as a consequence of its geographic location, originally occupying most of the coastal strip of the Brazilian territory, a place that sheltered and consolidated the permanence of 80% of the Brazilian population and the infrastructure for its development (Rocha et al. 2004b). Only about 11% of this biome remains preserved in Brazil, with very few native forested fragments, while the rest of the fragments consist of secondary forest (Ribeiro et al. 2009). It is necessary to expand these protected areas, as well as their interconnections to the formation of biological corridors, essential for maintaining and expanding the existing biodiversity bases.

Supplementary material

The following online material is available for this article:

Appendix

Acknowledgements

We thank Marcos Bilate and Rodrigo de Oliveira Lula Sales for review and suggestions. We are grateful to Aldo Caccavo; Bruna Pagliani; Bruno Miranda; Carina Azevedo; Carolina Silva-Barros; Cristiane Régis; Daniel Almada; Hudson Lemos; Jéssica Erasmi; Jessica Fratani; João Marcelo; Leonardo Lopes; Natália Souto; Natasha Radsack; Pablo Gonçalves; Pedro C. Prado; Pedro H. Cabral; Theo Aroeira; Vagner dos Santos; Macaé Fora de Estrada (MFE); PARNA de Jurubatiba/ICMBio staff for fieldwork help. We are especially grateful to Ana C. Petry and Pablo Gonçalves for logistical support. This project is part of the program Pesquisas Ecológicas de Longa Duração (PELD): Mudanças Climáticas Globais e o Funcionamento dos Ecossistemas Costeiros da Bacia de Campos: uma perspectiva espaço-temporal (Long Term Ecological Research program: Global Climate Changes and the Functioning of Coastal Ecosystems in the Bacia de Campos: A space-time perspective), iniciated in August 2013 and subsidized by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). In addition, it also integrates the program: Levantamento e Aspectos Biológicos da Herpetofauna do Parque Nacional da Restinga de Jurubatiba, RJ, Brasil, subsidized by Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro (FAPERJ). Both promoting institutions were extremely important for the development of this research. The specimens were collected under permit #38378-11, granted by the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio).

Author Contributions

Luiz Fernando Carmo: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Ingrid Ribeiro Miguel: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Pedro H. Pinna: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Daniel Silva Fernandes: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Manoela Woitovicz-Cardoso: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ASSUMPÇÃO, J. & NASCIMENTO, M.T. 2000. Estrutura e composição floristica de quatro formações vegetais de restinga no complexo lagunar Grussai/Iquipari, São João da Barra, RJ, Brasil. Acta Botanica Brasilica 14(3): 301-315.

ARAUJO, D.S.D., SCARANO, F.R., KURTZ, B.C., ZALUAR H, L.T., MONTEZUMA, R.C.M. & OLIVEIRA, R.C. 1998. Comunidades vegetais do Parque Nacional de Restinga de Jurubatiba. In Ecologia das lagoas costeiras do Parque Nacional da Restinga de Jurubatiba e do município de Macaé (F.A. Esteves, ed.). Rio de Janeiro: NUPEM-UFRJ, p.37-62.

BASTAZINI, C.V., MUNDURUCA, J.F.V., ROCHA, P.L.B. & NAPOLI, M.F. 2007. Which environmental variables better explain changes in anuran community composition? A case study in the restinga of Mata de São João, Bahia, Brazil. Herpetologica 63(4): 459-471.

BRASIL. Instituto Chico Mendes de Conservação da Biodiversidade – ICMBio. Ministério do Meio Ambiente (MMA). 2016. Parques Nacionais do Brasil. Downloaded on 31 January 2018. p.1-20.

BURNHAM, K.P. & OVERTON, W.S. 1979. Robust estimation of population size when capture probabilities vary among animals. Ecology 60(5): 927-936.

CANAVERO, A., CARREIRA, S., LANGONE, J.A., ACHAVAL, F., BORTEIRO, C., CAMARGO, A., ROSA, I., ESTRADES, A., FALLABRINO, A., KOLENC, F., LÓPEZ-MENDILAHARSU, M.M., MANEYRO, R., MENEGHEL, M., NUÑEZ, D., PRIGIONI, C.C. & ZIEGLER, L. 2010. Conservation status assessment of the amphibians and reptiles of Uruguay. Iheringia, Série Zoologia 100(1): 5-12.

CANELAS, M.A.S. & BERTOLUCI, J. 2007. Anurans of the Serra do Caraça, southeastern Brazil: species composition and phenological patterns of calling activity. Iheringia, Série Zoologia 97(1): 21-26.

CARDOZO, D.E., BALDO, D., PUPIN, N., GASPARINI, J.L. & HADDAD, C.F.B. 2018. A new species of *Pseudopaludicola* (Anura, Leiuperinae) from Espírito Santo, Brazil. PeerJ 6:e4766.

CECHIN, S.Z. & MARTINS, M. 2000. Eficiência de armadilhas de queda (*pitfall traps*) em amostragem de anfíbios e répteis no Brasil. Revista Brasileira de Zoologia 17(3): 729-740.

COGLIATTI-CARVALHO, L., NUNES DE FREITAS, A.F., ROCHA, C.F.D. & VAN SLUYS, M. 2001. Variação na estrutura e na composição de Bromeliaceae em cinco zonas de restinga no Parque Nacional da Restinga de Jurubatiba, Macaé, RJ. Revista Brasileira de Botânica 24(1): 1-9.

COLWELL, R.K. 2013. EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Persistent URL: <purl.oclc.org/estimates>.

COSENDEY, B.N., ROCHA, C.F.D. & MENEZES, V.A. 2016. Population density and conservation status of the Teiid lizard *Cnemidophorus littoralis*, an endangered species endemic to the sandy coastal plains (restinga habitats) of Rio de Janeiro state, Brazil. Journal of Coastal Conservation 20(2). DOI 10.1007/s11852-016-0421-4.

COSTA, W.J.E.M. 2008. *Rivulus jurubatibensis*, a new aplocheiloid killifish from the southeastern Brazilian coastal plains (Cyprinodontiformes: Rivulidae). Ichthyological Exploration Freshwaters 19(2): 135-140.

CRUZ, C.A.G. & PEIXOTO, L. 1983. Uma nova especie de *Hyla* do estado do Espirito Santo, Brasil (Amphibia, Anura, Hylidae). Revista Brasileira de Biologia 42: 721-724.

DEAN, W. 1997. With Broadax and Firebrand: The Destruction of the Brazilian Atlantic Forest. University of California Press, pp.504.

DIAS, I.R., MEDEIROS, T.T., NOVA, M.F.V. & SOLÉ, M. 2014. Amphibians of Serra Bonita, southern Bahia: a new hotpoint within Brazil's Atlantic Forest hotspot. ZooKeys 449: 105-130.

DUELLMAN, W.E. 1999. Patterns of distribution of amphibians: A Global Perspective. The Johns Hopkins University Press, Baltimore.

ESTEVES F.A. & LACERDA L.D. 2000. Ecologia de restingas e lagoas costeiras. Computer & Publish Editoração Gráfica. Núcleo de Pesquisas Ecológicas de Macaé (NUPEM/UFRJ), pp. 394.

ESTEVES, F.A. 2011. Do Índio Goitacá à economia do petróleo: Uma viagem pela história e ecologia da maior restinga protegida do Brasil. Ed. Essentia, Campos dos Goytacazes, RJ, pp. 232.

FALKENBERG, D.B. 1999. Aspectos da flora e vegetação secundária da restinga de Santa Catarina, Sul do Brasil. INSULA Revista de Botânica 28: 1-30.

FISHER, M.C., HENK, D.A., BRIGGS, C.J., BROWNSTEIN, J.S., MADOFF, L.C., MCCRAW, S.L. & GURR, S.J. 2012. Emerging fungal threats to animal, plant and ecosystem health. Nature 484: 186-194.

FREIRE, M.S.B.1990. Levantamento florístico do Parque Estadual das Dunas de Natal. Acta Botânica Brasílica, 4(2): 41-59.

FROST, D.R. 2018. Amphibian Species of the World: an Online Reference. Version 6.0. American Museum of Natural History, New York, USA. Available online at: http://research.amnh.org/vz/herpetology/amphibia/. (last access on 07/04/2018).

GAREY, M.V., PROVETE, D.B., MARTINS, I.A., HADDAD, C.F.B. & ROSSA-FERES, D.C. 2014. Anurans from the Serra da Bocaina National Park and surrounding buffer area, southeastern Brazil. Check List: Jounal of species list and distribuition 10(2): 308-316.

GONDIM-SILVA, F.A.T., ANDRADE, A.R.S., ABREU, R.O., NASCIMENTO, J.S., CORRÊA, G.P., MENEZES, L., TREVISAN, C.C., CAMARGO, S.S. & NAPOLI, M. F. 2016. Composition and diversity of anurans in the Restinga of the Conde municipality, northern coast of the state of Bahia, Northeastern Brazil. Biota Neotropica 16(3): e20160157. http://www.biotaneotropica.org.br/v16n3/pt/abstract?article+bn01016032016. (last accessed on: 25/08/2017).

HADDAD, C.F.B. & PRADO C.P.A. 2005. Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. *BioScience* 55(3): 207-217.

HADDAD, C.F.B., TOLEDO, L.F., PRADO, C.P.A., LOEBMANN, D., GASPARINI, J L. & SAZIMA, I. 2013. Guia dos anfíbios da Mata Atlântica – Diversidade e Biologia. Anolis Books Editora, pp. 544.

HENRIQUES, R.P.B., ARAÚJO, D.S.D. & HAY, J.D. 1986. Descrição e classificação dos tipos de vegetação da restinga de Carapebus, Rio de Janeiro. Revista Brasileira de Botânica 9: 173-189.

HEYER, W.R., RAND, A.S., CRUZ, C.A.G., PEIXOTO, O.L. & NELSON, C.E. 1990. Frogs of Boracéia. Arquivos de Zoologia 31(4): 231-410.

HOWARD, S.D. & BICKFORD, D.P. 2014. Amphibians over the edge: silent extinction risk of Data Deficient species. Diversity and Distributions 20(7): 837-846.

IUCN (INTERNATIONAL UNION FOR CONSERVATION OF NATURE). 2018. IUCN Red List of threatened species. Version 2018.2. http://www.iucnredlist.org/ (last access on 30/11/2018).

IZECKSOHN, E. 1959. Uma nova espécie de "Hylidae" da Baixada Fluminense, Estado do Rio de Janeiro, Brasil. Revista Brasileira de Biologia 19: 259-264.

IZECKSOHN, E. 1976. Uma nova espécie de *Leptodactylus* do Estado do Rio de Janeiro, Brasil (Amphibia, Anura, Leptodactylidae). Revista Brasileira de Biologia 36: 527-530.

JENKINSON, T.S., BETANCOURT ROMÁN, C.M., LAMBERTINI, C., VALENCIA-AGUILAR, A., RODRIGUEZ, D., NUNES-DE-ALMEIDA, C.H.L., RUGGERI, J., BELASEN A.M., DA SILVA-LEITE, D., ZAMUDIO, K.R., LONGCORE, J.E., TOLEDO, F.L. & JAMES, T.Y. 2016. Amphibian-killing chytrid in Brazil comprises both locally endemic and globally expanding populations. Molecular Ecology 25: 2978-2996.

LACERDA, L.D., ARAUJO, D.S.D. & MACIEL, N.C. 1993. Dry coastal ecosystems of the tropical Brazilian coastal in dry coastal-ecosystems: Africa, Asia, Oceania (E. Van der Maarel, ed.). Elsevier, Amsterdam, pp. 477-493.

LEMOS, H.M. & GONÇALVES, P.R. 2015. DINÂMICA POPULACIONAL DE CERRADOMYS GOYTACA TAVARES, PESSÔA & GONÇALVES, 2011 (RODENTIA: CRICETIDAE), Uma espécie endêmica de restingas. Oecologia Australis 19(1): 195-214.

LOYOLA, R.D., LEMES, P., BRUM, F.T., PROVETE, D.B. & DUARTE, L.D.S. 2014. Clade-specific consequences of climate change to amphibians in Atlantic Forest protected areas. Ecography 37: 65-2.

MANEYRO, R. & LANGONE, J.A. 2001. Categorización de los anfibios del Uruguay. Cuadernos de Herpetología 15 (2): 107-118.

MARGULES, C.R & PRESSEY R.L. 2000. Systematic conservation planning. Nature 405: 243-253.

MCDIARMID, R.W. 1994. Preparing amphibians as scientific specimens. In Measuring and monitoring biological diversity: standard methods for amphibians (Heyer W.R. *et al*., eds.). Washington, D.C. Smithsonian Institution Press, pp. 289-297.

MYERS, N., MITTERMEIER, R.A., MITTERMEIER, C.G., FONSECA, G.A.B. & KENT, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403: 853-858.

NARVAES, P., BERTOLUCI, J. & RODRIGUES, M.T. 2009. Composição, uso de hábitat e estações reprodutivas das espécies de anuros da floresta de restinga da Estação Ecológica Juréia-Itatins, sudeste do Brasil. Biota Neotropica 9(2): http://www.biotaneotropica.org.br/v9n2/pt/abstract?article+bn02009022009. (last accessed on: 30/09/2017).

OLIVEIRA, J.C.F. & ROCHA, C.F.D. 2015. Journal of coastal conservation: a review on the anurofauna of Brazil's sandy coastal plains. How much do we know about it?. Journal of Coastal Conservation 19: 35-49.

OLIVEIRA, J.C.F., WINCK, G.R., PEREIRA-RIBEIRO, J. & ROCHA, C.F.D. 2017. Local Environmental Factors Influence the Structure of Frog Communities on the Sandy Coastal Plains of Southeastern Brazil. Herpetologica 73(4): 307-312.

PEIXOTO, O.L. 1988. Sobre o "status" taxonômico de *Hyla catharinae alcatraz* B. Lutz 1973, com a descrição de uma nova espécie para o grupo *perpusilla* (Amphibia, Anura, Hylidae). Acta Biologica Leopoldensia. 10: 253-267.

PEIXOTO, O.L. 1995. Associação de anuros a bromeliáceas na Mata Atlântica. Revista Universidade Rural, Serie: Ciências Da Vida 17(2): 75-83.

PELOSO, P.L.V., FAIVOVICH, J., GRANT, T., GASPARINI, J.L. & HADDAD, C.F.B. 2012. An extraordinary new species of *Melanophryniscus* (Anura, Bufonidae) from southeastern Brazil. American Museum Novitiates 3762:1-32.

PIMENTA, B. & PEIXOTO, O.L. 2004. *Chiasmocleis lacrimae* (errata version published in 2015). The IUCN Red List of Threatened Species: e.T57753A85993475. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T57753A11680689.en. (last access on 18/01/2018)

RIBEIRO, M.C., METZGER, J.P., MARTENSEN, A.C., PONZONI, F.J. & HIROTA, M.M. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142:1141-1153.

ROCHA, C.F.D., VAN SLUYS, M., HATANO, F.H., BOQUIMPANI-FREITAS, L., MARRA, R.V. & MARQUES, R.V. 2004a. Relative efficiency of anuran sampling methods in a restinga habitat (Jurubatiba, Rio de Janeiro, Brazil). Brazilian Journal of Biology, 64 (4): 879-884.

ROCHA, C.F D., BERGALLO H.G., ALVES, M.A.S. & VAN SLUYS, M. 2004b. A Restinga de Jurubatiba e a conservação dos ambientes de restinga do Estado do Rio de Janeiro. In Pesquisas de Longa Duração na Restinga de Jurubatiba: Ecologia, História Natural e Conservação (C.F.D. Rocha; F.A. Esteves & F.R. Scarano, eds). Rima Editora, São Carlos 1: 341-352.

ROCHA, C.F.D., VAN SLUYS, M., BERGALLO, H.G. & ALVES, M.A.S. 2005. Endemic and threatened tetrapods in the restingas of the biodiversity corridors of Serra do Mar and of the Central da Mata Atlântica in eastern Brazil. Brazilian Journal of Biology 65(1):159-168.

ROCHA, C.F.D., BERGALLO, H.G., VAN SLUYS, M., ALVEZ, M.A.S. & JAMEL, C.E. 2007. Os remanescentes dos habitats de restinga na Floresta Atlântica do estado do Rio de Janeiro, Brasil: perda de habitat e risco de desaparecimento. Brazilian Journal of Biology 67(2): 263-273.

ROCHA, C.F.D., HATANO, F.H., VRCIBRADIC, D. & VAN SLUYS, M. 2008. Frog species richness, composition and β-diversity in coastal Brazilian restinga habitats. Brazilian Journal of Biology 68(1): 101-107.

SCARANO, F.R. 2002. Structure, function and floristic relationships of plant communities in stressful habitats marginal to the Brazilian Atlantic Rainforest. Annals of Botany 90: 517-524.

SCHINEIDER, J.A.P. & TEIXEIRA R.L. 2001. Relacionamento entre anfíbios anuros e bromélias da Restinga de Regência, Linhares, Espirito Santo, Brasil. Iheringia, Série Zoologia, Porto Alegre 91: 41-48.

SCOTT JR., NORMAN, J. 2001. Técnicas estándar para inventarios y monitoreos. Inventario completo de especies. p. 73-80. In Medición y monitoreo de la diversidad biológica. Métodos estandarizados para anfibios. (W.R. Heyer, M.A. Donnelly; R.W. McDiarmid; L.A.C. Hayek & M.S. Foster, eds). Editorial Universitaria de la Patagonia, Comodoro Rivadavia, Argentina, pp. 349.

SEGALLA, M.V., CARAMASCHI, U., CRUZ, C.A.G., GRANT, T., HADDAD, C.F.B., GARCIA, P.C.A., BERNECK, B.V.M. & LANGONE, J.A. 2016. Brazilian amphibians: list of species. Herpetologia Brasileira 5: 34-46.

SILVA, H.R., BRITO-PEREIRA M.C., CARAMASCHI, U. & CERQUEIRA, R. 1988. Anfíbios anuros da Restinga de Maricá: levantamento e observações preliminares sobre a atividade reprodutiva das espécies registradas. Anais do Seminário Regional de Ecologia 6: 295-306.

SILVA, H.R., CARVALHO, A.L.G. & BITTENCOURT-SILVA, G.B. 2008. Frogs of Marambaia: a naturally isolated restinga and Atlantic Forest remnant of southeastern Brazil. Biota Neotropica 8(4): 167-174. http://www.biotaneotropica.org.br/v8n4/pt/abstract?article+bn01808042008. (last accessed on: 15/07/2017).

SILVA, D.P., GOMES, I.A.M. & CRUZ, M.A.L. 2012. Vegetação de restinga: aspectos do impacto provocado pelo desenvolvimento sócio-econômico da região norte-fluminense e alternativas para sua valorização. InterSciencePlace 23(1):71-83.

SILVEIRA, A.L., SALLES, R.O.L. & PONTES, R.C. 2009. Amphibia, Anura, Bufonidae, *Rhinella pygmaea*: Distribution extension and geographic distribution map. Check List 5(3): 749-752.

STUART, S.N., CHANSON, J.S., COX, N.A., YOUNG, B.E., RODRÍGUEZ, A.S.L., FISHMAN, D.L. & WALLER, R.W. 2004. Status and trends of amphibian declines and extinctions worldwide. Science 306: 1783-1786.

TABARELLI, M., AGUIAR, A.V., RIBEIRO, M.C. & METZGER J.P. 2010. Prospects for biodiversity conservation in the Atlantic Forest: Lessons from aging human-modified landscapes. Biological Conservation 143(10): 2328-2340.

TEIXEIRA, R.L., SCHINEIDER, J.A.P. & ALMEIDA, G.I. 2002. The occurrence of amphibians in bromeliads from southeastern Brazilian resting habitat, with special reference to *Aparasphenodon brunoi* (ANURA, HYLIDAE). Brazilian Journal of Biology 62(2): 263-268.

TELLES, S.B.S., MENEZES, V.A., MAIA-CARNEIRO, T., DORIGO, T.A., WINCK, G.R. & ROCHA, C.F.D. 2012. Anurans from the "Restinga" of Parque Natural Municipal de Grumari, state of Rio de Janeiro, southeastern Brazil.Check List 8(6): 1267-1273.

VAN SLUYS, M., ROCHA, C.F.D., HATANO, F.H., BOQUIMPANI-FREITAS, L. & MARRA, R.V. (2004): Anfíbios da Restinga de Jurubatiba: composição e história natural. In Pesquisas de longa duração na restinga de Jurubatiba: ecologia, história natural e conservação (C.F.D. Rocha, F.A. Esteves & F.R. Scarano, eds.). – RiMa, São Carlos, SP, p.165-318.

VASCONCELOS, T.S., DOS SANTOS, T.G., HADDAD, C.F.B. & ROSSA-FERES, D.C. 2010. Climatic variables and altitude as predictors of anuran species richness and number of reproductive modes in Brazil. Journal of Tropical 26 (4): 423-432.

VASCONCELOS T.S., NASCIMENTO, B.T.M. & PRADO V.H.M. 2018. Expected impacts of climate change threaten the anuran diversity in the Brazilian hotspots. Ecology and Evolution 8: 7894-7906. https://doi.org/10.1002/ece3.4357.

VILELA, V.M.F.N., BRASSALOTI, R.A. & BERTOLUCI, J. 2011. Anuran fauna of the restinga forest of the Parque Estadual da Ilha do Cardoso, Southeastern Brazil: species composition and breeding site utilization. – Biota Neotropica 11(1) 83-93

WACHLEVSKI, M. & ROCHA, C.F.D. 2010. Amphibia, Anura, restinga of Baixada do Maciambu, municipality of Palhoça, state of Santa Catarina, southern Brazil. – Check List 6: 602-604.

WILKINSON, M., MEASEY, J., SILVANO, D. & CRUZ, C.A.G. 2004. *Chthonerpeton braestrupi*. The IUCN Red List of Threatened Species. e.T59535A11960054. http://dx.doi.org/10.2305/IUCN.UK.2004.RLTS.T59535A11960054.en. (last accessed on: 18/01/2017).

Received: 09/01/2019
Revised: 13/02/2019
Accepted: 26/02/2019
Published online: 28/03/2019

Biota Neotropica 19(2): e20180633, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Occurrence of concavities on the lorica of two species of *Testudinella* (Rotifera, Monogononta, Testudinellidae)

***Paula Nunes Coelho*[1]*, *Louizi Souza Magalhães Braghin*[2], *Fabio Amodêo Lansac-Tôha*[2] & *Raoul Henry*[1]**

[1]Universidade Estadual Paulista, Instituto de Biociências, Departamento de Zoologia, 18618-970, Botucatu, SP, Brasil
[2]Universidade Estadual de Maringá, Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Maringá, PR, Brasil
**Corresponding author: Paula Nunes Coelho, e-mail: paulinhancoelho@gmail.com*

COELHO, P.N., BRAGHIN, L.S.M., LANSAC-TÔHA, F.A., HENRY, R. **Occurrence of concavities on the lorica of two species of *Testudinella* (Rotifera, Monogononta, Testudinellidae)**. Biota Neotropica. 19(2): e20180633. http://dx.doi.org/10.1590/1676-0611-BN-2018-0633

Abstract: *Testudinella* is a taxon of Rotifera broadly distributed in Brazil. In a recent collection in marginal lakes connected to the Paranapanema River (São Paulo, Brazil), we found specimens of *Testudinella mucronata* and *T. patina* with concavities on their lorica. The objective of this study was to describe the concavities observed in individuals of both species and discuss their possible causes. Plankton samples were collected at two sampling stations, in the pelagic (PZ) and littoral (LZ) zones of the Panema and Coqueiral lakes; both of these lakes are ultra-oligotrophic and not polluted. For *T. mucronata*, 4% of individuals with concavities were found in the PZ of Coqueiral lake and 50% in the LZ of Panema lake. In *T. patina*, the proportion of specimens with concavities corresponded to 34% in the LZ of Panema lake and 17% in PZ of Coqueiral lake. In this study, we discussed that low water temperatures, predation events and/or recent hatching are factors that may justify the concavities in the lorica of specimens of our work.

Keywords: *Freshwater environments, marginal lakes, morphology, rotifers, polymorphism.*

Ocorrência de concavidades na lórica de duas espécies de *Testudinella* (Rotifera, Monogononta, Testudinellidae)

Resumo: *Testudinella* é um táxon de Rotifera amplamente distribuído no Brasil. Em recentes coletas em lagos marginais conectados ao rio Paranapanema (São Paulo, Brasil), nós encontramos espécimes de *Testudinella mucronata* e *T. patina* com concavidades em suas lóricas. O objetivo do estudo foi descrever as concavidades observadas em indivíduos de ambas as espécies e discutir suas possíveis causas. Amostras de plâncton foram coletadas em duas estações de amostragem, na zona pelágica (PZ) e litorânea (LZ) dos lagos Panema e Coqueiral; ambos os lagos são ultra-oligotróficos e não poluídos. Para *T. mucronata*, 4% dos indivíduos com concavidades foram encontrados na PZ do lago Coqueiral e 50% na LZ do lago Panema. Em *T. patina*, a proporção de espécimes com concavidades correspondeu a 34% na LZ do lago Panema e 17% na PZ do lago Coqueiral. Nesse estudo, nós sugerimos que baixas temperaturas da água, eventos de predação e/ou recente eclosão são fatores que podem justificar as concavidades na lórica dos espécimes do nosso trabalho.

Palavras-chave: *Ambientes de água doce, lagos marginais, morfologia, rotíferos, polimorfismo.*

Introduction

Rotifera are small animals with two peculiar structures called ciliated corona and mastax (Wallace et al. 2006). The representatives of this Phylum are distributed globally in both marine and freshwater ecosystems (e.g., Eriksen 1968; Sarma & Elias-Gutierrez 1997; Sharma & Sharma 2005; Fontaneto et al. 2008). Segers (2008) reported 2,031 described species for Rotifera; currently this number is probably greater due to numerous recent descriptions (e.g., Segers & Walsh 2017).

Many monogonont rotifers are well known to respond to environmental factors with morphological variations in their body. This phenomenon is known as polymorphism and is usually caused by kairomones from predators, variations in water temperature and food availability (see review of Gilbert 2017; 2018). In general, polymorphism in rotifers has great relevance because morphological variations can influence life history, population dynamics and ecological interactions (Gilbert, 2017). Moreover, laboratory bioassays demonstrated that environmental pollution of metals can change the size and morphology of rotifers (Ríos-Arana et al. 2007, Xue et al. 2017).

Testudinella Bory de St. Vincent, 1822 (Monogononta, Testudinellidae) is a rotifer taxon composed of approximately 40 valid species (Segers 2007), of which 18 occur in Brazilian waters (Garraffoni & Lourenço 2012). Among these species, *Testudinella patina* (Hermann, 1783) and *T. mucronata* (Gosse, 1886) are found in lakes (e.g., Garcia et al 1998; Casanova et al. 2009; Maia-Barbosa et al. 2014) and many freshwater ecosystems throughout the country (see Garraffoni & Lourenço 2012).

During a study conducted in two marginal lakes to the Paranapanema River (São Paulo State, Brazil), we collected specimens of *T. patina* and *T. mucronata* with the presence of concavities on the lorica. In this work, we describe the cases and discuss possible causes of the presence of these concavities.

Material and Methods

1. Study area

Panema (23°31'29"S; 48°38'55.4"W) and Coqueiral lakes (23°29'24"S; 48°37'10.9"W) are marginal environments connected to the Paranapanema River at its mouth zone, flowing into the Jurumirim Reservoir (São Paulo State, Brazil) (Figure 1). The average depth is 1.2 m for Panema lake and 2.5 m for Coqueiral lake; these lakes have total areas of 0.07 and 0.582 km^2 (Silva & Henry 2013), respectively. Coqueiral lake is dominated by extensive coverage of macrophytes, while Panema lake has low abundance of aquatic vegetation.

2. Data collection and analysis

Samples were collected in April 2017 (rainy season), from two sampling stations, in the pelagic (PZ) and littoral zones (LZ) of each of the studied lakes. The water surface temperature was measured with a Toho Dentam thermometer, pH with a Micronal B-380 pH meter and the electrical conductivity with a Hach Mod. 2511 conductivimeter. The dissolved oxygen concentration of the water was determined by the method of Winkler, modified by Golterman et al. (1978). The total phosphorus and nitrogen concentrations were determined according to Strickland & Parsons (1960) and Mackereth et al. (1978), respectively. The chlorophyll-*a* concentration was determined using the 90% acetone extraction method (Golterman et al. 1978). The trophic state index was calculated to classify the trophic level in both zones of the lakes (Cunha et al. 2013).

Rotifers were collected with a plankton net (50 μm mesh size), by means of a vertical trawl in the water column. The filtered volume (V) was calculated by the formula $V = \pi.r^2.d$, where r is radius of plankton net opening and d is the distance traveled by the net. Water sampling was conducted in order to result a filtered final volume of 100 L. Subsequently, the organisms were fixed with a 4% formaldehyde solution saturated with sugar. The counting of sampled individuals was carried out in a minimum of four subsamples of 1 mL, in a Sedgewick-Rafter chamber under an optical microscope; abundance was expressed in terms of individuals.m^{-3}. The organisms were identified based on specialized literature (Koste 1978, Sharma 1990, Shiel 1995).

In order to estimate the frequency of individuals with concavities, two categories were established based on the external morphology for *Testudinella mucronata* (individuals were sorted based on the presence and absence of concavities; see Figure 2). Three categories were designated for *T. patina* (absence of concavities; Type 1 - presence of a concavity on the lorica; Type 2 - more than two concavities on the lorica; Figure 3). This nomenclature (i.e., Type 1 and Type 2) was used for Cladocera (see Melo et al. 2017) and adapted herein to apply to Rotifera as well.

A student's t-test was used to verify significant differences between limnological variables and zones of each lake. Normality assumptions were performed with the Shapiro-Wilk test. We used the level of statistical significance of 95% ($p<0.05$). Statistical analyses were performed in R software (R Development Core Team, 2016).

Results

In general, high mean values of water temperature and electric conductivity were recorded in Panema lake, while low dissolved oxygen concentrations were found in Coqueiral lake (Table 1). In both lakes, the pH was neutral. Total phosphorus and nitrogen concentrations were low in both lakes. In relation to the limnological variables between the compartments of the lakes (pelagic and littoral zone), there was no significant difference between both lakes ($p>0.05$). The trophic status indexes showed that the lakes are composed of ultra-oligotrophic waters, in both the pelagic and littoral areas (Table 1).

Concavities were observed in individuals of both species of rotifers. In the case of *Testudinella mucronata*, several concavities were observed on the lorica of the individuals (Figure 2). In both lakes, the densities of affected organisms reached 59 ind.m^{-3} in littoral zone of the Panema lake and 33 ind.m^{-3} (pelagic zone) in Coqueiral lake (Figure 4). For this species, 4% of individuals with concavities were found in the PZ of Coqueiral lake and 50% in the LZ of Panema lake. In *T. patina*, the concavities were classified as Type 1 and Type 2 (Figure 3). The total density of specimens with Type 1 and Type 2 concavities for this taxon were 24 ind.m^{-3} in Coqueiral lake and 118 ind.m^{-3} in Panema lake (Figure 5). The total proportion of organisms with both types of concavities observed in *T. patina* corresponded to 34% in LZ of Panema lake and 17% in the PZ of Coqueiral lake.

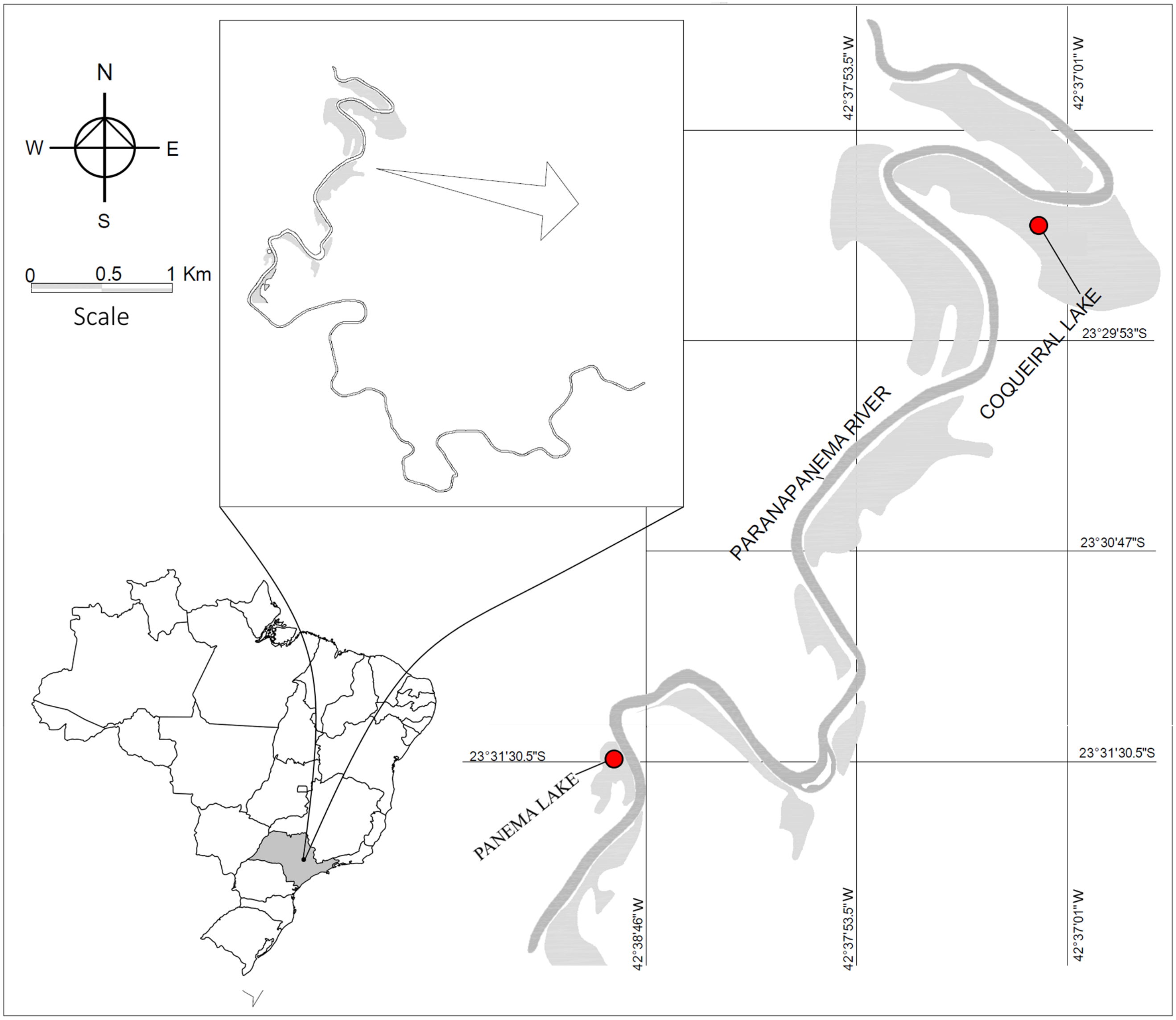


Figure 1. Localization of the sampling stations (red circle) in two marginal lakes on the Paranapanema River, São Paulo, Brazil.

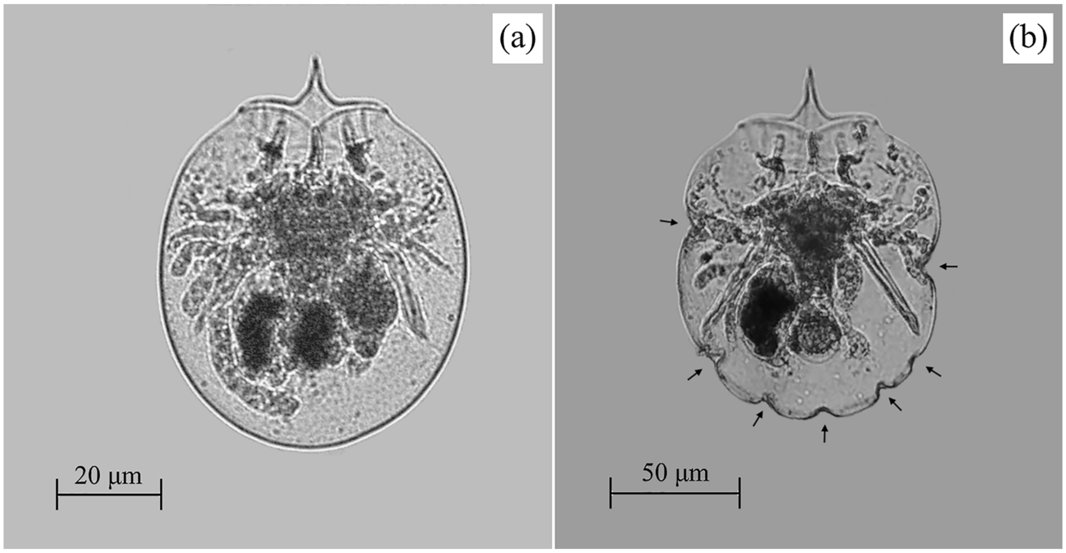


Figure 2. *Testudinella mucronata* with absence (a) and presence of concavities (b). The arrows indicate the locations of the concavities.

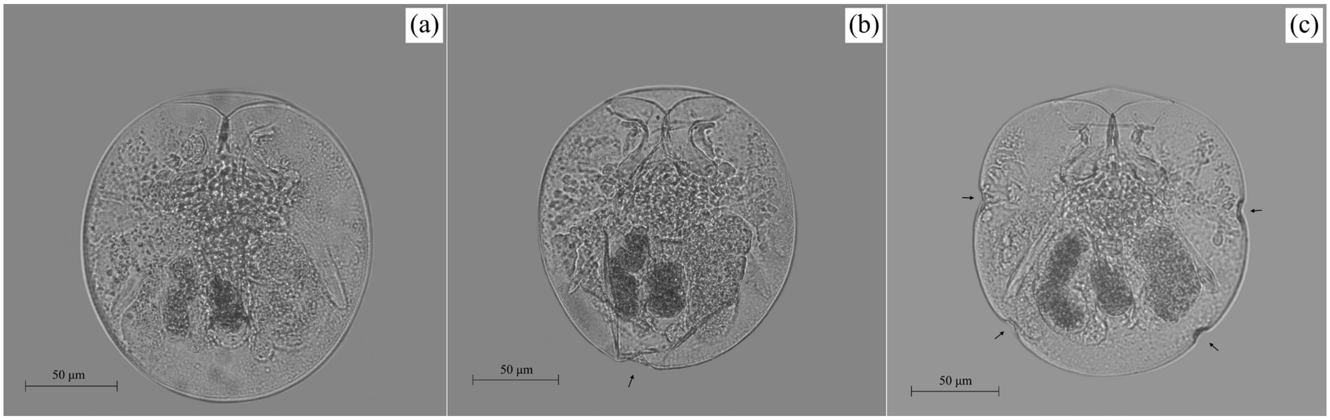


Figure 3. *Testudinella patina* with absence (a) presence of Type 1 (b) and Type 2 concavities (c). The arrows indicate the locations of the concavities.

Table 1. Mean and standard deviation of the physical and chemical variables of water in the pelagic and littoral zones of Coqueiral and Panema lakes (April 2017).

Variables	Pelagic		Littoral	
	Coqueiral	Panema	Coqueiral	Panema
Temperature (°C)	25.58 ± 0.25	26.6 ± 0.12	25.7 ± 0.05	26.2 ± 0.18
pH	7.0 ± 0.04	6.8 ± 0.04	6.8 ± 0.05	6.8 ± 0.05
Electric conductivity (µS.cm^{-1})	56.9 ± 1.4	92.5 ± 0.86	56.9 ± 0.69	94.1 ± 0.95
Dissolved oxygen (mg.L^{-1})	2.9 ± 0.05	5.1 ± 0.05	2.4 ± 0.29	4.9 ± 0.10
Total phosphorus (µg.L^{-1})	6.8 ± 3.08	7.4 ± 5.68	6.6 ± 3.43	5.7 ± 2.05
Total nitrogen (µg.L^{-1})	309.3 ± 47.81	355.6 ± 65.19	320.2 ± 64.48	401.9 ± 17.65
Trophic status index	47.3 ± 0.45	45.9 ± 0.86	45.1 ± 0.96	47.4 ± 0.62
Chlorophyll-*a* (µg.L^{-1})	0.69 ± 0.17	0.21 ± 0.08	0.19 ± 0.01	0.10 ± 0.03

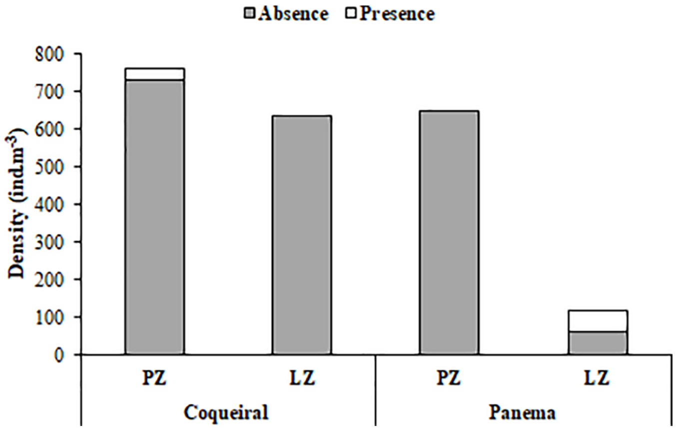


Figure 4. Density of individuals (ind.m^{-3}) with absence and presence of concavities in *Testudinella mucronata* collected in two marginal lakes (São Paulo State, Brazil). PZ = pelagic zone and LZ = littoral zone.

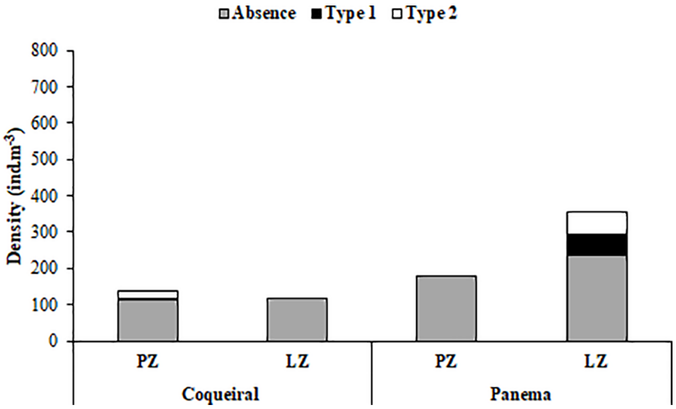


Figure 5. Density of individuals (ind.m^{-3}) with absence and presence of concavities of Type 1 and Type 2 in *Testudinella patina* collected in two marginal lakes (São Paulo State, Brazil). PZ = pelagic zone and LZ = littoral zone.

Discussion

Morphological alterations in zooplankton may be caused by human impacts on aquatic environments (e.g., Montú and Gloeden 1982, Dias et al. 1999, Souza et al. 2011, Melo et al. 2017). In Rotifera, for instance, Zurek (2006) recorded deformations on the spines of *Keratella cochlearis* (Gosse, 1851) caused by the exposure to sulfides or derivatives present in the water from a sulfur mine impoundment. Ecotoxicological tests have also shown that heavy metals and pesticides induce morphological changes in the rotifer *Plationus patulus* (Müller, 1786) (Ríos-Arana et al. 2007) and *Brachionus calyciflorus* Pallas 1766 (Alvarado-Flores et al. 2015). In our study, concentrations of phosphorus and total nitrogen in lakes were around 7 µg.L^{-1} and 355 µg.L^{-1}, respectively. When comparing these values with the reference conditions for nitrogen (300-350 µg.L^{-1}) and total phosphorus (10-15 µg.L^{-1}) established in subtropical reservoirs with different levels of enrichment (Cunha et al. 2012; Cunha & Calijuri 2011), we concluded that the lakes studied here are relatively nutrient depleted. In addition, the trophic status index showed that the lakes were classified in ultra-oligotrophic category, suggesting that the trophic state of the environments is not related to the presence of concavities in *Testudinella*. While we did not performed metal analyses, we believe that metals are not the cause of the concavities. Both lakes, despite their riverine connections, are not near any pollution sources and their environments are ultra-oligotrophic.

On the other hand, there are reports of polymorphism in some Rotifera species of the Brachionidae family (Stemberger & Gilbert 1984, see Gilbert 1999). This phenomenon can be induced by several environmental factors, including temperature variations (e.g., Gilbert 2018, Ge et al 2018). For example, some studies report that the temperature is responsible for lengthening spines of lorica in *B. falcatus* (Zacharias, 1898) and *K. cochlearis* (Bielañska-Grajner 1995; Ahad & Rao 2017). At low temperatures, the population growth rate of *K. cochlearis* decreases, resulting in the occurrence of organisms with longer caudal spines (Lindström & Pejler 1975). In the present study, there was no significant difference in limnological variables between the pelagic and littoral zones of the lakes. However, our study didn't include the temporal scale. The occurrence of concavities may also be due to the colder riverine influx, since both lakes are connected to the river channel. Studies carried out in lacustrine environments in the same region related the influence of the lateral influx of water from the river on the limnological variables and planktonic community (Casanova et al. 2009; Henry et al. 2011).

Others factors responsible for the morphological differences in rotifers are food availability and vulnerability to predation (e.g., Stemberger 1988; Garza-Mouriño et al 2005, Gilbert 2009). Gilbert (1967, 1980) reported that the formation of defensive lateral spines in *B. calyciflorus* were an induced response to the chemical presence (i.e., kairomone) of the predator *Asplanchna.* Similar results were observed in *B. quadridentatus* Hermann, 1783 (Gilbert 2001), *K. tropica* (Apstein, 1907) (Gilbert 2009) and *P. patulus macracanthus* (Daday, 1905) (Sarma et al 2011). In this study, we observed the occurrence of *Asplanchna brightwelli* Gosse, 1850 in both environments, suggesting that some individuals of *Testudinella* could develop concavities to avoid predation.

Lastly, concavities may be related to the stage of development. De Smet (2009) suggests that the presence of concavities (called by this author as "flattening") on the lorica of *T. elliptica* (Ehrenberg, 1834) is related to the development of the vitellarium. It is likely that the same occurrence is applicable to the organisms of both species of *Testudinella.* We do not discard the possibility that individuals in this study may be newly hatched.

In summary, here we described the presence of concavities in *T. mucronata* and *T. patina,* suggesting three possible causes for their formation: (i) temperature; (ii) predation; (iii) or recent hatching. However, additional studies are needed to investigate which of these possibilities are responsible for presence of concavities in our exemplars.

Acknowledgments

We are grateful to Reuben W. Nowell for the English revision. PNC was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Author Contributions

Paula Nunes Coelho: Collaborated with writing and successive revisions of the manuscript; idealized the study and performed the collections; identified the species.

Louizi Souza Magalhães Braghin: Collaborated with writing and successive revisions of the manuscript; identified the species.

Fabio Amodêo Lansac-Tôha: Collaborated with writing and successive revisions of the manuscript.

Raoul Henry: Idealized the study and performed the collections; collaborated with writing and successive revisions of the manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AHAD, N. & RAO, R.J. 2017. The nature and causes of cyclomorphosis in some species of *Brachionus-Brachionus falcatus* and *Brachionus forficula.* Int. J. Zool. Stud. 2(5): 31-35.

ALVARADO-FLORES, J., RICO-MARTÍNEZ, R., ADABACHE-ORTÍZ, A. & SILVA-BRIANO, M. 2015. Morphological alterations in the freshwater rotifer *Brachionus calyciflorus* Pallas 1766 (Rotifera: Monogononta) caused by vinclozolin chronic exposure. Ecotoxicology. 24(4): 915-925.

BIELAÑSKA-GRAJNER, I. 1995. Influence of temperature on morphological variation in populations of *Keratella cochlearis* (Gosse) in Rybnik Reservoir. Hydrobiologia. 313/314: 139-146.

CASANOVA, SMC, PANARELLI, EA & HENRY, R. 2009. Rotifer abundance, biomass, and secondary production after the recovery of hydrologic connectivity between a river and two marginal lakes (São Paulo, Brazil). Limnologica. 39(4): 292-301.

CUNHA, D.G.F. & CALIJURI, M.D.C. 2011. Limiting factors for phytoplankton growth in subtropical reservoirs: the effect of light and nutrient availability in different longitudinal compartments. Lake Reserv. Manag. 27(2):162-172.

CUNHA, D.G.F., CARMO CALIJURI, M. & LAMPARELLI, M.C. 2013. A trophic state index for tropical/subtropical reservoirs (TSItsr). Ecol. Eng. 60: 126-134.

CUNHA, D.G.F., OGURA, A.P. & CALIJURI, M.D.C. 2012. Nutrient reference concentrations and trophic state boundaries in subtropical reservoirs. Wat. Sci. Tech. 65(8): 1461-1467.

DE SMET, W.H. 2009. A review of the marine and brackish-water species of *Testudinella* (Rotifera: Monogononta, Testudinellidae), with the description of two new species. Zootaxa. 2092:1-20.

DIAS, C.D. 1999. Morphological abnormalities on *Acartia lilljeborgi* (Copepoda, Crustacea) in the Espírito Santo Bay (ES, Brazil). Hydrobiologia. 394: 249-251

ERIKSEN, B.G. 1968. Marine rotifers found in Norway, with descriptions of two new and one little known species. Sarsia. 33(1): 23-34.

FONTANETO, D., SEGERS, H. & MELONE, G. 2008. Marine rotifers from the Northern Adriatic Sea, with description of *Lecane insulaconae* sp. nov. (Rotifera: Monogononta: Lecanidae). J. Mar. Bio. Assoc. U. K. 88(2): 253-258.

GARCIA, A.P.P., LANSAC-TÔHA, F.A. & BONECKER, C.C. 1998. Species composition and abundance of rotifers in different environments of the floodplain of the upper Paraná river, Brazil. Revta Bras. Zool. 15(2): 327-343.

GARRAFFONI, A.R. & LOURENÇO, A.P. 2012. Synthesis of Brazilian Rotifera: an updated list of species. Check List. 8(3): 375-407.

GARZA-MOURIÑO, G., SILVA-BRIANO, M., NANDINI, S., SARMA, S. S. S. & CASTELLANOS-PAEZ, M. E. 2005. Morphological and morphometrical variations of selected rotifer species in response to predation: a seasonal study of selected brachionid species from Lake Xochimilco (Mexico). Hydrobiologia. 546(1): 169-179.

GE, Y., XI, Y., MA, J. & XU, D. 2018. Factors Influencing Morphological Characteristics of *Keratella cochlearis* in Lake Tingtang. Proc. Natl. Acad. Sci. India Sect B Biol. Sci. 88(1), 421-428.

GILBERT, J.J. 1967. *Asplanchna* and postero-lateral spine production in *Brachionus calyciflorus*. Arch. Hydrobiol. 64: 1-62.

GILBERT, J.J. 1980. Further observations on developmental polymorphism and its evolution in the rotifer *Brachionus calyciflorus*. Freshwater Biol. 10(3): 281-294.

GILBERT, J.J. 1999. Kairomone-induced morphological defenses in rotifers. In The ecology and evolution of inducible defenses (R. Tollrian & C.D. Harvell, eds). Princeton University Press, New Jersey, p. 127-141.

GILBERT, J.J. 2001. Spine development in *Brachionus quadridentatus* from an Australian billabong: genetic variation and induction by *Asplanchna*. Hydrobiologia. 446(1): 19-28.

GILBERT, J. J. 2009. Predator-specific inducible defenses in the rotifer *Keratella tropica*. Freshwater Biol. 54(9): 1933-1946.

GILBERT, J.J. 2017. Non-genetic polymorphisms in rotifers: environmental and endogenous controls, development, and features for predictable or unpredictable environments. Biol. Rev. Camb. Philos. Soc. 92(2): 964-992.

GILBERT, J.J. 2018. Morphological Variation and Its Significance in a Polymorphic Rotifer: Environmental, Endogenous, and Genetic Controls. BioScience. 68(3): 169-181.

GOLTERMAN, H.L., CLYMO, R.S. & OHNSTAD, M.A.M. 1978. Methods for physical and chemical analysis of freshwaters. Blackwell Scientific, London (vol 2).

HENRY, R., PANARELLI, E.A., CASANOVA, S.M.C., GRANADO, D.C., MORTARI, R.C. & ABRA, J. 2011. Plankton richness and abundance in several different hydrological situations in lakes lateral to a river: a case study in the mouth zone of a tributary into a tropical Reservoir. Oecologia Australis. 15(3): 537-558.

LINDSTRÖM, K., & PEJLER, B. 1975. Experimental studies on the seasonal variation of the rotifer *Keratella cochlearis* (Gosse). Hydrobiologia. 46(2-3): 191-197.

MACKERETH, F.J.H., HERON, J. & TALLING, J.F. 1978. Water analysis: some revised methods for limnologists. Titus Wilson & Son Ltd, Kendall.

MAIA-BARBOSA, P.M., MENENDEZ, R.M., PUJONI, D.G.F., BRITO, S.L., AOKI, A. & BARBOSA, F.A.R. 2014. Zooplankton (Copepoda, Rotifera, Cladocera and Protozoa: Amoeba Testacea) from natural lakes of the middle Rio Doce basin, Minas Gerais, Brazil. Biota Neotrop. 14(1): e20134040.

MELO, R.R., COELHO, P.N., SANTOS-WISNIEWSKI, M.J., WISNIEWSKI, C., MAGALHÃES, C.S. 2017. Morphological abnormalities in cladocerans related to eutrophication of a tropical reservoir. J. Limnol. 76: 94-102.

MONTÚ, M. & GLOEDEN, I. 1982. Morphological alterations in *Acartia tonsa* (Saco da mangueira, lagoa dos Patos, Brazil). Arq. Biol. Tecnol. 25: 361-369.

KOSTE, W. 1978. Rotatoria. Die Rädertiere Mitteleuropas, begründet von Max Voigt. Überordnung Monogononta. Gebrüder Borntaeger, Berlin, Stuttgart.

R Development Core Team, 2016. A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, Available online at: http://www.R-project.org (accessed 26.09.18).

RÍOS-ARANA, J.V., WALSH, E.J. & ORTIZ, M. 2007. Interaction effects of multi-metal solutions (As, Cr, Cu, Ni, Pb and Zn) on life history traits in the rotifer *Plationus patulus*. J. Environ. Sci. Health A. Tox. Hazard Subst. Environ. Eng. 42(10): 1473-1481.

SARMA, S. S. S. & ELIAS-GUTIERREZ, M. 1997. Taxonomic studies of freshwater rotifers [Rotifera] from Mexico. Pol. Arch. Hydrobiol. 3(44): 341-357.

SARMA, S.S.S., RESENDIZ, R.A.L. AND NANDINI, S. 2011. Morphometric and demographic responses of brachionid prey (*Brachionus calyciflorus* Pallas and *Plationus macracanthus* (Daday)) in the presence of different densities of the predator *Asplanchna brightwellii* (Rotifera: Asplanchnidae). Hydrobiologia. 662(1): 179-187.

SEGERS, H. 2007. Annotated checklist of the rotifers (Phylum Rotifera), with notes on nomenclature, taxonomy and distribution. *Zootaxa.* 1564: 1-104.

SEGERS, H. 2008. Global diversity of rotifers (Rotifera) in freshwater. Hydrobiologia. 595: 49-59.

SEGERS, H. & WALSH, E.J. 2017. The genus *Rhinoglena* (Rotifera, Monogononta, Ephiphanidae) in North America, with the description of two new species. Zootaxa. 4290(1): 113-122.

SHARMA, B.K. 1990. The genus *Testudinella* (Eurotatoria: Gnesiotrocha: Testudinellidae) in North-Eastern India. Hydrobiologia. 199: 29-33.

SHARMA, B.K. & SHARMA, S. 2005. Biodiversity of freshwater rotifers (Rotifera, Eurotatoria) from North-Eastern India. Mitt. Zool. Mus. Berl. 81(1): 81-88.

SHIEL, R.J. 1995. A guide to identification of rotifers, cladocerans and copepods from Australian inland waters. CRCFE Ident. Guide 3: 1–144.

SILVA, C. V. & HENRY, R. 2013. Aquatic macroinvertebrates associated with *Eichhornia azurea* (Swartz) Kunth and relationships with abiotic factors in marginal lentic ecosystems (São Paulo, Brazil). Braz. J. Biol. 73(1): 149-162.

SOUSA, F.D.R., ELMOOR-LOUREIRO, L.M.A. & SOUZA, M.B.G. 2011. Occurrence of abnormalities on labral keel of *Coronatella monacantha* (Cladocera, Anomopoda, Chydoridae) in a population from Ceará, Brazil. Braz. J. Biol. 71: 797-798.

STEMBERGER, R.S. & GILBERT, J.J. 1984. Spine development in the rotifer *Keratella cochlearis*: induction by cyclopoid copepods and *Asplanchna*. Freshwater Biol. 14(6): 639-647.

STEMBERGER, R.S. 1988. Reproductive costs and hydrodynamic benefits of chemically induced defenses in *Keratella testudo*. Limnol. Oceanogr. 33(4): 593-606.

STRICKLAND, J.D.H. & PARSONS, T.R. 1960. A manual of seawater analysis. J. Fish Res. Board Can. 125: 1-18.

WALLACE, R.L., SNELL, T.W., RICCI, C. & NOGRADY, T. 2006. Rotifera vol. 1: biology, ecology and systematics (2nd edition). In Segers H., & H. J. Dumont (eds), Guides to the Identification of the Microinvertebrates of the Continental Waters of the World, 23. Kenobi Productions, Gent, Belgium and Backhuys Academic Publishing BV. The Hague, The Netherlands.

XUE, Y.H., YANG, X.X., ZHANG, G. & XI, Y. L. 2017. Morphological differentiation of *Brachionus calyciflorus* caused by predation and coal ash pollution. Sci. Rep. 7(1): 15779.

ŻUREK, R. 2006. Response of rotifers to hydrochemical and biotic factors. Oceanol. Hydrobiol. St. 35(2): 121-139.

Received: 07/08/2018
Revised: 24/01/2019
Accepted: 29/01/2019

Biota Neotropica 19(2): e20180597, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Effect of oil palm (*Elaeis guineensis* Jacq.) plantations on individual-level diet variation of *Hylaeamys megacephalus* (G. Fisher 1814) (Rodentia, Cricetidae) in eastern Amazon Forest

Simone Almeida Pena[1] & Ana Cristina Mendes-Oliveira[2]*

[1]Universidade Federal do Pará, Museu Paraense Emílio Goeldi, Programa de Pós-Graduação em Zoologia,
Rua Augusto Corrêa, 01, CEP: 66075-110, Belém, PA, Brasil
[2]Universidade Federal do Pará, Instituto de Ciências Biológicas, Laboratório de Ecologia e Zoologia de
Vertebrados, Rua Augusto Corrêa , 01, CEP: 66075-110, Belém, PA, Brasil
**Corresponding author: Ana Cristina Mendes-Oliveira, e-mail: cris.mastozoologia@gmail.com*

PENA, S. A., MENDES-OLIVEIRA, A.C. **Effect of oil palm (*Elaeis guineensis* Jacq.) plantations on individual-level diet variation of *Hylaeamys megacephalus* (G. Fisher 1814) (Rodentia, Cricetidae) in eastern Amazon Forest**. Biota Neotropica. 19(2): e20180597. http://dx.doi.org/10.1590/1676-0611-BN-2018-0597

Abstract: In this study we described the diet of *Hylaeamys megacephalus* (G. Fisher, 1814) and investigated the degree of individual variation in the diet of this species among the Amazon Forest and the oil palm plantation. We analyzed the stomach contents of 36 individuals, of whom 11 were collected in the forest and 25 captured in the palm oil palm plantation. The *H. megacephalus* diet consisted of 18 food items, of which 12 were animal composition and eight were vegetable composition. The niche amplitude of the species was narrower in the forest area ($Ba_{forest} = 0.013$) compared to the palm tree plantation area ($Ba_{palm} = 0.478$). This shows that individuals have greater niche overlap in forest areas, while in the plantation areas the animals expand their food niche. In addition, the values of the mean of the individual diet in relation to the diet of the entire population were lower in the palm oil palm plantation environment ($IS_{palm} = 0.164$) than in the Forest environment ($IS_{forest} = 0.357$), indicating a high specialization in the palm oil plantation. These results indicate a population mechanism to reduce intraspecific competition in response to scarce resources.

Keywords: *Diet; food importance, individual specialization, niche amplitude, Rodentia.*

Efeito das plantações de dendê (*Elaeis guineensis* Jacq.) sobre a variação individual da dieta de *Hylaeamys megacephalus* (G. Fisher 1814) (Rodentia, Cricetidae) na Floresta Amazônica Oriental

Resumo: Neste estudo nós descrevemos a dieta de *Hylaeamys megacephalus* (G. Fisher, 1814) e investigamos o grau de variação individual na dieta desta espécie entre os hábitats de Floresta Amazônica e Plantação de Palmeira de Dendê. Analisamos o conteúdo estomacal de 36 indivíduos, dos quais 11 foram coletados na floresta e 25 capturados na plantação de palmeira de dendê. A dieta de *H. megacephalus* consistiu em 18 itens alimentares, dos quais 12 de composição animal e oito de composição vegetal. A amplitude de nicho da espécie foi mais estreita na área de floresta ($Ba_{forest} = 0{,}013$) comparada com a área de plantação de palmeira de dendê ($Ba_{palm} = 0{,}478$). Isto sugere que os indivíduos têm maior sobreposição de nicho nas áreas de floresta, enquanto que nas áreas de plantação os animais expandem seu nicho alimentar. Além disto, os valores da média de similaridade da dieta individual em relação à dieta de toda a população foi menor no ambiente de plantação de palmeira de dendê ($IS_{palm} = 0{,}164$) do que no ambiente de Floresta ($IS_{forest} = 0{,}357$), indicando uma alta especialização individual na plantação de dendê. Estes resultados indicam um mecanismo populacional para reduzir a competição intraespecífica em resposta à escassez de recursos.

Palavras-chave: *Dieta, importância alimentar, especialização individual, amplitude de nicho, Rodentia.*

Introduction

The individual specialization has been considered as an endogenous mechanism of population in response to several factors, such as environmental variations (eg. habitat changes, seasonality or daily periods), ecological pressures (eg. predation, competition, reproductive period) and resource polymorphisms (Skulason & Smith 1995, Bolnick et al. 2003). The resource polymorphism can be explained by the discrete morphological discrepancies related to intraspecific differences in resource use, which not only interferes with the size of a population's niche but may also influence the isolation reproductive behaviour of individuals, representing an intermediate stage of speciation (Smith & Skúlasson 1996). The individual specialization would be a mechanism to reduce intraspecific competition (Roughgarden 1972, Svanbäck & Persson 2004) in populations with high rates of individual specialization, where each individual typically uses a resource set significantly smaller than the set of resources used by the population as a whole and with less overlap of individual niches (Bolnick et al. 2003).

This scenario usually occurs when resources are meager, for example, where individuals vary their diet in order to decrease intraspecific competition (Svanbäck & Bolnick 2005). The opposite would occur when resources are abundant, where the overlap of individual niches is high and individuals would use a pool of resources close to the niche size of the population as a whole (Werner & Hall 1974, Bolnick et al. 2003). Most of these theories have been commonly studied in communities of fish, insects and amphibians (Araújo & Gonzaga 2007, Araújo et al. 2009, Hannah et al. 2013, Bolnick et al. 2014, Costa et al. 2015), but in mammalian species the knowledge about individual specialization and polymorphism of resources is scarce (Olsson et al. 2007, Martins et al. 2008, Camargo et al. 2013, Pires et al. 2013, Svanbäck et al. 2015).

In the Amazon, the conversion of natural ecosystems into oil palm monocultures (*Elaeis guineensis*) has expanded over the last 40 years (Fitzherbert et al. 2008, Butler & Laurence 2009, Wilcove & Koh 2010). The conversion of forest habitats to oil palm plantations causes loss of environmental complexity and reduction of ecosystem resources. Different from the original native forests, the oil palm plantations present a uniform spatial distribution and tree age structure (Fitzherbert et al. 2008) with exposed soils, reduced leaf litter (Yeboua, 2000, Nelson 2002), low-density understory, highly volatile microclimate (Chung et al. 2000), and a lower discontinuous canopy (Turner 2009). All these drastic structural changes induced by the conversion to oil palm plantation, have been affecting the fauna in different ways in the tropical forests all over the world (Danielsen & Heegaard 1995, Chung et al. 2000, Peh et al. 2006, Barlow et al. 2007, Butler & Laurence 2009, Yaap et al. 2010, Correa et al. 2015, Cunha et al. 2015, Ferreira et al. 2018).

Van Valen (1965) was one of the first ecologists to propose the individual-level niche variation as a consequence of different genotypes in the same population. Age classes and sex are factors that could influence the individual variability of niche use in a population (Shine 1991, Bolnick et al. 2003, Martins et al. 2008). However, differences in habitat and resource availability would also influence individual variations (Lomnicki 1980, Bourke et al. 1997). Individual-level niche variations have relevant effects, in the medium and long term, on diversification of species, since they affect the stability of population (Dieckmann & Doebeli 1999). Here we investigate the diet of the small rodent *Hylaeamys megacephalus* (G. Fisher 1814) and evaluated the degree of individual variation in response to the difference of habitats, including native Amazon Forest and oil palm plantation. We hypothesize that in the oil palm plantation the individual variation of the *Hylaeamys megacephalus* diet would be greater than in the forest, because of the lower supply of food resources in the first habitat.

Material and Methods

1. *Hylaeamys megacephalus*

The species *H. megacephalus* (G. Fisher 1814) is a small rodent from the Cricetidae Family, Subfamily Sigmodontinae (Figure 1). According to this study, adults weight an average of 58g ± 2g. They are considered as frugivorous/gramnivorous (Paglia et al. 2012) and are primarily terrestrial (Nitikman & Mares 1987) but also scansorial (Voss et al. 2001). Despite the low sampling of this species in Amazon Forest, it is probably widely distributed in this biome (Musser et al. 1998, Patton et al. 2000). In addition, its distribution is known for the Cerrado and the Atlantic Forest (Paglia et al. 2012, Percequillo et al. 2015), always associated with forest habitats (Mares et al. 1986, Ochoa et al. 1993, Percequillo et al. 2015).

2. *Study Site*

We collected the *H. megacephalus* (G. Fisher 1814) specimens within the 103,000 ha Agropalma private landholding (01˚55'57" S/02º24'4" S, 48˚45'49" W/48º48'2" W). The study area contains 39,000 ha of oil palm plantations and 64,000 ha of *terra firme* primary forest (Figure 1). The remaining forest patches interspersed with oil palm plantations ranged from 1,500 to 15,000 ha. The region of the study is currently a mosaic of open habitat areas and natural forest remnants under several other natural phytophysionomic types. The height of the forest canopy range from 25 to 35 m, dominated by some plant species, such as the *Attalea maripa* (inajá), *Cecropia distachya*, *Cordia scabrifolia*, *Tapiriri guianensis* (tapiriri), *Rollinia exsuca*, *Inga thibaudiana* (ingá) and *Vismia guianensis* (sealing). The climate in the region is tropical humid, according to Köppen's classification adapted by Peel et al. (2007), with a rainy season from December to May and a dry season from June to November. Mean temperature in the region is 26 °C and mean air humidity reaches up to 85% (Oliveira et al. 2002). The *Elaeis guineensis* has an economic life of 20 to30 years, reaching about 20 m in height. After that, the trees are cut for new planting, and the remains of the previous planting are stacked in lines interspersed with the new planting. These clumps of organic matter provide a good environment for invertebrate and small vertebrate fauna. The oil palm tree produces fibrous fruits most of the year, that are rich in beta-carotene which gives them an orange color (Luskin & Potts 2011).

3. *Specimens Collection and Procedures*

We sampled the small non-flying mammals in the study area in two field campaigns conducted in 2012, one in the rainy season and the other in the dry season. Each campaign lasted about 25 days. We installed 16 sampling points with a minimum of 1 km distance between them, with eight points located in the Forest and eight points located in the Oil Palm Plantation. At each sampling point we established a

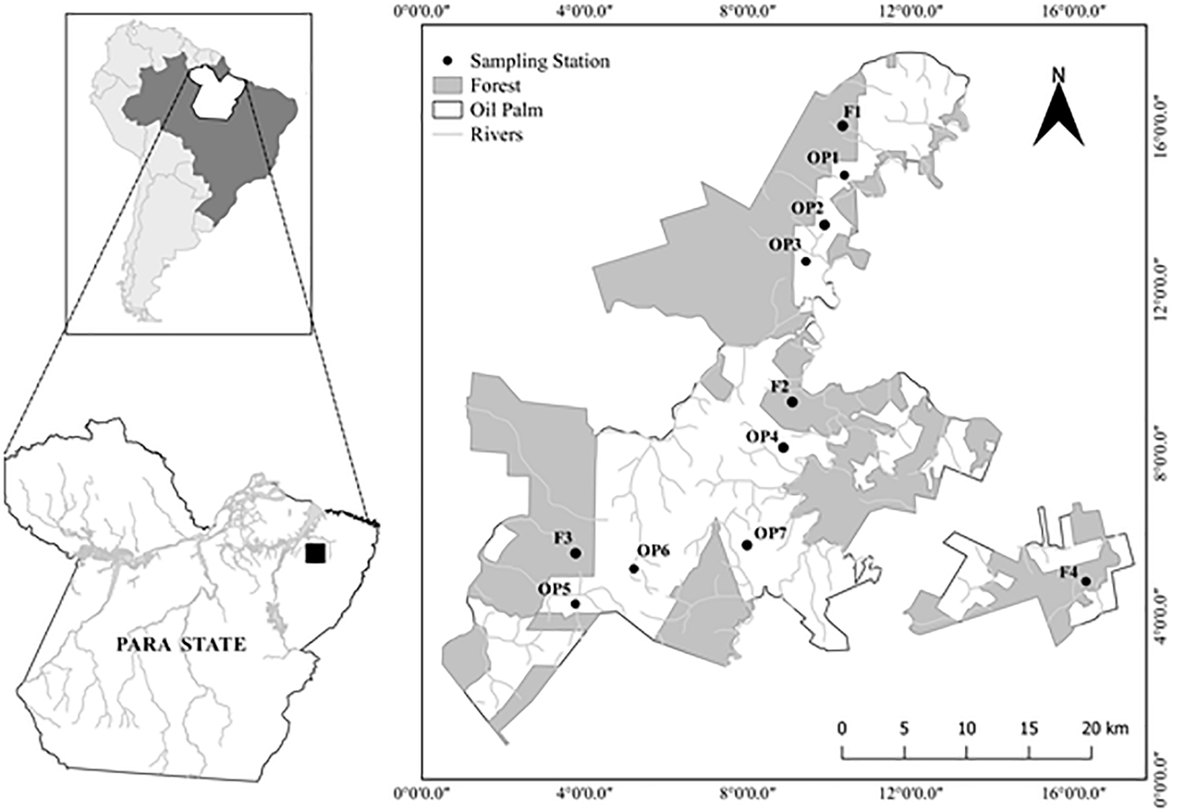


Figure 1. Location of the study area in the State of Pará, Brazil, delimiting the forest fragments (in gray) and areas of oil palm plantation (in white), and location of the sampling points (in black).

collection station, with a minimum distance of 500m from the edge of each habitat (forest or oil palm plantation). At each collection station we used pitfalls (Bury and Corn 1987; Ribeiro-Júnior et al. 2011) and live traps, like Sherman and Tomahawk (Voss and Emmons 1996). However, we used only *H. megacephalus* specimens collected in pitfalls, since the Sherman and Tomahawk traps were baited, and this would influence the results. At each collection station we implemented a pitfall line with a set of four buckets of 60 litters, buried with a distance of 15m between them. The sampling effort was 2,240 traps/nights, divided between the two types of habitats. All the points were sampled at the same time. We collected the specimens of *H. megacephalus* just in 11 points from the 16 points sampled (Figure 1). The collection of samples was authorized by ICMBio/SISBIO, through the permanent license number 4628-1 assigned to Ana Cristina Mendes-Oliveira.

We withdraw the gastrointestinal tract from subjects using the stomach and fecal contents for diet analysis. We measured the total weight of the stomach and intestine, and the dry weight of the stomach and intestinal contents using a high precision scale (20 g x 0.001 g). The content was displayed on a petri dish using a microscope. We calculate the frequency of occurrence (Hyslop 1980), and each food item was weighted to calculate the mass frequency (Hahn et al., 1997) . We sorted each item into categories within the groups of plants and animals. The only plant item identified was palm oil. Plant items included the following categories: plant fragments (bark, pulp and fiber), fragments of palm oil (bark, pulp, fiber and almond) and whole seeds. Items of animals were identified by order or by family.

4. *Data analyses*

We calculated the Frequency of Occurrence (FO*i*%) for each item. We also calculated the mass frequency of each food item on the total weight of all items of diet (Hynes 1950, Hyslop 1980). To evaluate the relative importance of each food item in the diet, we calculated the Food Index (IA*i*%) (Kawakami & Vazzoler 1980, Oliveira et al. 2004) using the formula: $IA_i\% = (FO_i\% * P_i\%/\sum FO_i\% * P_i\%) * 100$; Where the IA*i*% means food importance of item *i*, FO*i*% means frequency of occurrence of item *i*, and Pi% means the weight of item *i*. We calculated the IA*i* for each food item recorded in each habitat separately, which allowed us to analyze the food items consumed in each of the oil palm and forest habitats.

We evaluated the effect of the environment (oil palm and forest) on the niche width of the *H. megacephalus* species, using the Niche Amplitude calculation (Hurlbert 1978), which includes the value of the Levins index (Hurlbert 1978) ranging from 0 to 1. This index is equal to 0 when each animal consumes a single type of food category and equals 1 when the population consumes all available food categories similarly (Hulbert 1978, Fugi et al. 2008). Important to mention that the Levins´s measure does not allow for the possibility that the resource vary in abundance (Krebs, 1999), but it´s still very usefully to standardize the niche breath. To evaluate the variation in the diversity of items consumed by the population of *H. megacephalus*, the Shannon Diversity Index (H') (Zar 1984) was performed. The higher the H' value the greater the diversity of food items in the diet. We performed a t-test to analyze differences between each sampled environment. The t-test was performed in R (R Development Core Team 2016), using the diversity function of the Vegan package (Oksanen et al. 2013).

We calculated the individual dietary variation through the Proportional Similarity Index (PSI), which measures the overlap between the diet of individual *i* and the menu of the population as a whole (Bolnick et al. 2002, 2003). This index allowed us to compare the degree of individual specialization among the sexes and the habitats through the t-test. The measure of the degree of proportional similarity among individuals of a population (IS) is calculated by averaging the individual PS*i* values (Bolnick et al. 2002). The SI ranges from 0 (individual maximum specialization) to 1 (no individual specialization), and we consider values below 0.6, suggested by Bolnick et al. (2002), to represent high levels of individual specialization of the population. These values were calculated using the RInSp (R Development Core Team 2016) package.

Results

We collected 36 individuals (six in the dry season and 30 in the rainy season), of which 11 were collected in the forest and 25 specimens captured in palm oil planting. The *H. megacephalus* diet is composed of 18 food item of which 10 are of animal origin (Orthoptera, Coleoptera, Dermaptera, Heteroptera, Lepdoptera, Blattaria, Formicidae, Curculionidae, Cerambycidae and Araneae) and eight are of vegetal origin, as plants fragments (peel, pulp, vegetable fiber and whole seeds) and oil palm fragments (peel, pulp, fiber and almond) (Table 1).

In the whole study, we recorded 17 food items consumed in the oil palm plantation areas and 13 in the forest areas (Table 1). Of the total number of items consumed in the oil palm habitat, nine were of animal origin, Coleoptera being the most frequent and also the most important (Table 1). Among the eight ingested vegetable components, the palm pulp had the highest importance in the diet of the species in the oil palm habitat (Table 1). In the Forest environment, the most consumed animal item was also Coleoptera, among the six registered animal components. But unlike the oil palm plantation, the fibers and bark of native plant species were more important in the diet of *H. megacephalus* in the forest habitat. Whole seeds also had great importance in the diet, especially in the forest environment (Table 1).

The niche amplitude calculated for *H. megacephalus* in the forest habitat (Ba_{forest} = 0,013) was narrower than the oil palm plantation habitat (Ba_{palm} = 0,478). These results show that forest individuals tend to limit their niche and overlap their diet. The opposite occurs in the areas of oil palm plantation.

The diversity of dietary items of the forest individuals (H'_{forest} = 1.039) did not differ significantly from the diet of the oil palm plantation (H'_{palm} = 0.907) (t = 0.686, df = 34, p = 0.497). However, the analysis of ANOSIN showed differences between the diet composition and abundance of oil palm and forest samples (p = 0.038, r = 0.206).

When we evaluated the individuals separately, we observed that in the oil palm plantation the values of PSI are lower than in the forest habitat, which means less overlapping of diet in relation to the diet of the whole population, than the individuals in the oil palm plantation. The t-test (t = -1.964; df = 32; p = 0,048) present an effect of the environment on individual diet specialization of the population (Figure 2). The average values of the degree of proportional similarity between the diet of the individuals, considering the entire population (IS), were lower in the oil palm (IS_{palm} = 0,164) than in the forest (IS_{forest} = 0,357), indicating greater individual specialization of the diet in oil palm plantation.

Table 1. Diet composition of the individuals of the species *Hylaeamys megacephalus*. Legend: frequency of occurrence (FO*i*%) and food importance index (IA*i*%) of each item in the two different habitats (oil palm plantation and forest).

Food category	**Oil palm (*n*=25)**		**Forest (*n*=11)**	
Items	**FO*i*%**	**IA*i*%**	**FO*i*%**	**IA*i*%**
Class Insecta				
Order				
Orthoptera	0	0	18,2	2,666
Coleoptera	36	1,3	36,4	8
Dermaptera	4	0,022	0	0
Suborder				
Heteroptera	16	0,244	9,1	1
Lepidoptera	4	0,005	0	0
Blattaria	12	0,05	9,1	0,666
Families				
Formicidae	12	0,083	18,2	1,333
Curculionidae	4	0,005	0	0
Cerambycidae	4	0,005	0	0
Class Arachnida				
Order				
Araneae	28	0,389	18,2	2
Plants (fragments)				
Peel	36	0,85	63,6	16,333
Pulp	20	3,446	18,2	4
Vegetable fiber	44	1,773	72,7	37,333
Whole seeds	40	2,835	45,5	16,666
Oil palm (fragments)				
Peel	36	0,65	9,1	0,333
Pulp	20	85,413	0	0
Fibra	52	2,529	9,1	1,666
Almond	20	0,333	36,4	8

Discussion

We concluded that the changes in the environment has an effect on the *H. megacephalus* diet, wherein the oil palm plantation has an increase in the individual variation of the diet. This would be a mechanism for reducing intrapopulation competition in response to scarce resources (Roughgarden 1972, Svanback & Persson 2004). The increase in individual variability reflected the increase in the niche amplitude of the population as a whole, in the area of oil palm plantation. In the Forest, theoretically, individuals tend to overlap their diet and decrease their niche breadth. The broader food niche observed in the

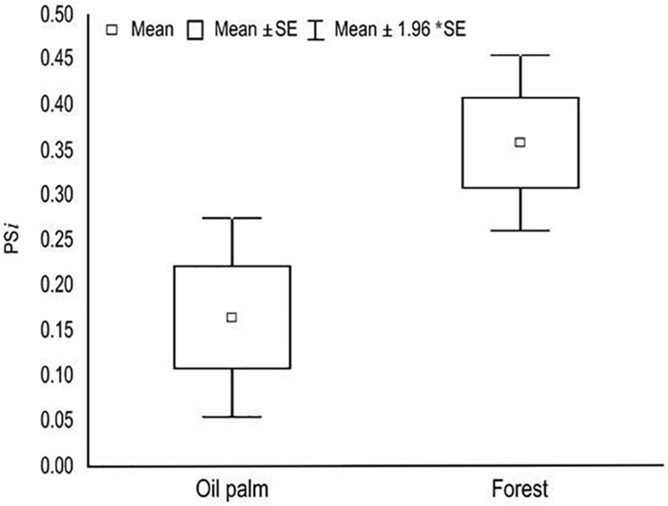


Figure 2. Analysis of T-test comparing the variability in the diet of between different habitats (oil palm plantation and forest).

plantation environment can also be determined by strong intraspecific competition as a result of a higher population density, leading to a behavioral change as a result of depletion of the preferred resources. The description of these patterns for mammals, especially for rodents, may be useful for a better understanding of the mechanisms that promote evolutionary diversification through individual specialization (Svanbäck & Bolnick 2007). The studied population is seen as a generalist population composed of individual specialists. Thus, this work reinforces the need to evaluate diet individually in studies that consider evaluating the impacts on the use of these resources (Bolnick et al., 2003). It is noteworthy that the increase in the amplitude of niche population was disproportionally greater than the increase in individual niche amplitudes, indicating that individual niches remain relatively limited when the niche of the population expands on the oil palm habitat.

The reduction in the complexity of the oil palm plantation habitat compared to the forest reflects on the increase of the individual variability of *H. megacephalus*. This individual specialization is considered an endogenous mechanism of the population, in response to several factors, such as environmental variations (Bolnick et al. 2003, Skulason & Smith 1995). Differences in habitat and resource availability can influence individual variations (Lomnicki 1980, Bourke et al. 1997). In this case, the individual specialization could reduce intraspecific competition (Roughgarden 1972, Svanbäck & Persson 2004). However, in the medium and long-term, the high individual-level niche variations can have as consequence the diversification of the species, as they affect the stability of the population (Dieckmann & Doebeli 1999).

In this study, we suggest the change of the frugivorous/gramnivorous diet defined to *H. megacephalus* by Paglia et al (2006) to frugivorous/insectivorous. We observe that these animals feed on large quantities of plant items such as bark, pulp, plant fiber and seeds, but also ingest a wide variety of animal food items including insects and arachnids. Other studies using close phylogenetically species, found arthropods in 100% of samples and some seeds and other parts of plants (Palma 1996). Our results suggest that when these items were not available, *H. megacephalus* increased the consumption of other items. This was evidenced by the frequency of occurrence of the palm kernel consumed in the forest area. Due to the difficult investigation of the small rodent diet, as these animals fragment food into very small parts (Hansson 1970), information about their eating habits is still limited. Few studies, often superficial, elucidate little about food ecology and impacts on communities. For the vast majority of rodent species, it is not known what the true importance of food resources is and how these items vary in their diet.

Although we have not done feasibility tests of the seeds ingested, we suggest a dispersion potential of this species, as the IA*i*% values for the whole seed item was the third highest among all food items consumed. The high consumption of seeds by terrestrial rodents in the neotropics makes this group of fauna essential in the maintenance and recovery of forest habitats (Forget 1992), not only as dispersers, but controlling some dominant plant species, allowing greater biodiversity. Several small mammals are being considered as potential dispersers in the neotropics (Pimentel & Tabarelli 2004). Consumption of fruits and seeds, albeit in variable proportions, indicates a strong dispersal potential for small mammals, although information on the dietary memories of some genera may be scarce and, in some cases, associated with phylogenetically close species (Lessa & Geise 2010, Casella & Cáceres 2006, Lessa & Costa 2009).

In general, we demonstrated that the niche used by the *H. megacephalus* species is narrower in the forest since the individual dietary variation is smaller. While in the oil palm plantation, where the PSI values are lower, indicating a smaller individual niche overlap, the whole niche of the population expanded. However, the richness and composition of the items consumed by the individuals of both habitats are not different. It means that populations of *H. megacephalus* in oil palm plantation as well as in forest habitats are feeding on almost the same food items. Individuals residing in the oil palm plantation further diversify the use of these food items among themselves. In this way, we can say that there is an impact of the oil palm plantation on the individual variability of the *H. megacephalus* species diet. We reinforce the importance of considering individual variability in the diet and niche amplitude studies, since if we considered all specimens as ecologically equivalents (DeAngelis & Gross 1992) in the *H. megacephalus* population, we could erroneously conclude on the comparison of intake between habitats. The description of these patterns for mammals, especially for rodents, may be useful for a better understanding of the mechanisms that promote evolutionary diversification through individual specialization (Svanbäck & Bolnick 2007).

Acknowledgments

We kindly acknowledg to the Conservation International (CI) and the AGROPALMA Company for the logistical support to carry out the research. To Dr. Eli Gurgel of the Laboratory of Botany of the Museu Paraense Emílio Goeldi, for the help in the identification of the vegetal items of the diet. To Marcely Valois and Mariano Brandão of the Laboratory of Invertebrates of the Federal University of Pará for the help in the identification of the animal items of the diet. Also to Jesse Carlton for the final English revision.

Author Contributions

Simone Almeida: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Ana Cristina Mendes-Oliveira: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ARAÚJO, M.S., BOLNICK, D.I., MARTINELLI, L.A., GIARETTA, A.A. & DOS REIS, S.F. 2009. Individual-level diet variation in four species of Brazilian frogs. Journal of Animal Ecology, 78, 848–856.

ARAÚJO, M.S. & GONZAGA, M.O. 2007. Individual specialization in the hunting wasp *Trypoxylon* (Trypargilum) *albonigrum* (Hymenoptera, Crabronidae). Behav Ecol Sociobiol 61:1855–1863.

BARLOW, J., GARDNER, T.A., ARAUJO, I.S., ÁVILA-PIRES, T.C., BONALDO, A.B., COSTA, J.E., ESPOSITO, M.C., FERREIRA, L.V., HAWES, J., HERNANDEZ, M.I.M., HOOGMED, M.S., LEITE, R.N., LO-MAN-HUNG, N.F., MALCOLM, J.R., MARTINS, M.B., MESTRE, L.A.M., MIRANDA-SANTOS, R., NUNES-GUTJAHR, A.L., OVERAL, W.L., PARRY, L., PETERS, S.L., RIBEIRO-JUNIOR, M.A., DA SILVA, M.N.F., SILVA-MOTA, C. & PERES, C.A. 2007. Quantifying the biodiversity value of tropical primary, secondary and plantation forest. PNAS, 20 (1040):18555-18560.

BOLNICK, D.I., YANG, L.H., FORDYCE, J.A., DAVIS, J.M. & SVANBÄCK, R. 2002. Measuring individual-level resource specialization. Ecology 83:2936–2941.

BOLNICK, D.I., SVANBÄCK R., FORDYCE, J.A., YANG, L.H., DAVIS, J.M., HULSEY, C.D. & FORISTER, M.L. 2003. The Ecology of Individuals: Incidence and Implications of Individual Specialization. The American Naturalist vol. 161, nº 1, pp.1-28.

BOLNICK, D. I., SNOWBERG, L.K., HIRSCH, P.E., LAUBER, C.L., KNIGHT, R., CAPORASO, J.G. & SVANBÄCK, R. 2014. Individuals' diet diversity influences gut microbial diversity in two freshwater fish (threespine stickleback and Eurasian perch). Ecology Letters, 17: 979–987.

BOURKE, P., MAGNAN P. & RODRIQUEZ M.A. 1997. Individual variations in habitat use and morphology in brook charr. Journal of Fish Biology 51:783–794.

BURY, R.B. & CORN, P.S. 1987. Evaluation of pitfall trapping in northwestern forests: trap arrays with drift fences. J. Wildl. Manage 51:112-119.

BUTLET, R.A. & LAURANCE, W.F. 2009. Is oil palm the next emerging threat to the Amazon? Tropical Conservation Science, 2:1-10.

CASELLA, J. & CÁCERES, N.C. 2006. Diet of four small mammal species from Atlantic forest patches in south Brazilian. Neotropical Biology Conservation 1: 5-11.

CAMARGO, N.F., RIBEIRO, J.F., DE CAMARGO, A.J.A. & VIEIRA, E.M. 2013. Intra- and inter-individual variation show distinct trends as drivers of seasonal changes in the resource use of a neotropical marsupial. Biological Journal of the Linnean Society, 111, 737–747.

CORREA, F.S., JUEN, L., RODRIGUES, L.C., SILVA-FILHO, H.F. & SANTOS-COSTA, M.C. 2015. Effects of oil palm plantations on anuran diversity in the eastern Amazon. Animal Biology 65: 321–335.

COSTA, A., SALVIDIO, S., POSILLICO, M., MATTEUCCI, G., DE CINTI, B. & ROMANO, A. 2015. Generalisation within specialization: inter-individual diet variation in the only specialized salamander in the world. Sci. Rep, 5, 13260.

CUNHA, E.J., MONTAG, L.F.A. & JUEN, L. 2015. Oil palm crops effects on environmental integrity of Amazonian streams and heteropteran (Hemiptera) species diversity. Ecol. Indic., 52, 422-429.

CHUNG, A.Y.C., EGGLETON, P., SPEIGTH, M.R., HAMMOND, P.M. & CHEY, V.K. 2000. The diversity of beetle assemblages in different habitat types in Sabah, Malaysia. Bulletin of Entomological Research, 90:475-496.

DANIELSEN, F. & HEEGAARD, M. 1995. Impact of logging and plantation development on species diversity: a case study from Sumatra. In: Sandbukt, O. (Ed) Management of tropical forest: towards an integrated perspective. Oslo: University of Oslo, Centre for Development and the Environment.

DE ANGELIS, D.L. & GROSS, L.J. 1992. Individual-Based Models and approaches in Ecology: Populations, Communities and Ecosystems. Chapman and Hall, NY.

DIECKMANN, U. & DOEBELI, M. 1999. On the origin of species by sympatric speciation. Nature 400:354–357.

FERREIRA, M.C., BEGOT, T.O., PRUDENTE, B.S., JUEN, L. & MONTAG, L.F.A. 2018. Effects of oil palm plantation on habitats structure and fish assemblages in Amazon streams. Environmental Biology of Fishes 101.4: 547-562.

FITZHERBERT, E.B., STRUEBIG, M.J., MOREL, A., DANIELSEN, F., BRÜHL, C.A., DONALD, P.F. & PHALAN, B. 2008. How will oil palm expansion affect biodiversity? Trends Ecol. Evol., 23, 538-545.

FORGET, P.M., 1992. Seed removal and seed fate in *Gustavia superba* (Lecythidaceae). Biotropica, 24: 408-414.

FUGI, R., LUZ-AGOSTINHO, K.D.G. & AGOSTINHO, A.A. 2008. Trophic interaction between an introduced (peacock bass) and a native (dogfish) piscivorous fish in a Neotropical impounded river. Hydrobiologia, 607: 143–150.

HAHN, N.S., ANDRIAN, I.F.; FUGI, R. & ALMEIDA, V.L.L. 1997. Ecologia trófica. Pp. 209-228. *In*: Vazzoler, A. E. A. M.; Agostinho, A. A. & Hahn, N. S. (Eds.). A planície de inundação do Alto rio Paraná: aspectos físicos, biológicos e socioeconômicos. Maringá, Eduem, 460p.

HANNAH, B., ZANDEN, V., BJORNDAL, K.A. & BOLTEN, A.B. 2013. Temporal consistency and individual specialization in resource use by green turtles in successive life stages. Oecologia, 173(3):767-77.

HANSSON, L. 1970. Methods of morphological diet microanalysis in rodents. Oikos, 21: 255–266.

HULBERT, S. H. 1978. The measurement of niche overlap and some of its relatives. Ecology, 59: 67-77.

HYNES, H.B.N. 1950. The food of freshwater sticklebacks (*Gasterosteus aculeatus* and *Pygosteus pungitius*) with a review of methods used in studies of the food of fishes. J. Anim. Ecol. 19, 36-58.

HYSLOP, E.J. 1980. Stomach contents analysis-a review of methods and their application. The Fisheries Society of the British Isles. 17, 41 1-429.

KAWAKAMI, E. & VAZZOLER, G. 1980. Método gráfico e estimativa de índice alimentar aplicado no estudo de alimentação de peixes. Boletim do Instituto Oceanográfico, 29: 205-207.

KREBS, C.J. 1999. Ecological Methodology. University of British Columbia, 619 pp.

LESSA, L.G. & COSTA, F.N. 2009. Diet and seed dispersal by five marsupials (Didelphimorphia: Didelphidae) in a Brazilian cerrado reserve. Mammal Biology 75: 10-16.

LESSA, L.G. & GEISE L. 2010. Hábitos alimentares de marsupiais didelfídeos brasileiros: análise do estado de conhecimento atual. Oecologia Australis, 14(4): 901-910.

LOMNICKI, A. 1980. Regulation of population density due to individual differences and patchy environment. Oikos 35:185–193.

LUSKIN, M.S. & POTTS, M.D. 2011. Microclimate and habitat heterogeneity through the oil palm lifecycle. Basic and Applied Ecology 12:540-551.

MARES, M.A.; ERNEST, K.A. & GETTINGER, D.A. 1986. Small mammal community structure and composition in the Cerrado province of central Brazil. *J.* Trop. Ecol. 2:289– 300.

MARTINS, E.G., ARAÚJO, M.S., BONATO, V. & DOS REIS, S.F. 2008. Sex and Season Affect Individual Level Diet Variation in the Neotropical Marsupial *Gracilinanus microtarsus* (Didelphidae). Biotropica 40(1): 132–135.

MUSSER, G.G.; CARLETON, M.D.; BROTHERS, E. & GARDNER, A.L. 1998. Systematic studies of Oryzomyine rodents (Muridae, Sigmodontinae): diagnoses and distributions of species formerly assigned to *Oryzomys* "*capito.*" Bull. Am. Mus. Nat. Hist. 236:1– 376.

NELSON, B.P.N., RHEBERGEN, T., BERTHELSEN, S., WEBB, M.J., BANABAS, M., OBERTHÜR, T., DONOUGH, C.R., RAHMADSYAH, INDRASUARA, K. & LUBIS, A. 2002. Soil Acidification under Oil Palm: Rates and Effects on Yield. Slides 22–25.

NITIKMAN, L.Z. & MARES, M.A. 1987. Ecology small mammal in a gallery forest of central Brazil. Annals of Carnegie Museum 56 (1-2):75-99.

OCHOA, G.J.C., MOLINA, C. & GINER, S. 1993. Inventario y estudio comunitario de los mamíferos del Parque Nacional Canaima, con una lista de las espécies registradas para la Guayana Venezolana. Acta Cient. Venez. 44:245– 62.

OKSANEN, J., BLANCHET, F.G., FRIENDLY, M., KINDT, R., LEGENDRE, P., MCGLINN, D., MINCHIN, P.R., O'HARA, R.B., SIMPSON, G.L., SOLYMOS, P., STEVENS, M.H.H., SZOECS, E. & WAGNER, H. 2013. Package 'vegan'. *Community ecology package, version*, *2*(9).

OLIVEIRA, L.L., FONTINHAS, R.L., LIMA, A.M.M. & LIMA, R.J.S. 2002. Mapas dos parâmetros climatológicos do Estado do Pará: umidade, temperatura e insolação, médias anuais. Anais do XIII Congresso Brasileiro de Meteorologia. Sociedade Brasileira de Meteorologia, Fortaleza.

OLIVEIRA, A.K., ALVIM, M.C.C., PERET, A.C. & ALVES, C.B.M. 2004. Diet shifts related to body size of the pirambeba *Serrasalmus brandtii* Lütken, 1875 (Osteichthyes, Serrasalminae) in the Cajuru Reservoir, São Francisco River basin, Brazil. Braz. J. Biol. 64:117-124.

OLSSON, J., QUEVEDO, M., COLSON, C. & SVANBÄCK, R. 2007. Gut length plasticity in perch: into the bowels of resource polymorphisms. Biological Journal of the Linnean Society, 90, 517–523.

PAGLIA, A.P., FONSECA, G.A.B., RYLANDS, A.B., HERRMANN, G., AGUIAR, L.M.S., CHIARELLO, A.G., LEITE, Y.L.R., COSTA, L.P., SICILIANO, S., KIERULFF, M.C.M., MENDES, S.L., TAVARES, V.C., MITTERMEIER, R.A. & PATTON, J.L. 2012. Lista Anotada dos Mamíferos do Brasil. 2ª Ed. Occasional Papers in Conservation Biology, Number 6, Conservation International, Arlington, VA.

PALMA, A.R.T. 1996. Separação de nichos entre pequenos mamíferos de Mata Atlântica. M.S. thesis, Universidade Estadual de Campinas, Campinas, Brazil (in Portuguese).

PATTON, J.L., DA SILVA, M.N.F. & MALCOLM, J.R. 2000. Mammals of the Rio Juruá and the evolutionary and ecological diversifi cation of Amazonia. Bull. Am. Mus. Nat. Hist. 244:1– 306.

PEH, K.S.H., SODHI, N.S., DE JONG, J., SEKERCIOGLU, C.H., YAP, C.A.M. & LIM, S.L.H. 2006. Conservation value of degraded habitats for forest birds in southern peninsular malaysia. divers. distrib. 12:572-581.

PEEL, M.C., FINLAYSON, B.L. & MCMAHON, T.A. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrol Earth Syst Sci 11(5):1633–1644.

PERCEQUILLO, A.R. 2015. Genus *Hylaeamys* Weksler, Percequillo, and Voss, 2006, *In*: Patton, J.L., PARDIÑAS, U.F.G. & D'Elía, G. (Eds). Mammals of South America – Rodents. Volume 2. The University of Chicago Press, Ltd., London. p. 335-346.

PIMENTEL, D.S. & TABARELLI, M. 2004. Seed dispersal of the palm *Attalea oleifera* in a remmant of the Brazilian Atlantic Forest. Biotropica 36: 74-84.

PIRES, M.M., MARTINS, E.G., ARAÚJO, M.S. & DOS REIS, S.F. 2013. Between-individual variation drives the seasonal dynamics in the trophic niche of a Neotropical marsupial. Austral Ecology*,* 38: 664-671.

R DEVELOPMENT CORE TEAM 2016 R: A language and environment for statistical computing. *R Foundation for Statistical Computing*, Vienna, Austria. Available at: http://www.R-project.org.

ROUGHGARDEN, J. 1972. Evolution of niche width. American Naturalist 106:683–718.

SHINE, R. 1991. Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. American Naturalist 138:103–122.

SKULASON, S. & SMITH, T.B. 1995. Resource polymorphisms in vertebrates. Trends in Ecology & Evolution 10:366–370.

SMITH, T.B. & SKULASON, S. 1996. Evolutionary significance of resource polymorphisms in fishes, amphibians, and birds. Annual Review of Ecology and Systematics 27:111–133.

SVANBÄCK, R. & PERSSON, L. 2004. Individual specialization, niche width and population dynamics: implications for trophic polymorphisms. Journal of Animal Ecology 73: 973–982.

SVANBÄCK, R. & BOLNICK, D.I. 2005. Intraspecific competition affects the strength of individual specialization: an optimal diet theory model. Evolutionary Ecology Research **7**, 993-1012.

SVANBÄCK, R. & BOLNICK, D.I. 2007. Intraspecific competition drives increased resource use diversity within a natural population. Proceedings of the Royal Society, Biological Sciences, 274: 839-844.

SVANBÄCK, R., QUEVEDO, M., OLSSON, J. & EKLÖV, P. 2015. Individuals in food webs: the relationships between trophic position, omnivory and amongindividual diet variation. Oecologia, 178:103–114.

TURNER, E.C. & FOSTER, W.A. 2009. The impact of forest conversion to oil palm on arthropod abundance and biomass in Sabah, Malaysia. J. of Trop. Ecol., 25:23-30.

VAN VALEN, L. 1965. Morphological variation and width of ecological niche. American Naturalist 99:377–389.

VOSS, R.S., LUNDE, D.P. & SIMMONS, N.B. 2001. The mammals of Paracou, French Guiana: A neotropical lowland rainforest fauna - part 2. Nonvolant species. Bulletin of the American Museum of Natural History, v. 263, p. 3-236.

WERNER, E.E. & HALL, D.J. 1974. Optimal foraging and the size selection of prey by the bluegill sunfish (*Lepomis macrochirus*). Ecology 55:1042–1052.

WILCOVE, D.S. & KOH, L.P. 2010. Addressing the threats to biodiversity from oil palm agriculture. Biodiversity and Conservation, 19:999-1007.

YAAP, B., STRUEBIG, J., PAOLI, G. & KOH, L.P. 2010. Mitigating the biodiversity impacts of oil palm development. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 5(19):1-11.

YEBOUA, K. & BALLO, K. 2000. Caracteristiques chimiques du sol sous palmeraie. Cahiers d'études et de recherches francofones/Agricultures, 9:73-76.

ZAR, J.H. 1984. Biostatistical analysis, 2nd ed. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Received: 13/06/2018
Revised: 11/02/2019
Accepted: 21/02/2019
Published online: 28/03/2019

Biota Neotropica 19(2): e20180609er, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Erratum

In the article "Dragonflies and damselflies (Insecta: Odonata) from a Cerrado area at Triângulo Mineiro, Minas Gerais, Brazil" with the DOI code number http://dx.doi.org/10.1590/1676-0611-bn-2018-0609, published at Biota Neotropica 19(1):e20180609:

Where it was written:

Tramea binotata (Rambur, 1842)

Should read:

Tramea calverti Muttkowski, 1910

In page 4, where it was written:

In general, the number of species in a single assemblage (pool) varies strongly in Brazilian Cerrado habitats, from as small as 26 and 31 species (Almeida et al. 2013, Vilela et al. 2016) to as numerous as 50-80, generally at sites with more sampled areas (Calvão et al. 2013, Carvalho et al. 2013, Dutra & De Marco 2015, Ferreira-Peruquetti & Fonseca-Gessner 2013) (Table 3). The number of species recorded in our study can be considered intermediary based on the small sampling effort, and the fact that we cover only half of the preserved area, indicating that the PANMC can present a rich pool of odonates, when compared to other places in Minas Gerais (Almeida et al. 2013, Souza et al. 2013). Additionally, there are many distinct Cerrado biotopes found around the aquatic habitats in the RLNMC, e.g., cerrado strictu sensu and campos de murundus, creating a gradient that could increase the diversity of odonates (Bedê et al. 2015).

Should read:

Overall, the richness in an odonate community has a great disparity in Brazilian Cerrado habitats. In some areas, species richness is low, between 26 and 31 species (Almeida et al. 2013, Vilela et al. 2016). In other areas, the richness of species is relatively high, between 50 and 80 species, probably due to the fact that these areas present more sampling points, which increases the species richness (Calvão et al. 2013, Carvalho et al. 2013, Dutra & De Marco 2015, Ferreira-Peruquetti & Fonseca-Gessner 2013) (Table 3). The richness recorded in our study fits better on an intermediary level taking in account the small sampling effort, and the fact that we cover only half of the preserved area. These results indicate that the PANMC has the potential to hold a rich pool of odonates, in comparison to other places in Minas Gerais (Almeida et al. 2013, Souza et al. 2013). Moreover, around the aquatic habitats in the LRNMC, there are other different Cerrado phytophysiognomies, such as, cerrado strictu sensu and campos de murundus. These habitats can build a slope of environmental heterogeneity, which can boost the odonate diversity (Bedê et al. 2015, Souza et al. 2017).

In page 9, where it was written:

SOARES, D.M., NASCIMENTO, A.R.T., SILVA, L.C. & DE-PINHO-JÚNIOR, G.V. 2015. Natural Regeneration and Biological Invasion by Pinus caribaea Morelet in Two Vereda Sites: Woody Vegetation Response. Am. J. Plant Sci. 6(17): 2708–2717.

SOUZA, M.M., SOUZA, B., PEREIRA, M.C.S.A. & MACHADO, A.B.M. 2013. List of Odonates from Mata do Baú, Barroso, Minas Gerais, Brazil. Check List 9(6):1367–1370.

Should read:

SOARES, D.M., NASCIMENTO, A.R.T., SILVA, L.C. & DE-PINHO-JÚNIOR, G.V. 2015. Natural Regeneration and Biological Invasion by Pinus caribaea Morelet in Two Vereda Sites: Woody Vegetation Response. Am. J. Plant Sci. 6(17): 2708–2717.

SOUZA, M.M., PIRES, E.P., BRUNISMANN, A.G., MILANI, L.R. & PINTO, A.P. 2017. Dragonflies and damselflies (Odonata) from the wetland of the Rio Pandeiros, northern region of Minas Gerais State, Brazil, with a description of the male of Archaeogomphus vanbrinki Machado (Anisoptera: Gomphidae) Int J Odonatol 20(1):13-26.

SOUZA, M.M., SOUZA, B., PEREIRA, M.C.S.A. & MACHADO, A.B.M. 2013. List of Odonates from Mata do Baú, Barroso, Minas Gerais, Brazil. Check List 9(6):1367–1370.

Biota Neotropica 19(2): e20180601, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

A predictive index based on environmental filters for the bioassessment of river basins without reference areas in Atlantic Forest biome, Brazil

Natália Freitas de Souza[1,2]*, Darcílio Fernandes Baptista[1] & Daniel Forsin Buss[1]

[1]Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Laboratório de Avaliação e Promoção da Saúde Ambiental, Rio de Janeiro, RJ, Brasil
[2]Universidade Federal do Rio de Janeiro, Departamento de Ecologia, Rio de Janeiro, RJ, Brasil
**Corresponding author: Natália Freitas de Souza, e-mail: souzanf00@gmail.com*

SOUZA N.F., BAPTISTA, D.F., BUSS D.F. **A predictive index based on environmental filters for the bioassessment of river basins without reference areas in Atlantic Forest biome, Brazil**. Biota Neotropica. 19(1): e20180601. http://dx.doi.org/10.1590/1676-0611-BN-2018-0601

Abstract: Biological assessments that use the reference condition approach are based on the concept of comparing a site's observed biology to sites where disturbance is minimal or absent. However, in many regions of the world, such areas are scarce or nonexistent. In this study, an alternative approach proposed by Chessman and Royal for bioassessment without reference areas based on environmental filters was tested in Brazil. This approach assumes that key environmental features act in the selection of potential colonists, from a regional pool of taxa, based on the ecological traits (tolerances) possessed by each taxon. We developed the approach by: 1) determining the regional pool, based on a large Atlantic Forest biome database; 2) selecting environmental filters (elevation, original vegetation and soil type); and 3) including information on the tolerance and preferences of aquatic insects to these filters. With this information we were able to determine the expected taxon under natural conditions and compare with observed taxon, developing a predictive index (Observed/Expected). Although the model was intended to predict the fauna in regions without reference sites, we included reference areas to test the model responsiveness, precision and sensitivity. Our results indicated that the index was able to discriminate impairment classes ($F=56.9$; $p<0,001$), it has high precision due to low standard deviation across reference sites values ($SD=0.098$) and high sensitivity due the correlation with environmental variables that are sensitive to human alteration ($r=0.74$, $p<0.01$). Also, it was strongly correlated with multimetric indices developed for multiple watersheds in the state, showing agreement between the methods in relation to ecological quality classification. Even though the predictive index had performed well in our study, we make some considerations that may help to improve its sensitivity of similar methods that are being tested using the environmental filters approach.

Keywords: *biomonitoring, environmental management, aquatic insects, macroinvertebrate, neotropical region.*

Índice preditivo baseado em filtros ambientais para o biomonitoramento de rios em bacias sem áreas de referência no bioma Mata Atlântica, Brasil

Resumo: Avaliações biológicas que usam a abordagem de condição de referência se baseiam na comparação entre a biota observada em locais teste e a presente em locais íntegros ou minimamente impactados. Entretanto, em muitas regiões do mundo, estes locais são escassos ou inexistentes. Neste estudo, a abordagem alternativa proposta por Chessman e Royal, que consiste no biomonitoramento baseado em filtros ambientais sem o uso de áreas de referência, foi testado para o Brasil. Esta abordagem assume que características ambientais chave agem na seleção de colonizadores potenciais, a partir do *pool* regional de táxons, baseado nos traços ecológicos (tolerância) de cada táxon. A abordagem foi desenvolvida através da: (1) determinação do pool regional, baseado em uma ampla base de dados do bioma Mata Atlântica; 2) seleção de filtros ambientais (elevação, vegetação original e tipo de solo); e 3) inclusão de informações sobre a tolerância e preferência ambiental dos insetos aquáticos à esses filtros. Essas informações possibilitam determinar os táxons esperados sob condições naturais (ausência de impacto) e comparar com os táxons observados em cada local, desenvolvendo um índice preditivo (Observado/Esperado). Embora esta abordagem proponha a predição da fauna na ausência de áreas de referência, estas áreas foram incluídas com o propósito de testar a resposta, precisão e sensibilidade desta abordagem. Os resultados indicam que o índice desenvolvido foi capaz de distinguir diferentes classes de impacto ($F=56.9$; $p<0,001$), obteve alta precisão, o que foi observado com o baixo desvio padrão obtido para os valores de locais de referência ($SD=0.098$) e alta sensibilidade, observado através da correlação significativa com variáveis ambientais sensíveis a alterações antrópicas ($r=0.74$, $p<0.01$). Além disso, o índice foi fortemente correlacionado com índices multimétricos desenvolvidos especificamente para diferentes regiões hidrográficas, mostrando concordância entre diferentes métodos no que se refere a avaliação da integridade ecológica. Apesar do bom desempenho do índice preditivo neste estudo, nós fizemos algumas considerações para melhorar a sensibilidade e o potencial da abordagem de filtros ambientais em aplicações futuras.

Palavras-chave: *avaliação biológica, gestão ambiental, insetos aquáticos, macroinvertebrados, região neotropical.*

Introduction

Biological assessments are generally based on measurements of attributes of biological assemblages, which are often characterized and expressed as indices. Different types of indices have been developed (Hawkins et al. 2010, Feio & Poquet 2011, Herman & Nejadhashemi 2015), but most of these methods share the need to use a benchmark (i.e. a reference condition), from which the measured biological condition will be compared. The impairment of a site is defined as how much their biological attributes differ from those found at the benchmark's condition.

Ideally, reference benchmark conditions represent historical, pristine conditions (Hawkins et al. 2010). However, in many regions of the world due to a long history of anthropogenic alteration of the aquatic ecosystems, those conditions are scarce or absent. In such cases, where "minimally disturbed" or "historical condition" sites (sensu Stoddard et al. 2006) are missing, some protocols relax the reference criteria and use "best attainable" or "least disturbed" sites as reference (Stoddard et al. 2006). The misuse of this practice can generate a series of problems (Chessman 2006, Labay et al. 2015, Elias et al. 2015). First, these definitions are often arbitrary and inconsistent, thus impractical to apply them in broad spatial scales (Cao & Hawkins 2011). Second, these sites have human-altered hydrological, physical and chemical conditions, that also affects their corresponding biological attributes. Thus, an index based on those sites may differ from one developed using natural benchmarks. This practice should be applied with great prudence, otherwise can influence indices by incorporating increasing degradation into the modelling.

The need to implement bioassessment programs in regions where reference sites are absent or scarce have fostered the development and testing of alternative methods (Chessman & Royal 2004, Carter & Fend 2005, Stranko et al. 2005, Chessman 2006, Blocksom & Johnson 2009, Hawkins et al. 2010, Birk et al. 2012, Schoolmaster et al. 2013, Labay et al. 2015, Milošević et al. 2016, Elias et al. 2016). Some researchers point that an approach that does not require the use of reference sites should be explored (Olden et al. 2006, Feio et al. 2009, Feio & Poquet 2011, Elias et al. 2015). Recent literature reviews on bioassessment (Dolédec & Statzner 2010, Hawkins et al. 2010) classified Chessman and Royal's (2004) approach, the Observed Proportion of Potential – OPP, as promising, but lacking sufficient validation. This approach is based on the environmental filters concept (Poff 1997), which assumes that key environmental features act in the selection of potential colonists, from a regional pool of taxa, based on the ecological traits (tolerances) possessed by each taxon. The premise of the approach is ecologically intuitive: a taxon from the regional pool (i.e. known to possibly occur in a given area) that possess environmental tolerances that fit the 'natural' environmental conditions would occur in a site if those 'natural' conditions are found. If the environmental conditions are changed in a way they extrapolate a taxon's tolerance, they act as filters excluding the taxon from that site. The use of multiple key environmental filters would allow predicting the composition of the community potentially occurring in a site (Poff 1997, Chessman & Royal 2004, Stranko et al. 2005, Chessman 2006). Anthropogenic impacts can be viewed as either modifying the natural filters (allowing more, fewer, or different taxa to pass) or creating additional filters (Chessman & Royal 2004). Thus, the comparison of the observed taxa with the expected taxa provides a measure of the impairment level of a site. It results in an Observed/ Expected index (O/E index), similar to the type used in a RIVPACS and AUSRIVAS (Feio & Poquet 2011), but Chessman and Royal's approach identify taxa expected to occur in each typology based on life history information, rather than through reference-based predictive modeling. This can be considered typological approach (sensu Hawkins et al. 2010), because the expected taxa are identical to all streams within each type listed.

In this study, we test the potential for environmental filters approach to provide a basis for bioassessment of regions without reference sites. The environmental filters predicted the natural potential distribution of aquatic insect families, which was used as benchmarks for comparisons with observed assemblages. The predictive index was tested for its ability to determine the impairment condition of streams sampled in southeast Brazil. Specifically, to assess index performance we tested for its (1) responsiveness, in order to verify the ability to discriminate impairment classes; (2) precision, to know the precision of filters approach worked; (3) sensitivity to stressor gradient through evaluation of the relationship between the OPP and environmental variables that are highly sensitive to human alteration. Then, we compared OPP with existing multimetric indices in order to verify the agreement between methods in relation to ecological quality classification.

Material and Methods

1. *Study Area*

This study was carried out with data collected from seven of the nine main river basins of Rio de Janeiro state, southeast Brazil (Figure 1). The geomorphology of the state is composed of coastal plains separated by hills and two mountain chains that run parallel to the ocean (Serra do Mar, ranging from elevations 0-2,000 m.a.s.l and Serra da Mantiqueira, ranging from 800-2,500 m.a.s.l). The state's main river, rio Paraiba do Sul, runs in the valley formed between the two mountain chains at an elevation of about 800 m.a.s.l. According to a recent review of Köppen's climate classification for Brazil most of Rio de Janeiro state´s mid-to-lowland portions (44%) is classified as tropical with a summer rainy season (Aw type), and the mountainous regions and plateaus classified as humid subtropical zones with hot summer, without dry seasons (Cfa type) or with a dry winter (Cwa type) (Alvares et al. 2013). The temperature oscillates between 15oC and 28oC and the mean annual rainfall is around 1000-1500mm. The Atlantic Forest, which originally covered virtually the entire region, now represents less than 12% of its original extent, and is mostly spread in the higher parts of the mountains and in remnants interspersed with agriculture and pasture (Ribeiro et al. 2011).

2. *Development of the predictive model*

A model to predict the natural distribution of macroinvertebrate based on environmental filters was built using three main types of information: 1) the regional pool of macroinvertebrate taxa; 2) the environmental filters; 3) the preference and tolerance of each taxon to the three filters.

The 'regional pool' of aquatic insects was based on a database of both published and unpublished information. This database consisted of over 400,000 individuals representing 100 macroinvertebrates families from 370 stream sites distributed in five neighboring states (Rio Grande

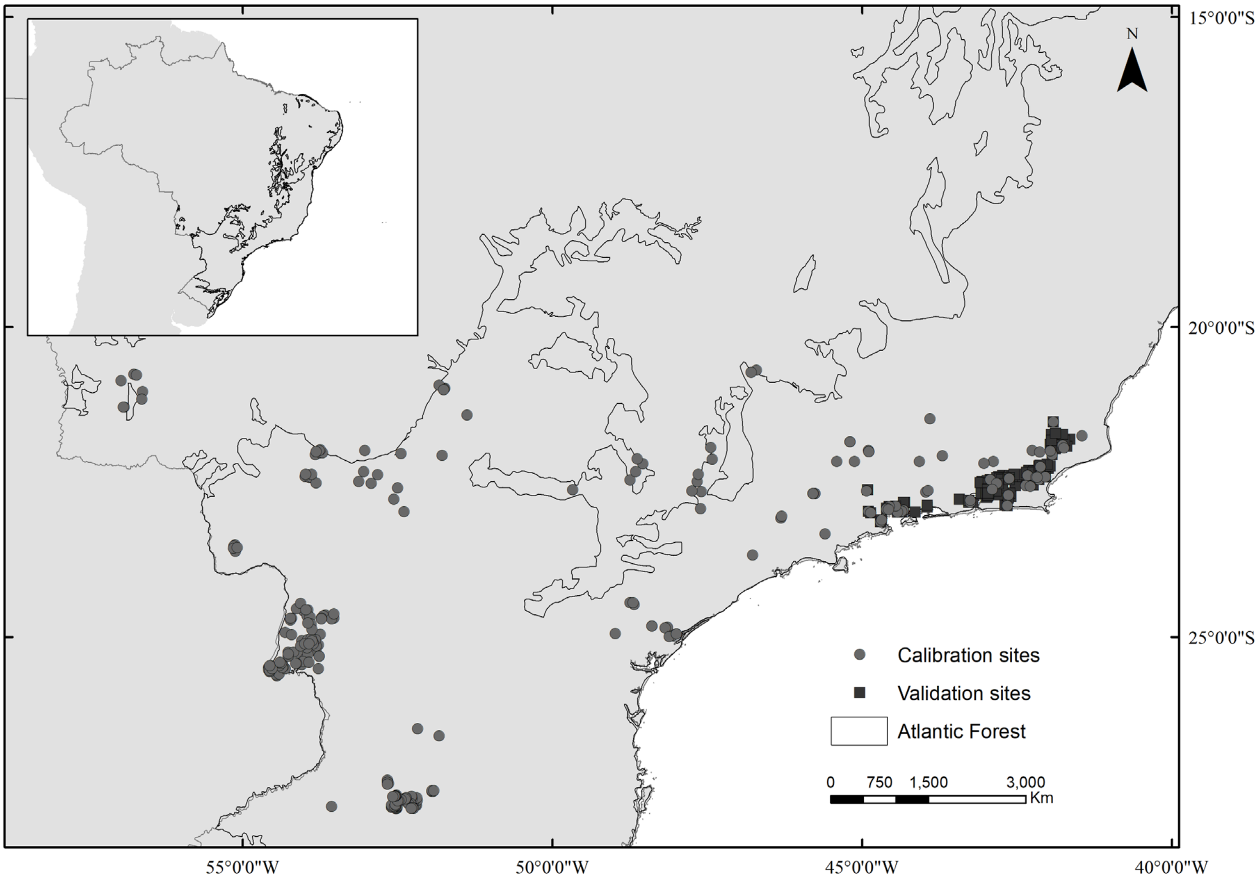


Figure 1. Locations of the macroinvertebrate sampling sites.

do Sul, Paraná, Mato Grosso do Sul, São Paulo and Rio de Janeiro) in south and southeast Atlantic Forest region. To define the regional pool, we identified the set of families that occurs in a region using the database. All families within the database were included as part of the regional pool and the list of families were confirmed with local experts. In next step, we excluded the rare families, which occurred in less than 10% of total sites in the database. These families that occurs with low frequencies could reduce model performance, because we couldn't define their preferences and environmental tolerances accurately or their absence in the following analyses are probably due to rarity rather anthropogenic degradation.

The environmental filters were used to generate a potential natural macroinvertebrate assemblage for each site by selecting from the regional pool the taxon which had the possibility (based on its environmental tolerance) that allowed it to occupy each test site. To obtain the local pool, we used a series of binary filters. Thus, a taxon was considered as a potential colonizer at a given site if it was listed in the regional pool and the site's characteristics were compatible with the environmental range described for the taxon. If a given taxon were filtered (excluded) by a single environmental attribute it was not considered to be potentially present in that location ("one-out-all-out" principle). We obtained a list of families potentially present in each combination of environmental filters (Appendix 1).

Ideally, the environmental filters should be relevant to the biological assemblages, must have enough available information regarding their biological tolerance ranges, and must not be strongly affected by stressors (Chessman & Royal 2004, Chessman 2006, Walsh 2006). In our study, the environmental variables that held these characteristics were elevation, original vegetation and soil type. These three filters are not directly influenced by human activities, they have been reported to influence aquatic insects assemblage distributions (Walsh 2006, Dudgeon 2012, Olson & Hawkins 2012), and there was enough information to map them and to calculate the tolerance range values for aquatic insects. The elevation range was divided into low (0-200m.a.s.l.), medium (>200-800m.a.s.l.) and high (>800m.a.s.l.), because these categories were reported as holding different aquatic insects assemblages in our studied region (Baptista et al. 2001, Henriques-Oliveira & Nessimian 2010). Original vegetation (Atlantic ombrophilous dense and semi-deciduous forest) and soil type (Cambisols, Ferralsols and Podzols) followed IBGE (http://mapas.ibge.gov.br).

The preferences and environmental tolerances of each taxon regarding the elevation range, vegetation and soil type were based on information obtained in the database and numerous publications. We used the database to define occurrence frequency of taxa in each category within the environmental filters. We used the database to define occurrence frequency of taxa in each category within the environmental filters. Families which the occurrence was equal or more than 10% of the streams in that category was considered potentially present in the category of the environmental filter (Appendix 1). So, the probabilities were transformed to binary presence (1) and absent (0). We used the literature to avoid errors and the ones containing more information were Baptista et al. (2001); Hepp & Santos (2009); Henriques-Oliveira & Nessimian (2010); Suriano et al. (2011); Siqueira et al. 2012).

All taxa were identified to the lowest taxonomic level possible (mostly to genus level), but data were aggregated to family level for the modelling. The family level was chosen due to the lack of information about the preferences and environmental tolerances (ecological traits) on genus or lower levels. Also, this taxonomic level was shown to present similar responses to impairment as lower levels in Brazil (Buss & Vitorino 2010) and, due to practical reasons, family-level is recommended as a starting point for bioassessments in regions with taxonomic and resources constraints, such as Brazil (Buss et al. 2015). Other studies developing predictive modelling based on environmental filters (Chessman & Royal 2004, Stranko et al. 2005, Chessman 2006, Walsh et al. 2010, Davies et al. 2012) have found that those approaches may be applied successfully using family level.

3. *Database used to apply and test the model*

The dataset used to apply and test the model consisted of 106,088 individuals representing 67 families and 10 orders of insects, from 146 sites sampled in Rio de Janeiro state. The records from these 146 sites were excluded from model development so that they could be independently used to assess the model performance. Aquatic insects were sampled in streams of 1st to 4th orders (of which less than 15% were of 5nd to 6th orders), representing elevations from sea level to 1,700 m.a.s.l. and under different land uses (reference, pasture, agriculture or urban). The great majority of the samples were taken avoiding the wet season (thus, sampled from April to October) between the years of 2005 and 2010. All samples were taken and processed by the same research team. In the field, twenty samples (around 20m^2) were collected proportionally to the microhabitats (substrates) available in each stream reach using a Kick sampler (30x30cm; 500 µm mesh size), following the multi-habitat method (Barbour et al. 1999). The percentage of available habitats was previously estimated by visual inspection and substrates with less than 5% of the site area were not sampled. Samples were obtained from a reach length of approximately 20 times the channel width. Samples were conserved in the field in 80% ethanol and taken to the laboratory for further inspection. In the laboratory, samples were washed to remove coarse organic matter, such as leaves and twigs and the remaining material was placed in a sub-sampler measuring 64 x 36 cm, divided into 24 quadrats, each measuring 10.5 x 8.5 cm, with area of 89.25 cm^2 (European patent number 2572576). Eight quadrats were chosen at random and processed entirely, following the procedures described in Oliveira et al. (2011a).

In the field, water was analyzed for pH (MPA 210p LabConte) and dissolved oxygen (mg/L; YSI 550A). Cooled samples were taken to the lab for further analysis. The parameters ammonia (mg/L NH_3), nitrate (mg/L NO_3), and total phosphorus (mg/L P-total) were analyzed using a spectrophotometer (HACH DR2500), following Standard Methods protocols (APHA 2000). Sampling sites were also classified in the field using the visual-based habitat assessment protocol (HAP; Barbour et al. 1999). The HAP analyzes ten environmental parameters, such as substrate availability for colonization by benthic fauna, water velocity and embeddedness, channel condition, sediment deposition, margin stability and riparian vegetation extent and condition. For each parameter a score between 0 and 20 is assigned. Sites are classified according to the mean score obtained in the HAP, as follows: 0-5 “Poor”, 5.1-9.9 “Marginal”, 10-14.9 “Suboptimal” and 15-20 indicating an “Optimal” environmental condition (Barbour et al. 1999). Landscape variables (elevation, original vegetation and soil types) were obtained for each site using the ArcGIS 10.3 software and their corresponding digitalized 1:500,000 maps (IBGE http://mapas.ibge.gov.br).

Although our aim in this study was to develop a model to predict the fauna in regions lacking proper reference sites, we included reference areas to test the model. We hypothesize that if the model is robust enough it should yield higher scores to reference sites in comparison to other impairment conditions. Impairment classes were assigned based on physical, chemical and environmental parameters, the latter following Barbour et al. (1999). Sites were classified as reference if water had dissolved oxygen >6.0 mg/L, an “Optimal” or “Good” environmental condition according to the HAP, no sign of channelization locally or upstream and if <25% area upstream land-use were urban (based on recent satellite images). Most reference sites were within or in buffer zones of protected areas and were thus classified as “minimally disturbed” (sensu Stoddard et al. 2006). Impaired sites were classified if they had “Poor” condition according to HAP and if recent satellite images showed >40% of upstream area was affected by urban areas and/or agriculture. Intermediate sites had characteristics between these two classes. When in the field we noticed some intermediate sites had been reforested or were in process of recovery. Those sites were classified as “best attainable conditions” (sensu Stoddard et al. 2006), and we used this subset of intermediate sites to refine the model testing.

4. *Comparison of observed and expected taxa*

The suite of families observed at each site was compared with the suite that was attributed as potential colonizers of that site. This ratio (number of observed within the expected/total expected) is termed as “observed proportion of potential” (OPP; Chessman & Royal 2004). The OPP scores range from 0 to 1, where scores close to zero indicate few expected families were observed and scores close to one indicate the opposite. The lower the OPP score the higher the impairment level of a site.

5. *Testing the model*

The testing of the OPP was done threefold, aiming to verify: (1) responsiveness, if the OPP scores statistically discriminate sites of the four impairment classes (minimally disturbed reference, best attainable conditions, intermediate and impaired) using an ANOVA followed by a Tukey post-hoc; (2) precision, using standard deviation of scores across reference sites; (3) sensitivity, if OPP scores correlated with environmental variables that are highly sensitive to human alteration, which allow to define a stressor gradient; (4) agreement between the methods in relation to ecological quality classification, if OPP scores correlated with multimetric indices previously developed for river basins where those sites were located. To verify if the OPP scores were correlated to environmental variables, first a PCA were calculated using environmental variables (ammonia, nitrate, P-total, and the HAP index) for 110 sites where all those informations were available. Prior to analysis, data were standardized by subtracting each value from its mean and dividing it by its standard deviation to reduce the effects of different scales used in the variables. Second, Pearson correlations were calculated using the OPP scores for each site and the obtained PCA first-axis values (environmental variables considered should be positively correlated in the PCA first-axis). To verify if the OPP scores were correlated with multimetric indices, Pearson correlations were calculated between the OPP score for each site and the indices: GMMI (Guapiaçu-Macacu Multimetric Index; Oliveira et al. 2011b), for 38 sites sampled in this basin; PPPMI (Paquequer-Piabanha-Preto Multimetric Index; Baptista et al. 2011), for 22 sites; MISB (Serra da Bocaina Mutimetric Index; Baptista et al. 2013) for 16 sites; IMMM (Macaé Multimetric Index; unpublished data) for 29 sites; and ECMI (East Coast Multimetric Index; Pereira et al. 2016), for 20 sites. Twenty-one sites were sampled in basins without prior developed indices and were not included in this analysis.

Results

Based on the criteria to determine impairment conditions, the 146 sampled sites were classified as follows: 35 reference sites, 20 with best attainable conditions, 55 intermediate and 36 sites classified as impaired. Physical and chemical parameters suggest a gradient of impairment related to organic origin (as the values of ammonia and P-total indicate) and to non-point source pollution and/or related to environmental degradation (based on the HAP index; Table 1). The HAP index classified reference sites as "optimal" or "good", while impaired sites were classified as "regular" or "poor" environmental condition.

1. *Environmental filters*

From the 146 stream sites analyzed in this study, cambisols had the higher number of sites (104) among soil types, followed by podzols and ferralsols (26 and 16 sites, respectively). The great majority of sites (136) belonged to ombrophylous dense forest dominion, with the ten remaining belonging to the Atlantic semi-deciduous forest dominion. The elevation range separated sites in more even numbers: 51 sites from 0-200m.a.s.l., 60 sites in the 200-800m.a.s.l range and 35 sites >800m.a.s.l. It is important to notice that the distribution of streams do not follow the percentiles of each environmental condition in the state, being an artefact of the database. The three environmental filters were combined to determine the potential of occurrence of aquatic insects. Sixty-seven families were identified as having the natural potential to occur at one or more sites. The combination with the higher number of families with potential to occur was cambisols covered by ombrophilous dense forest and in the 200-800m.a.s.l. elevational range (53 families; Table 2). The combinations with the lower numbers of families with potential to occur were those at low elevation (0-200m.a.s.l) covered by Atlantic semi-deciduous forest, both in ferralsols and podzols soil types (35 families; Table 2). Sampled sites represented most possible filters combinations. All combinations had at least one site sampled, with the exception of sites >800m.a.s.l. in Atlantic semi-deciduous forest on podzols and ferralsols, and in Atlantic ombrophilous dense forest on ferralsols (Table 2).

2. *Testing the model*

The OPP scores for the 146 sites ranged from 0.02 to 0.75. Reference sites had a high number of occasions where the expected families were also observed (E+O+, Table 3). This contributed for the highest OPP scores obtained by sites of this class (percentiles 25%-75% = 0.46 and 0.60, respectively; Figure 2). BAC sites scores were statistically similar to those of reference sites (ANOVA Tukey post-hoc test p=0.11), although 75% of BAC scores were lower than the median score of reference sites (Figure 2). On the other hand, in impaired sites, a high number of expected families were not observed (E+O-, Table 3). The lower number of E+O+ occasions resulted in lower OPP scores for impaired sites (percentiles 25%-75% = 0.12 and 0.32, respectively). Intermediate sites scored between the two extremes (ANOVA Tukey post-hoc test p< 0.01 for all those pairs of data; Figure 2). The scores of reference sites had low standard deviation (0.54, SD= 0.098) which indicates high precision of filters approach. In general, sensitive taxa (Corydalidae, Grypopterygidae, Perlidae, Psephenidae and Pyralidae) and the shredders – group particularly vulnerable to riparian deforestation (e.g., Calamoceratidae, Leptoceridae and Tipulidae) – were observed with higher frequency in reference sites (Table 3). Those taxa, among others, had distinct presence patterns between reference and impaired sites. For example, Grypopterygidae was observed in 34 of the 35 reference sites it had a natural potential of occurring, but it was observed in only two of the 36 impaired sites it was expected. Some very abundant tolerant or moderately tolerant taxa were observed in most sites they were expected to occur (e.g., Elmidae, Chironomidae, Simuliidae, Baetidae, Leptohyphidae e Hydropsychidae), regardless the impairment condition (reference, BAC, intermediate or impaired; Table 3). In very few occasions, some families unassigned by the filters for occurring at a site were observed (E-O+, Table 3).

Table 1. Mean values (and standard deviation) of the physical, chemical and environmental parameters measured for stream sites classified as reference, best attainable condition (BAC), intermediate and impaired.

	Reference	BAC	Intermediate	Impaired
Habitat Assessment Protocol (HAP)	17.38 (±2.39)	15.48 (±3.62)	11.71 (±4.77)	4.02 (±2.04)
HAP classes	optimal-good	optimal-good	good-poor	regular-poor
NH3-N (mg/L)	0.02 (±0.02)	0.02 (±0.02)	0.07 (±0.25)	0.54 (±0.93)
P-total (mg/L)	0.50 (±0.22)	0.36 (±0.07)	0.46 (±0.22)	0.87 (±0.69)
NO3 (mg/L)	1.99 (±1.71)	2.60 (±1.63)	1.90 (±1.28)	1.59 (±1.27)
Dissolved oxygen (mg/L)	6.83 (±1.85)	7.80 (±1.08)	7.15 (±1.83)	5.38 (±2.40)
pH	6.90 (±0.45)	6.68 (±0.29)	7.04 (±0.51)	6.96 (±0.45)

Table 2. Number of sampled sites and number of families with potential of occurring, according to environmental filters (soil types, original vegetation cover and elevation ranges).

Soil types	Original vegetation cover	Elevation (m)	Number of stream sites sampled	Number of families with potential of occurring
Cambisols	Atlantic ombrophilous dense forest	<200	31	40
		200-800	39	53
		>800	34	51
Ferralsols	Atlantic ombrophilous dense forest	0-200	4	36
		200-800	7	47
		>800	0	46
Ferralsols	Atlantic semi-deciduous forest	<200	1	35
		200-800	4	43
		>800	0	43
Podzols	Atlantic ombrophilous dense forest	<200	12	40
		200-800	8	51
		>800	1	50
Podzols	Atlantic semi-deciduous forest	<200	3	35
		200-800	2	45
		>800	0	44

Table 3. Number of occasions of aquatic insect families with/without a natural potential of occurrence in a site based on environmental filters (E+, E-), and observed/not- observed (O+, O-), at reference (**bold letters**) and impaired sites (*italic letters*). "E+O+" (number of occasions the family with potential was collected), "E+O-" (number of occasions the family with potential was not collected); "E-O+" (number of occasions the family with no potential was collected); "E-O-" (number of occasions the family with no potential was not collected).

	E+O+	*E+O+*	**E+O-**	*E+O-*	**E-O+**	*E-O+*	**E-O-**	*E-O-*
Aeshnidae	12	3	12	20	1	0	10	13
Baetidae	35	26	0	10	0	0	0	0
Belostomatidae	9	2	21	31	1	0	4	3
Blephariceridae	13	0	17	31	0	0	5	5
Caenidae	5	1	20	25	2	0	8	10
Calamoceratidae	34	4	1	32	0	0	0	0
Calopterygidae	21	18	14	18	0	0	0	0
Ceratopogonidae	18	13	17	23	0	0	0	0
Chironomidae	35	36	0	0	0	0	0	0
Coenagrionidae	9	5	26	31	0	0	0	0
Collembola	0	0	0	0	2	1	33	35
Cordullidae	3	1	18	22	1	3	13	10
Corixidae	0	0	4	5	0	0	31	31
Corydalidae	21	5	14	31	0	0	0	0
Culicidae	0	0	0	0	1	0	34	36
Dixidae	5	2	24	31	2	0	4	3
Dryopidae	4	0	20	23	2	1	9	12
Dytiscidae	1	0	23	23	1	0	10	13
Ecnomidae	0	0	2	3	0	0	33	33
Elmidae	35	27	0	9	0	0	0	0
Empididae	27	11	8	25	0	0	0	0
Ephrydidae	1	2	28	31	0	0	6	3
Euthyplociidae	1	0	17	20	1	0	16	16

Continuation Table 3.

	E+O+	*E+O+*	**E+O-**	*E+O-*	**E-O+**	*E-O+*	**E-O-**	*E-O-*
Gerridae	0	0	0	0	3	0	32	36
Glossosomatidae	1	1	23	22	1	1	10	12
Gomphidae	17	12	18	24	0	0	0	0
Grypopterygidae	31	2	4	34	0	0	0	0
Gyrinidae	8	3	16	20	2	1	9	12
Hebridae	0	0	0	0	0	0	35	36
Helicopsychidae	24	3	11	33	0	0	0	0
Helotrephidae	9	2	1	8	9	1	16	25
Hydrobiosidae	16	1	15	35	2	0	2	0
Hydrophilidae	0	0	0	0	19	5	16	31
Hydropsychidae	35	24	0	12	0	0	0	0
Hydroptilidae	9	2	26	34	0	0	0	0
Leptoceridae	30	12	5	24	0	0	0	0
Leptohyphidae	35	23	0	13	0	0	0	0
Leptophlebiidae	31	15	4	21	0	0	0	0
Libellulidae	16	18	19	18	0	0	0	0
Lutrochidae	21	4	14	32	0	0	0	0
Megapodagrionidae	13	3	22	33	0	0	0	0
Mesoveliidae	1	0	9	11	7	1	18	24
Naucoridae	24	7	11	29	0	0	0	0
Noteridae	0	0	0	0	0	0	35	36
Notonectidae	0	0	0	0	0	0	35	36
Odontoceridae	13	3	11	20	3	0	8	13
Oligoneuriidae	0	0	9	10	0	0	26	26
Perilestidae	0	2	9	8	2	1	24	25
Perlidae	35	8	0	28	0	0	0	0
Philopotamidae	21	10	14	26	0	0	0	0
Pleidae	11	0	24	36	0	0	0	0
Polycentropodidae	4	2	31	34	0	0	0	0
Protoneuridae	3	1	16	21	2	1	14	13
Psephenidae	23	6	12	30	0	0	0	0
Psychodidae	4	11	31	25	0	0	0	0
Ptilodactylidae	0	0	20	21	2	0	13	15
Pyralidae	24	8	11	28	0	0	0	0
Scirtidae	0	0	0	0	2	0	33	36
Sericostomatidae	15	5	20	31	0	0	0	0
Simuliidae	34	31	1	5	0	0	0	0
Staphylinidae	16	9	19	27	0	0	0	0
Stratiomyidae	0	0	1	2	1	4	33	30
Syrphidae	0	0	0	0	0	0	35	36
Tabanidae	5	0	19	23	0	0	11	13
Tipulidae	25	9	10	27	0	0	0	0
Vellidae	30	13	5	23	0	0	0	0
Xyphocetronidae	0	0	0	0	1	0	34	36

Bold letters: Reference sites; *Italic letters: Impaired sites*.

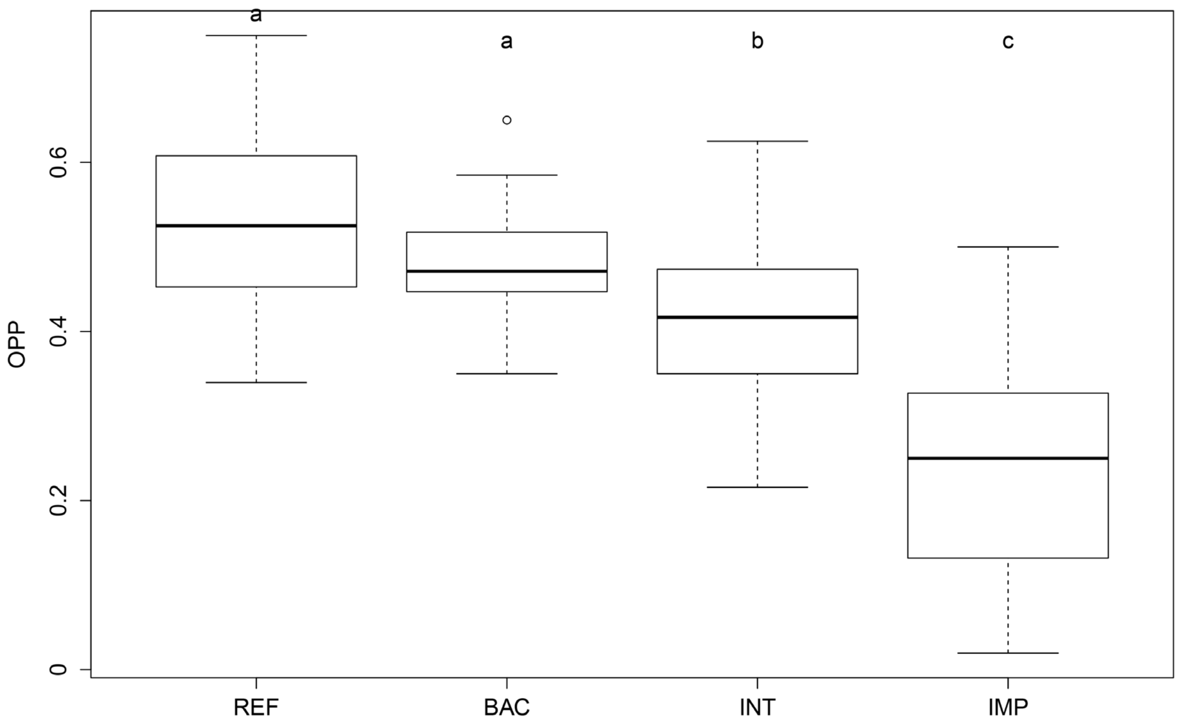


Figure 2. OPP scores calculated for the four impairment classes: Reference (Ref.), Best Attainable Condition sites (BAC), Intermediate (Inter.), and Impaired sites (Imp.). Different letters above the box-plots indicate significant difference, according to Tuckey post-hoc tests (ANOVA F=56.9; p<0,001).

The axis 1 of the environmental PCA represented 54.04% of the variance and it was the only significant one, according to the broken-stick model. PCA axis 1 discriminated Reference and BAC sites from intermediate and impaired conditions (Mann-Whitney test using PCA axis 1 scores, $p<0.01$ for all pairs except Ref x BAC, $p = 0.56$). The parameters ammonia and P-total had the highest eigenvalues (>0.50) for this axis. The OPP scores responded to this gradient of impairment conditions and were significantly correlated with the environmental PCA axis 1 values ($r=0.74$, $p<0.01$). The OPP scores were also highly correlated to all subsets of biological data, expressed by the multimetric indices calculated for each basin (Table 4).

Table 4. Pearson correlations (R and p-level) between the OPP scores and the multimetric indices for each stream basin.

Multimetric index	n	r	p-level
GMMI	38	0.82	<0.01
PPPMI	22	0.95	<0.01
MISB	16	0.72	<0.01
IMMM	29	0.70	<0.01
ECMI	20	0.92	<0.01

n = number of sites.

Discussion

The OPP index developed in this study was sensitive to discriminate a gradient of impairment conditions (reference, best attainable, intermediate and impaired sites; Figure 2). The OPP scores were also correlated with a set of environmental variables, and it was strongly correlated with all multimetric indices developed for multiple watersheds in the state (Table 4). This latter result was unexpected, since those multimetric indices were developed separately for each watershed, incorporating their particular environmental and biological conditions. Chessman and Royal (2004) found that OPP was significantly correlated with different disturbance measures and with another biological index (SIGNAL2; Stream Invertebrate Grade Number Average Level). Chessman et al. (2006) reported the OPP had higher sensitivity to distinguish the impairment gradient than the AUSRIVAS O/E index (Australian River Assessment System Observed over Expected). Even though the OPP had performed very well in our study, we believe it could be improved if some of the following aspects is implemented: using a taxonomic level lower than family-level; using more refined environmental filters; and by incorporating more information about the auto-ecology of aquatic insects taxa.

1. *Considerations on taxonomic level*

The taxonomic level to be used for bioassessment and monitoring purposes is a topic of ongoing debate (e.g., Buss & Vitorino 2010, Mueller et al. 2013). Some researchers argue that the identification of aquatic insects to species-level have a higher sensitivity to detect small differences among sites (Heino 2014), but some others argue that in biomonitoring programs datasets are summarized in indices, which do not necessarily require species data and are often robust to taxonomic aggregation (Buss & Vitorino 2010, Whittier & Van Sickle 2010, Mueller et al. 2013). The original OPP index in Australia was also developed in family-level (Chessman & Royal 2004). Those authors argued that this level was chosen because of the lack of taxonomic keys, due to practical reasons (lower cost and skill requirements), and because most bioassessments in the region has operated at family level. In Brazil and other parts of Latin America the situation is the same. The identification to the species-level is not always possible due to the limited taxonomic knowledge for many insect groups, and although taxonomic keys are being developed and becoming available (e.g., Domínguez & Fernandez 2009, Mugnai et al. 2010, Hamada et al. 2014) there is still a lack of taxonomic keys for most regions and an insufficient and decreasing number of experts in taxonomy. Still, if these limitations may be overcome, the use of lower taxonomic levels (e.g., genus) could hypothetically increase the precision of the model. Some neotropical families are composed by genera that differ considerably regarding their tolerance to stressors and ecological preferences (e.g., Chironomidae – Roque et al. 2010; Baetidae – Buss & Salles 2007). In such cases, a family may have been assigned as having the "potential of occurrence" based on the distribution and preferences of a given genus 'A', while the collected genus in one location was the 'B', incorrectly increasing the OPP score for that site. Higher taxonomic levels (e.g., family) could be used more consistently for families composed by few genera and/or where the genera share similar ecological traits. Since the tolerance and biological traits of many species or genera are still missing (see discussion below), an interesting starting point could be using mixed taxonomic levels – using the criteria above for the decision – instead of losing information by using data aggregated to family level.

2. *Considerations on environmental filters*

In our study, OPP scores for some reference areas were relatively low (OPP = 0.34 the lowest score for this condition), juxtaposing with some impaired condition scores (OPP = 0.46, the highest score for this condition). For the OPP, we used environmental filters at a "landscape-" or "regional-scale" (elevation, original vegetation and soil types). In part, we chose these filters based on Omernik's (1987) ecoregional approach, which have already been shown to have a good response by the macroinvertebrate (Verdonschot & Nijboer 2004) and fish fauna (Pinto et al. 2009). Also, these features are not subject to human disturbance, which is an ideal condition for the use of the filters approach (Poff 1997, Walsh 2006). On the other hand, some researchers argue that macroinvertebrate faunal predictions based on filters should be based on multiple spatial scales, mixing both "regional" and more "local" features (Poff 1997, Olden et al. 2006). This is supported by research based on diversity partitioning analyses, which indicate that both scales help to explain macroinvertebrate assemblage distribution in Brazil (Ligeiro et al. 2010, Macedo et al. 2014). However, the use of local environmental filters has some limitations. There is an increasing difficulty for extracting patterns from large databases since local information are not widely available and may not be extracted by GIS procedures – rather, they must be gathered on site – and most importantly, that local features are strongly affected by anthropogenic impacts, even relatively mild ones. The problem of using predictor variables that are affected by human disturbance is that the modelling will incorporate part of the disturbance, and it may misclassify sites with some degradation as being undegraded (Walsh 2006). To correct this, simulation models of human influences may enable to predict natural features and they could be used instead of the observed features (Chessman et al. 2006). The use of GIS-based information coupled with Self-organizing maps (SOM) techniques may aid further the prediction of local-level features based on larger-scale data (e.g., Davies et al. 2000, Snelder et al. 2011).

3. *Considerations on taxa preferences and tolerances*

The virtual lack of information on biological traits hinders the application of environmental filters approach in many regions (Helm et al. 2015). In recent years, there was a greater focus on this subject worldwide (Van den Brink et al. 2011, Culp et al. 2011, Mueller et al. 2013). Still, more data on the autecological characteristics, ecological preferences and biological traits are necessary, especially for neotropic benthic fauna. The way forward includes the compilation of bio/ecological traits in large databases, such as those developed for Europe (Bonada & Dolédec 2011, Schmidt-Kloiber and Hering 2015), North America (Vieira et al. 2006), South America (Tomanova & Usseglio-Polatera 2007), and New Zealand (Dolédec et al. 2006). Several researchers highlight the potential of using biological traits as metrics for bioassessments, since they can potentially reveal additional information concerning ecosystem properties beyond taxonomic composition (Poff et al. 2006, Dolédec & Statzner 2008, Culp et al. 2011).

In our study, we found few occasions where an unexpected family by the environmental filters analysis was, in fact, observed (E-O+, Table 3). Chessman & Royal (2004) argue that such cases may occur because of the model was based on inappropriate filters or incorrect information on biological traits, or according to Helm et al. (2015) they can indicate invasion of non-native species from different geographic regions or opportunistic species that historically do not occupy this particular habitat. Very little information is available on the historical distributions of the aquatic insect assemblages included in this study. However, this should be a concern when using this approach for the whole macroinvertebrate assemblages, especially given the many cases of mollusk and mosquito exotic species invasions in South America (e.g. Thomaz et al. 2014).

4. *Considerations on reference sites*

Based on our judgment, the "best attainable areas" (BAC) in this study would be classified as intermediate condition, or reference "on the fringe". The OPP approach was sensitive to detect this subtle change: although BAC sites had OPP scores statistically similar to reference, their scores were lower than most reference sites' scores. Also, both conditions were statistically different than intermediate and impaired conditions (Figure 2). These results indicate that best management practices (BMP) in these areas, such as maintaining or recovering the riparian zone and the natural stream habitat features, were efficient to partially sustain the aquatic insect fauna. We hope this can stimulate and guide managers toward BMPs, even though the recovery could be slow (Meals et al. 2010), and limited (Harding et al. 1998).

5. *Further developments on environmental filters approach*

Some alternative approaches were developed for the assessment of streams without reference areas. In general, those methods select from the population of sites the "least disturbed" ones – using physical, chemical and/or biological data – and use them as benchmarks. This can be achieved by using a pre-defined percentile of sites (Blocksom & Johnson 2009) or by multivariate analyses with abiotic data to determine the "best" sites (Birk et al. 2012), or even through partitioned least squares regressions to identify the highest biotic scores along an impairment gradient (Carter & Fend 2005). Those approaches, however, include some disturbance in their reference database, thus reducing the sensitivity of indices to detect impairment in test sites. More recently, methods based on artificial intelligence (e.g., species distribution models) were employed to predict "null assemblages" from large historical databases and then compared with contemporary distributions to determine the ecological status of streams (Davies et al. 2012, Labay et al. 2015, Milošević et al. 2016). These tools rely on large, comparable and sufficiently representative databases, something that unfortunately are not available for many biological groups and regions around the globe. Still, similar approaches based on environmental filters, like the OPP, are promising alternatives for the bioassessment without true reference sites, and they should be tested further.

Supplementary material

The following online material is available for this article:
Appendix 1

Acknowledgments

We thank the research team from LAPSA/IOC/FIOCRUZ that helped building the database used in this study. We are also thankful to F.O. Roque and L. Hepp for sharing part of the data for the Atlantic forest database. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001, CNPq/PROEP No 400107/2011-2, PAEF IOC-008-FIO-15-8 and PAEF IOC-023-FIO-18-2-4.

Author Contributions

Natália Freitas de Souza - Substantial contribution in the concept and design of the study, contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Darcílio Fernandes Baptista - Contribution to manuscript preparation and to critical revision, adding intellectual content.

Daniel Forsin Buss - Substantial contribution in the concept and design of the study, contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALVARES, C. A., J. L. STAPE, P. C. SENTELHAS, J. L. DE MORAES GONÇALVES, AND G. SPAROVEK. 2013. Kooppen's climate classification map for Brazil. Meteorologische Zeitschrift 22:711–728.

BAPTISTA, D. F., D. F. BUSS, L. F. M. DORVILLÉ, AND J.L. NESSIMIAN. 2001. Diversity and habitat preference of aquatic insects along the longitudinal gradient of the macaé river basin, Rio de Janeiro , Brazil. Revista Brasileira de Biologia 61:249–258.

BAPTISTA, D. F., R. S. G. DE SOUZA, C. A. VIEIRA, R. MUGNAI, A. S. SOUZA, AND R. B. S. DE OLIVEIRA. 2011. Multimetric index for assessing ecological condition of running waters in the upper reaches of the Piabanha-Paquequer-Preto Basin, Rio de Janeiro, Brazil. Zoologia (Curitiba) 28:619–628.

BAPTISTA, D. F., A. L. HENRIQUES-OLIVEIRA, R. OLIVEIRA, R. MUGNAI, J. L. NESSIMIAN, AND D. F. BUSS. 2013. Development of a benthic multimetric index for the Serra da Bocaina bioregion in Southeast Brazil. Brazilian Journal of Biology 73:573–583.

BARBOUR, M. T., J. GERRITSEN, B.D. SNYDER, AND J. B. STRIBLING. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers. USEPA, Washington.

BIRK, S., L. VAN KOUWEN, AND N. WILLBY. 2012. Harmonising the bioassessment of large rivers in the absence of near-natural reference conditions - a case study of the Danube River. Freshwater Biology 57:1716–1732.

BLOCKSOM, K. A., AND B. R. JOHNSON. 2009. Development of a regional macroinvertebrate index for large river bioassessment. Ecological Indicators 9:313–328.

BONADA, N., AND S. DOLÉDEC. 2011. Do mediterranean genera not included in Tachet et al. 2002 have mediterranean trait characteristics? Limnetica 30:129–142.

BUSS, D. F., D. M. CARLISLE, T. S. CHON, J. CULP, J. S. HARDING, H. E. KEIZER-VLEK, W. A. ROBINSON, S. STRACHAN, C. THIRION, AND R. M. HUGHES. 2015. Stream biomonitoring using macroinvertebrates around the globe: a comparison of large-scale programs. Environmental Monitoring and Assessment 187:4132.

BUSS, D. F., AND F. F. SALLES. 2007. Using Baetidae species as biological indicators of environmental degradation in a Brazilian river basin. Environmental Monitoring and Assessment 130:365–372.

BUSS, D. F., AND A. S. VITORINO. 2010. Rapid Bioassessment Protocols using benthic macroinvertebrates in Brazil: evaluation of taxonomic sufficiency. Journal of the North American Benthological Society 29:562–571.

CAO, Y., AND C. P. HAWKINS. 2011. The comparability of bioassessments: a review of conceptual and methodological issues 1. Journal of the North American Benthological Society 30:680–701.

CARTER, J. L., AND S. V. FEND. 2005. Setting Limits: The Development and Use of Factor-Ceiling Distributions for an Urban Assessment Using Macroinvertebrates. American Fisheries Society symposium 47:179–191.

CHESSMAN, B. C. 2006. Prediction of riverine fish assemblages throught the concept of environmental filters. Marine and Freshwater Research 57:601–609.

CHESSMAN, B. C., L. A. THURTELL, AND M. J. ROYAL. 2006. Bioassessment in a harsh environment: A comparison of macroinvertebrate assemblages at reference and assessment sites in an Australian Inland River System. Environmental Monitoring and Assessment 119:303–330.

CHESSMAN, B. C., AND M. J. ROYAL. 2004. Bioassessment without reference sites: use of environmental filters to predict natural assemblages of river macroinvertebrates. Journal of the North American Benthological Society 23:599–615.

CULP, J. M., D. G. ARMANINI, M. J. DUNBAR, J. M. ORLOFSKE, N. L. POFF, A. I. POLLARD, A. G. YATES, AND G. C. HOSE. 2011. Incorporating traits in aquatic biomonitoring to enhance causal diagnosis and prediction. Integrated Environmental Assessment and Management 7:187–97.

DAVIES, N. M., R. H. NORRIS, AND M. C. THOMS. 2000. Prediction and assessment of local stream habitat features using large-scale catchment characteristics. Freshwater Biology 45:343–369.

DAVIES, P. E., M. J. STEWARDSON, T. J. HILLMAN, J. R. ROBERTS, AND M. C. THOMS. 2012. Sustainable Rivers Audit 2: The ecological health of rivers in the Murray–Darling Basin at the end of the Millennium Drought (2008–2010). Murray-Darling Basin Authority, Australia.

DOLÉDEC, S., N. PHILLIPS, M. SCARSBROOK, R. H. RILEY, AND C. R. TOWNSEND. 2006. Comparison of structural and functional approaches to determining landuse effects on grassland stream invertebrate communities. Journal of the North American Benthological Society 25:44–60.

DOLÉDEC, S., AND B. STATZNER. 2008. Invertebrate traits for the biomonitoring of large European rivers: An assessment of specific types of human impact. Freshwater Biology 53:617–634.

DOLÉDEC, S., AND B. STATZNER. 2010. Responses of freshwater biota to human disturbances: contribution of J-NABS to developments in ecological integrity assessments. Journal of the North American Benthological Society 29:286–311.

DOMÍNGUEZ, E. AND H.R. FERNÁNDEZ. 2009. Macroinvertebrados bentónicos sudamericanos: Sistemática y Biología. Fundación Miguel Lillo, Argentina.

DUDGEON, D. 2012. Responses of benthic macroinvertebrate communities to altitude and geology in tributaries of the Sepik River (Papua New Guinea): the influence of taxonomic resolution on the detection of environmental gradients. Freshwater Biology 57:1794–1812.

ELIAS, C. L., A. R. CALAPEZ, S. F. ALMEIDA, AND M. J. FEIO. 2015. Determining useful benchmarks for the bioassessment of highly disturbed areas based on diatoms. Limnologica - Ecology and Management of Inland Waters 51:83–93.

ELIAS, C. L., CALAPEZ, A. R., ALMEIDA, S. F. P., CHESSMAN, B., SIMÕES, N., & FEIO, M. J. Predicting reference conditions for river bioassessment by incorporating boosted trees in the environmental filters method. 2016. Ecological indicators 69: 239-251.

FEIO, M. J., R. H. NORRIS, M. A. S. GRAÇA, AND S. NICHOLS. 2009. Water quality assessment of Portuguese streams: Regional or national predictive models?. Ecological Indicators 9:791–806.

FEIO, M. J., AND J. M. POQUET. 2011. Predictive Models for Freshwater Biological Assessment: Statistical Approaches, Biological Elements and the Iberian Peninsula Experience: A Review. International Review of Hydrobiology 96:321–346.

HAMADA, N., NESSIMIAN, J.L. AND QUERINO, R.B. 2014. Insetos aquáticos na Amazônia brasileira: taxonomia, biologia e ecologia. 1ed. Manaus: Editora INPA. 724p.

HARDING, J.S., E.F. BENFIELD, P.V. BOLSTAD, G.S. HELFMAN, AND E.B.D. JONES. 1998. Stream biodiversity: The ghost of land use past. Proceedings of National Academy of Sciences 95(25): 14843–14847.

HAWKINS, C. P., J. R. OLSON, AND R. A. HILL. 2010. The reference condition: predicting benchmarks for ecological and water-quality assessments. Journal of the North American Benthological Society 29:312–343.

HEINO, J. 2014. Taxonomic surrogacy, numerical resolution and responses of stream macroinvertebrate communities to ecological gradients: Are the inferences transferable among regions?. Ecological Indicators 36:186–194.

HELM, A., M. ZOBEL, A. T. MOLES, R. SZAVA-KOVATS, AND M. PÄRTEL. 2015. Characteristic and derived diversity: implementing the species pool concept to quantify conservation condition of habitats. Diversity and Distributions 21:711–721.

HENRIQUES-OLIVEIRA, A. L., AND J. L. NESSIMIAN. 2010. Aquatic macroinvertebrate diversity and composition in streams along an altitudinal gradient in Southeastern Brazil. Biota Neotropica 10:115–128.

HEPP, L. U., AND S. SANTOS. 2009. Benthic communities of streams related to different land uses in a hydrographic basin in southern Brazil. Environmental Monitoring and Assessment 157:305–318.

HERMAN, M. R., AND A. P. NEJADHASHEMI. 2015. A review of macroinvertebrate- and fish-based stream health indices. Ecohydrology & Hydrobiology:1–15.

LABAY, B. J., HENDRICKSON, D. A., COHEN, A. E., BONNER, T. H., KING, R. S., KLEINSASSER, W. LINAM, AND K. O. WINEMILLER. 2015. Can Species Distribution Models Aid Bioassessment when Reference Sites are Lacking? Tests Based on Freshwater Fishes. Environmental Management 56:835–846.

LIGEIRO, R., A. S. MELO, AND M. CALLISTO. 2010. Spatial scale and the diversity of macroinvertebrates in a Neotropical catchment. Freshwater Biology 55:424–435.

MACEDO, D. R., R. M. HUGHES, R. LIGEIRO, W. R. FERREIRA, M. A. CASTRO, N. T. JUNQUEIRA, D. R. OLIVEIRA, K. R. FIRMIANO, P. R. KAUFMANN, P. S. POMPEU, AND M. CALLISTO. 2014. The relative influence of catchment and site variables on fish and macroinvertebrate richness in cerrado biome streams. Landscape Ecology 29:1001–1016.

MEALS, D.W., S.A. DRESSING, T.E. DAVENPORT. 2010. Lag time in water quality response to best management practices: A review. Journal of Environmental Quality 39:85–96.

MILOŠEVIĆ, D., D. ČERBA, J. SZEKERES, B. CSÁNYI, B. TUBIĆ, V. SIMIĆ, AND M. PAUNOVIĆ. 2016. Artificial neural networks as an indicator search engine: The visualization of natural and man-caused taxa variability. Ecological Indicators 61:777–789.

MUELLER, M., J. PANDER, AND J. GEIST. 2013. Taxonomic sufficiency in freshwater ecosystems: effects of taxonomic resolution, functional traits, and data transformation. Freshwater Science 32:762–778.

MUGNAI, R., J.L. NESSIMIAN, D.F. BAPTISTA. 2010. Manual de identificação de macroinvertebrados aquáticos do Rio de Janeiro. Ed. Technical Books. Rio de Janeiro.

OLDEN, J. D., N. L. POFF, AND B. P. BLEDSOE. 2006. Incorporating ecological knowledge into ecoinformatics: An example of modeling hierarchically structured aquatic communities with neural networks. Ecological Informatics 1:33–42.

OLIVEIRA, R. B., R. MUGNAI, C.M. CASTRO, AND D. F. BAPTISTA. 2011a. Determining subsampling effort for the development of a rapid bioassessment protocol using benthic macroinvertebrates in streams of Southeastern Brazil. Environmental Monitoring and Assessment 175:75–85.

OLIVEIRA, R. B., D. F. BAPTISTA, R. MUGNAI, C. M. CASTRO, AND R. M. HUGHES. 2011b. Towards rapid bioassessment of wadeable streams in Brazil: Development of the Guapiaçu-Macau Multimetric Index (GMMI) based on benthic macroinvertebrates. Ecological Indicators 11:1584–1593.

OLSON, J. R., AND C. P. HAWKINS. 2012. Predicting natural base-flow stream water chemistry in the western United States. Water Resources Research 48:1–19.

OMERNIK, J. M. 1987. Ecoregions of the Conterminuous United States. Annals of the Association of American Geographers 77:118–125.

PINTO, B. C. T., F. G. ARAUJO, V. D. RODRIGUES, AND R. M. HUGHES. 2009. Local and ecoregion effects on fish assemblage structure in tributaries of the Rio Paraíba do Sul, Brazil. Freshwater Biology 54:2600–2615.

POFF, N. L. 1997. Landscape filters and species traits: towards mechanistic understanding and prediction in stream ecology. Journal of the North American Benthological Society 16:391–409.

POFF, N. L., J. D. OLDEN, N. K. VIEIRA, D. S. FINN, M. P. SIMMONS, AND B. C. KONDRATIEFF. 2006. Functional trait niches of North American lotic insects: traits-based ecological applications in light of phylogenetic relationships. Journal of the North American Benthological Society 25:730–755.

PEREIRA, P. S., SOUZA, N. F., BAPTISTA, D. F., OLIVEIRA, J. L., BUSS, D. F. 2016. Incorporating natural variability in the bioassessment of stream condition in the Atlantic Forest biome, Brazil. Ecological Indicators, 69, 606-616.

RIBEIRO, M. C., A. C. MARTENSEN, J. P. METZGER, M. TABARELLI, F. SACARANO AND M. J. FORTIN. 2011. The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. Biodiversity hotspot: Distribution and Protection of Conservation Priority Areas. Springer Berlin Heidelberg.

ROQUE, F. O., T. SIQUEIRA, L. M. BINI, M. C. RIBEIRO, L. R. TAMBOSI, G. CIOCHETI, AND S. TRIVINHO-STRIXINO. 2010. Untangling associations between chironomid taxa in Neotropical streams using local and landscape filters. Freshwater Biology 55:847–865.

SCHMIDT-KLOIBER, A., AND D. HERING. 2015. www.freshwaterecology.info - An online tool that unifies, standardises and codifies more than 20,000 European freshwater organisms and their ecological preferences. Ecological Indicators 53:271–282.

SIQUEIRA, T., L. M. BINI, F. O. ROQUE, S. R. MARQUES COUCEIRO, S. TRIVINHO-STRIXINO, AND K. COTTENIE. 2012. Common and rare species respond to similar niche processes in macroinvertebrate metacommunities. Ecography 35:183–192.

SNELDER, T. H., N. LAMOUROUX, AND H. PELLA. 2011. Empirical modelling of large scale patterns in river bed surface grain size. Geomorphology 127:189–197.

STODDARD, J. L., D. P. LARSEN, C. P. HAWKINS, R. K. JOHNSON, AND R. H. NORRIS. 2006. Setting expectations for the ecological condition of streams: the concept of reference condition. Ecological Applications 16:1267–76.

STRANKO, S. A., M. K. HURD, AND R. J. KLAUDA. 2005. Applying a large, statewide database to the assessment, stressor diagnosis, and restoration of stream fish communities. Environmental Monitoring and Assessment 108:99–121.

SURIANO, M. T., A. A. FONSECA-GESSNER, F. O. ROQUE, AND C. G. FROEHLICH. 2011. Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. Environmental Monitoring and Assessment 175:87–101.

THOMAZ, S. M., K. E. KOVALENKO, J. E. HAVEL, AND L. B. KATS. 2014. Aquatic invasive species: general trends in the literature and introduction to the special issue. Hydrobiologia 746:1–12.

TOMANOVA, S., AND P. USSEGLIO-POLATERA. 2007. Patterns of benthic community traits in neotropical streams: relationship to mesoscale spatial variability. Fundamental and Applied Limnology/Archiv für Hydrobiologie 170:243–255.

VAN DEN BRINK, P. J., A. C. ALEXANDER, M. DESROSIERS, W. GOEDKOOP, P. L. GOETHALS, M. LIESS, AND S. D. DYER. 2011. Traits-based approaches in bioassessment and ecological risk assessment: Strengths, weaknesses, opportunities and threats. Integrated Environmental Assessment and Management 7:198–208.

VERDONSCHOT, P. F.M., AND R. C. NIJBOER. 2004. Testing the European stream typology of the Water Framework Directive for macroinvertebrates. Hydrobiologia 516:35–54.

VIEIRA, N. K. M., N. L. POFF, D. M. CARLISLE, S. R. MOUTON, M. L. KOSKI, AND B. C. KONDRATIEFF. 2006. A Database of Lotic Invertebrate Traits for North America. U.S. Geological Survey, Virginia. (Available from: http://pubs.water.usgs.gov/ds187)

WALSH, C. J. 2006. Biological indicators of stream health using macroinvertebrate assemblage composition: A comparison of sensitivity to an urban gradient. Marine and Freshwater Research 57:37–47.

WALSH, C. J., E. M. WALLIS, AND M. J. STEWARDSON. 2010. Macroinvertebrate Filters Project stage 3: revision and refinement of the Filters V2 macroinvertebrate analytical model. Murray-Darling Basin Authority, Australia.

WHITTIER, T. R., AND J. VAN SICKLE. 2010. Macroinvertebrate tolerance values and an assemblage tolerance index (ATI) for western USA streams and rivers. Journal of the North American Benthological Society 29:852–866.

Received: 20/06/2018
Revised: 25/10/2018
Accepted: 17/11/2018
Published online: 11/04/2019

Biota Neotropica 19(2): e20180621, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Short Communication

Notes on *Vampyressa thyone* (Chiroptera: Phyllostomidae): distribution, genetics and hypopigmentation

Giovani Hernández-Canchola[1], Yire A. Gómez-Jiménez[1], Iván Hernández-Chávez[1],

Sara Carolina Lucero-Verdugo[1] & Livia León-Paniagua[1]*

[1]Universidad Nacional Autónoma de México, Facultad de Ciencias, Departamento de Biología Evolutiva,
Museo de Zoología, Av. Universidad 3000, 04510, Ciudad de Mexico, Mexico
**Corresponding author: Livia León-Paniagua, e-mail: llp@ciencias.unam.mx*

HERNÁNDEZ-CANCHOLA, G., GÓMEZ-JIMÉNEZ, Y.A., HERNÁNDEZ-CHÁVEZ, I., LUCERO-VERDUGO, S.C., LEÓN-PANIAGUA, L. **Notes on *Vampyressa thyone* Chiroptera: Phyllostomidae): distribution, genetics and hypopigmentation**. Biota Neotropica. 19(2): e20180621. http://dx.doi.org/10.1590/1676-0611-BN-2018-0621

Abstract: The frugivorous bats of the genus *Vampyressa* include five neotropical species, and some of them are considered relatively rare and uncommonly encountered. The northernmost distribution of this genus is in Mexico where only one species, the monotypic *Vampyressa thyone*, is found. Here, we report the Northwesternmost record for this species, extending its distribution range to the state of Guerrero, Mexico. This record extends the limit of the species distribution by more than 153 km. We also compared this specimens with other *V. thyone*, and using mitochondrial *cytochrome-b* sequences we detected three geographic clades within the species. The new Mexican specimen represent the most divergent *cytochrome-b* sequence within *V. thyone*. We recommend a taxonomic revision to validate the taxonomic status of the three groups detected within *V. thyone*, particularly the differentiated Mexican clade. Additionally, we report a case of hypopigmentation in another *V. thyone* collected in a mature tropical rainforest in Chiapas, Mexico.

Keywords: *Mexico; Northern little yellow-eared bat; pigmentation disorder; range extension; Stenodermatinae.*

Notas sobre *Vampyressa thyone* (Chiroptera: Phyllostomidae): distribución, genética e hipopigmentación

Resumen: Los murciélagos frugívoros del género *Vampyressa* agrupan a cinco especies neotropicales, algunas de ellas consideradas relativamente raras y poco comunes de encontrar. Este género alcanza su distribución más norteña en México en donde solo una especie monotípica, *Vampyressa thyone*, habita. Reportamos el registro al noroeste más alejado para esta especie, extendiendo su rango de distribución al estado de Guerrero, México. Este registro extiende el límite de distribución de la especie por más de 153 Km. También comparamos este espécimen con otros *V. thyone*, y utilizando secuencias mitocondriales de citocromo-b detectamos tres clados geográficos dentro de la especie. El nuevo espécimen mexicano representa la secuencia de citocromo-b más divergente dentro de *V. thyone*. Recomendamos una revisión taxonómica para validar el estatus taxonómico de los tres clados detectados dentro de *V. thyone*, y en particular para el clado mexicano más diferenciado. Adicionalmente, reportamos un caso de hipopigmentación en otro *V. thyone* colectado en una selva húmeda tropical en Chiapas, México.

Palabras clave: *México, murciélago norteño de orejas amarillas, desorden de pigmentación, extensión de distribución; Stenodermatinae.*

Introduction

The yellow-eared bats of the genus *Vampyressa* Wagner, 1843 consist of five different species, two of them recently described (Burgin et al. 2018). These bats are considered primarily or exclusively frugivorous, though little is known of their feeding habitats or ecology (Tavares et al. 2014). The distribution of this genus includes the Neotropical region, from Argentina to Mexico (Lim et al. 2003). However, in Mexico only one species of the genus is found, the Northern little yellow-eared bat *Vampyressa thyone* Thomas, 1909.

Vampyressa thyone is a small bat with a tragus, entire base of the ear, and upper edges of the ears yellow; and tail membrane short (6 -7 mm), edge lightly fringed at center (Reid 2009). This species roosts in leaves of trees and shrubs, and these bats are found in very wet habitats, such as streams in gallery forest (Téllez-Girón 2014). *Vampyressa thyone* is monotypic and is found in Bolivia, Brazil, Peru, Ecuador, Colombia, Venezuela, Guyana, French Guiana, Panama, Costa Rica, Nicaragua, Honduras, Belize, Guatemala and southern Mexico (Lim et al. 2003). The species has been reported only in the Mexican states of Campeche, Chiapas, and its northernmost records are located in the states of Veracruz and Oaxaca (Lim et al. 2003, Téllez-Girón 2014). It is not currently recognized in any threat category by Mexican authorities or the IUCN (SEMARNAT 2010, Tavares et al. 2015).

In Mexico and Central America it is uncommon (Reid 2009), and in Mexico it is considered rare and few individuals have been collected from only a few sites (Téllez-Girón 2014). Besides, the information related with the geographic range of *V. thyone* is still fragmented or incomplete, for example, new records available in biodiversity portals have not been included in IUCN maps (Tavares et al. 2015). It is for the foregoing that our goal was to update the knowledge about the distribution of *V. thyone* based on a new northwestern record and the available data of this species.

Materials and Methods

During a field expedition in Coacoyulichán, in the municipality of Cuautepec, Guerrero, Mexico (16°47'40.3''N; 98°55'59.9''W), at 418 meters above sea level (Figure 1) on April 16th 2014, when we used 3 mist nets (6, 6, and 15 meters during one night) set at ground level in a riparian forest on the side of a rocky river, we trapped a specimen of *V. thyone* and other phyllostomid and mormoopid bats. Coacoyulichán has a mean annual precipitation of 5.09 mm, a mean annual maximum temperature of 32.79 °C and a mean annual minimum temperature of 19.60 °C (SMN 2009).

We followed the recommendation and procedures proposed by the American Society of Mammalogists (Sikes et al. 2011), and we collected the specimen following Mexico's wildlife legislation (SEMARNAT SGPA/DGVS/08257). The specimen (field number GHC212) is deposited in the Mammal Collection of the Zoology Museum, UNAM (Facultad de Ciencias – Universidad Nacional Autónoma de México, Mexico City, Mexico, MZFC-M). We preserved the specimen as a skin with skeleton (MZFC-M 16012) (Figure 2A-B), and we preserved tissue samples (heart, liver, and kidney; TN 6214).

To corroborate the identification of this specimen and to compare with other *V. thyone*, we obtained external and cranial measurements of this specimen, and we also sequenced the mitochondrial gene *cytochrome-b* (*cyt-b*, 1,140 bp) following the protocols used by Hernández-Canchola and León-Paniagua (2017), which is available at GenBank (Accession number MK409639). We also downloaded the sequences available of other *V. thyone* (AY157048 – AY157054, DQ312428 – DQ312431) and related species (*Vampyressa pusilla*: AY157046 – AY157047, DQ312428; *Vampyressa melissa*: DQ312426 – DQ312427, FJ154185; *Mesophylla macconnelli*: AY157035 – AY157035; *Vampyriscus nymphaea*: DQ312415 – DQ312418; *Vampyriscus brocki*: AY157043, DQ312419 – DQ312421; *Vampyriscus bidens*: AY157044 – AY157045, AY157055, FJ154181; *Ectophylla alba*: AY157033). We selected the best scheme of partition and evolution model (*cyt-b*$_{[1]}$ = SYM+I+Γ; *cyt-b*$_{[2]}$ = HKY+I; *cyt-b*$_{[3]}$ = GTR+I) in PARTITIONFINDER 2 (Lanfear et al. 2017), and in MRBAYES 3.2.3 (Ronquist et al. 2012) we used 3 hot and 1 cold chains, in two independent runs of 10 million generations, sampling data every 1000 iterations. The final topology was obtained using a majority tree consensus and considering a burn-in of 10%. We checked the convergence of our results and a good sampling (ESS > 200) in TRACER 1.6. In Mega 6 (Tamura et al. 2013) we calculated the genetic distances between species, using the Kimura 2-parameter model.

To analyze geographic records of *V. thyone* available in biodiversity portals on the web, we downloaded this information from VertNet (http://portal.vertnet.org), UNIBIO (http://unibio.unam.mx), and Remib (http://www.conabio.gob.mx/remib/doctos/remib_esp.html), accessed on March 21st, 2018. We also included the geographic information from other specimens measured in this work: Mexico: Chiapas; Zona Arqueológica Monumento Natural Yaxchilán [16°53'53.6''N; 90°57'52.5''W] (MZFC-M 7102, 7108); Porción norte de la omega, Monumento Natural Yaxchilán [16°53'52.8''N; 90°58'25.1''W] (MZFC-M 7159); Campamento Arqueológico del INAH de Yaxchilán [16°54'3.5''N; 90°58'9.8''W] (MZFC-M 7187). Oaxaca; San Isidro, "La Gringa" [17°6'13''N; 94°7'21''W] (MZFC-M 7760); La Luz, carretera rumbo a Río Grande [16°6'36.4''N; 97°35'41.35''W] (MZFC-M 8651).

Results

Here, we report the new northwesternmost record of *Vampyressa thyone* and the first record in the Mexican state of Guerrero. The *V. thyone* reported here was an adult male with no scrotal testes, which was found with *Artibeus jamaicensis*, *A. lituratus*, *Carollia subrufa*, *Dermanura phaeotis*, *Glossophaga commissarisi*, *G. leachii*, *G. soricina* and *Pteronotus parnellii*.

In the genus *Vampyressa* there is a craniodental character, a small cusp in the lingual border of the longitudinal sulcus on posterior crown of upper canines (Tavares et al. 2014), but in the *V. thyone* specimens examined in the MZFC-M the accessory cusps seem to be more developed in males than in females. Nevertheless, in the new specimen the accessory cusp is even more developed than in the other males. This specimen in general also has the largest measurements of all measured specimens. External measurements, cranial measurements and weight of this specimen and all other *V. thyone* in the MZFC-M are shown in Table 1.

Our phylogenetic construction recovered the genera *Vampyriscus*, *Mesophylla* and *Vampyressa* as monophyletic. Besides, our results support that samples from Brazil and Paraguay recognized as *Vampyressa pusilla*, are an independent clade sister to *Vampyressa thyone*, which inhabits from Bolivia to Mexico. Nevertheless, we

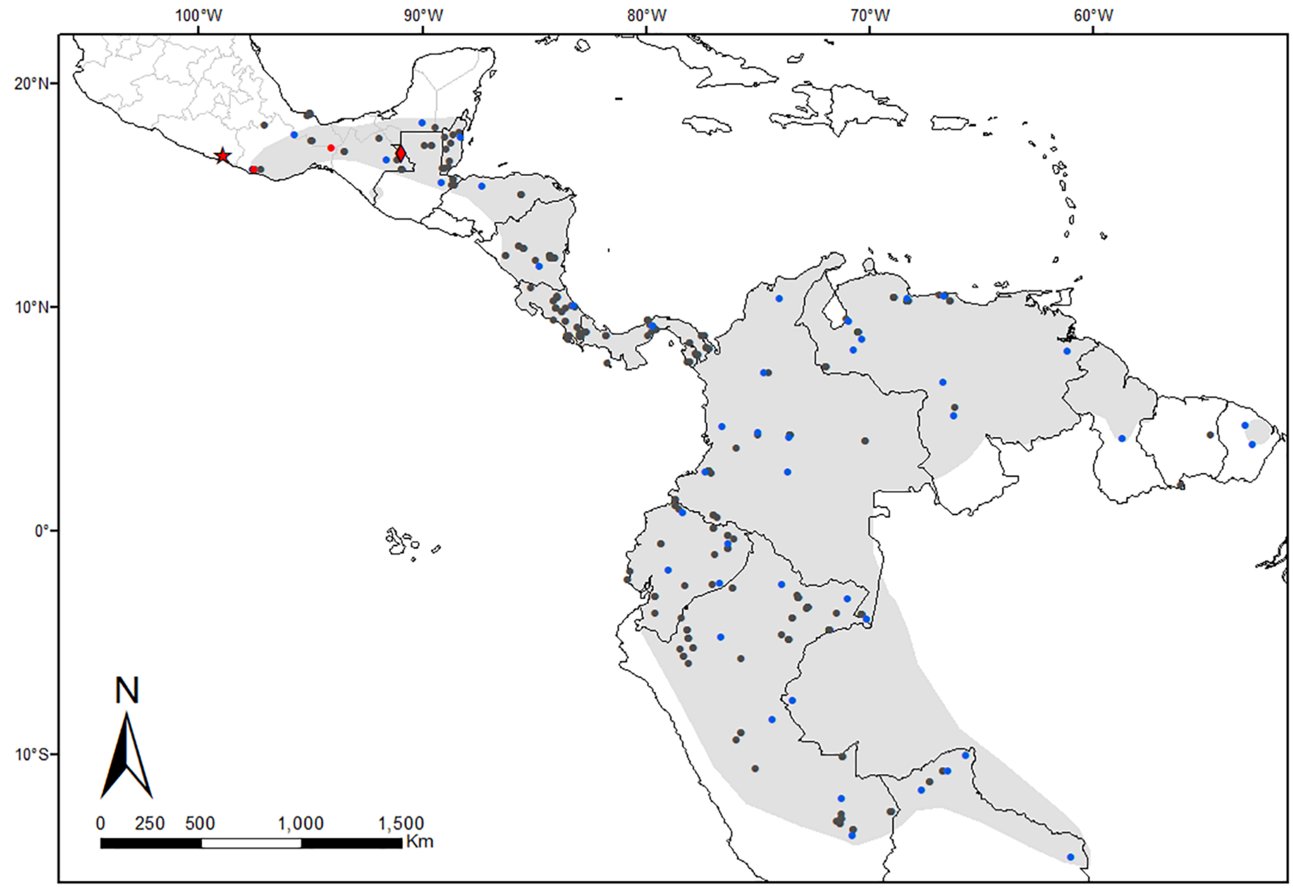


Figure 1. Geographic distribution of *Vampyressa thyone*. The gray area follows the geographic range from the IUCN Red List (Tavares et al. 2015). The blue dots are sites recorded by Lim et al. (2003) and Brosset and Charles-Dominique (1990), the gray dots are sites recorded in VertNet and UNIBIO, and the red symbols are specimens in the MZFC-M. The new Northwesternmost record is indicated with a star, and the hypopigmentation record with a diamond.

detected three genetic – geographic clades within *V. thyone*: a first clade with specimens from Peru to Panama, sister to a clade with specimens from Costa Rica to Honduras, and finally a third lineage, represented by the new Mexican record, sister to the two first clades (Figure 3). The genetic divergences ranged from 14.53 – 15.5 % between *V. thyone* and the genus *Vampyriscus*, 13.21 % with *Mesophylla*, 10.73 % with *Vampyressa melissa* and 11.41 % with *Vampyressa pusilla*. Within *V. thyone* the genetic distance between South and Central American clades was 2.1 %, and between the Mexican sample and South and Central American clades were 4.41 and 4.64 %, respectively.

In addition, there are records in VertNet and UNIBIO that have not been considered as marginal records of *V. thyone* by IUCN (Tavares et al. 2015): this species has been previously recorded in the Mexican states of Oaxaca and Veracruz; the specimen KU 143607 from Salado River, Oaxaca and specimens FMNH (127108 – 127111) and CNMA (TUX167 – TUX172, TUX315 – 322) from Sierra de los Tuxtlas, Veracruz, represent the northernmost records of this species. Furthermore, there are records from Suriname not previously considered by IUCN: specimens TTU 106030 and ROM 120356 (Figure 1). To include these records as part of *V. thyone* distribution is important to validate the identification of these specimens. We validated samples hosted in Mexican scientific collections (CNMA, MZFC-M), but we recommend the same for *V. thyone* hosted in USA (KU, FMNH, TTU, ROM).

During the review of specimens of *V. thyone* at the MZFC-M, we also detected an individual (MZFC-M 7108) (Figure 2C) with signals of hypopigmentation. The specimen is a female captured in the Zona Arqueológica Monumento Natural Yaxchilán, in the municipality of Ocosingo, Chiapas, Mexico (16°53'53.6"N; 90°57'52.5"W) that was collected on August 27th, 1998.

Discussion

The new specimen reported here represents the first record in the Mexican state of Guerrero, according to the most recent mammal checklist for the state (Espinosa-Martínez et al. 2017), the distribution of the samples used in the systematic revision of *V. thyone* (Lim et al. 2003) and in a previous range extension work (Brosset & Charles-Dominique 1990). This specimen extends the northwestern limit of the species' distribution by more than 153 Km and increases the bat diversity of Guerrero to 67 species. In Mexico, this state is considered one of the most diverse in terms of mammal species, despite reduced sampling effort by field researchers over the past 50 years due to social and security problems (Espinosa-Martínez et al. 2017). Increased sampling effort is necessary, mainly in Eastern Guerrero, because we suspect that other Stenodermatinae species with ecological requirements similar to *V. thyone* (such as *Chiroderma villosum* or *Platyrrhinus helleri*, which

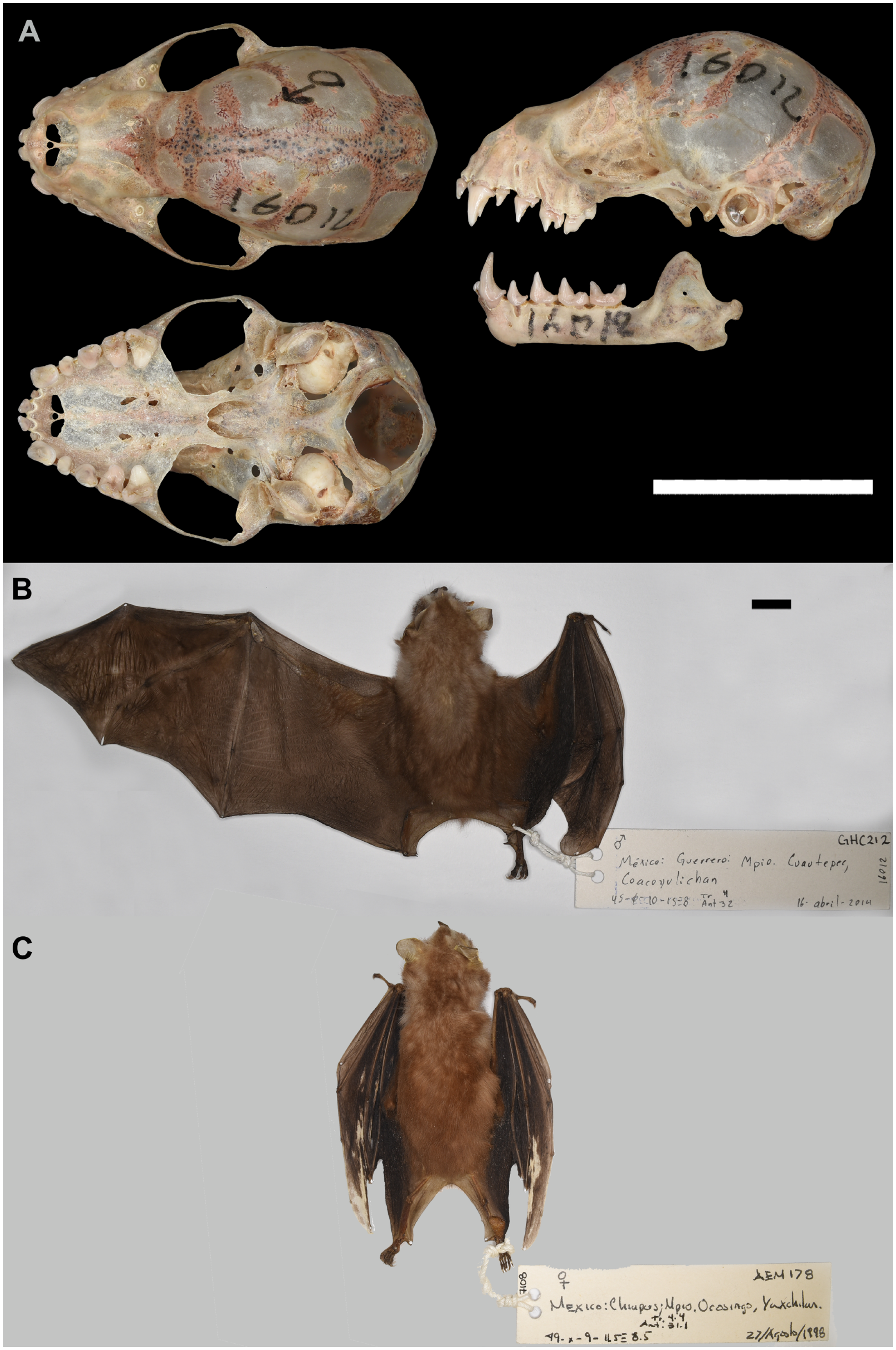


Figure 2. *Vampyressa thyone* reported in this study. The Northwesternmost specimen of *V. thyone* (MZFC-M 16012), from Coacoyulichán, Guerrero, Mexico: (A) Dorsal, ventral and lateral views of the skull and lateral view of the mandible (Scale bar = 1 cm); (B) Dorsal view of skin (Scale bar = 1 cm). (C) The hypopigmented specimen of *V. thyone* (MZFC-M 7108), from Zona Arqueológica Monumento Natural Yaxchilán, Chiapas, Mexico. The white spots are located on both wings.

Table 1. Measurements (mm) and weight (g) of the *Vampyressa thyone* at the MZFC-M.

Specimen	Sex	GLS	ZB	IOB	MB	GWM	MTL	mTL	FL	W
MZFC-M 7102	M	17.8	10.56	4.65	8.70	7.40	5.40	6.10	-	-
MZFC-M 7108+	F	17.70	10.69	4.74	9.10	7.76	5.78	6.13	31.1	8.5
MZFC-M 7159	F	18.46	10.67	4.88	9.15	7.72	5.70	6.29	33.0	8.0
MZFC-M 7187	M	18.49	10.75	4.71	9.01	7.75	5.43	6.33	31.3	7.0
MZFC-M 7760	M	18.66	-	4.78	9.44	8.06	6.05	6.42	34.0	8.5
MZFC-M 8651	M	18.56	-	4.48	9.16	7.88	5.84	6.37	31.9	8.0
MZFC-M 16012*	M	18.70	11.30	4.73	9.10	7.96	5.89	6.39	32.0	8.0

+ Hypopigmentation record and * New Northwesternmost record. GLS: greatest length of skull, ZB: zygomatic breadth, IOB: interorbital breadth, MB: mastoid breadth, GWM: greatest width across upper molars, MTL: maxillary toothrow length, mTL: mandibular toothrow length, FL: forearm length, and W: weight.

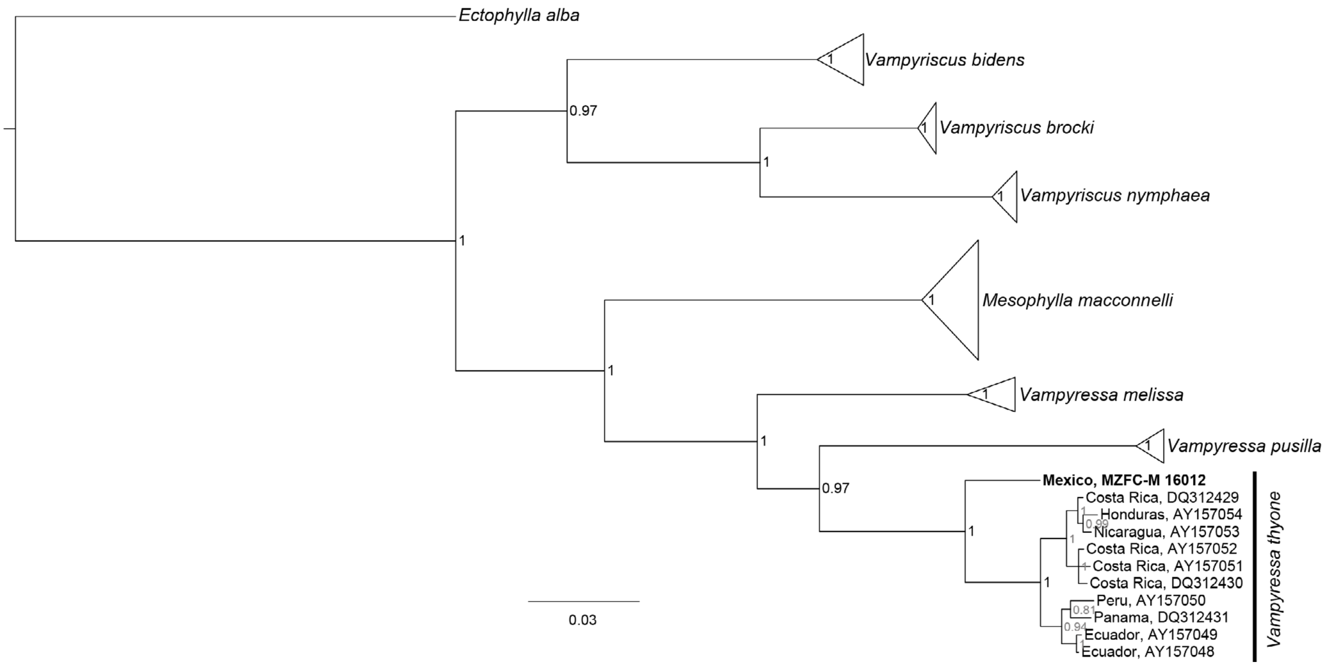


Figure 3. Topology obtained using a Bayesian inference that shows the phylogenetic relationships of *V. thyone* and related species. The height of enclosing triangles is proportional to the number of samples they contain, and the numbers at the nodes represent posterior probabilities.

have their limits until Oaxaca state; Pacheco R., 2014; Tellez-Giron, 2014), could inhabit Guerrero due to the continuity of habitats and absence of geographical barriers.

The topology of our Bayesian inference is similar to previous phylogenies (Porter & Baker 2004, Hoofer & Baker 2006), despite mixing both sets of data and including new sequences reported by Velazco & Patterson (2008). On the other hand, the genetic clades with geographic agreement within *V. thyone* have been reported in other Stenodermatinae bats, such as the genus *Platyrrhinus* (Velazco & Patterson 2008) and *Sturnira* (Hernández-Canchola & León-Paniagua 2017), and in some cases those clades represent different species. Besides, the genetic distance of the new Mexican specimen is the highest within *V. thyone*, so we recommend an integrative taxonomic revision of the three groups detected, to properly define species boundaries.

The specimen with pigmentation disorder represents the first record of hypopigmentation in *V. thyone*, and this atypical coloration of the wings has been reported in many other Neotropical bats species (Zalapa et al. 2016, Lucati & López-Baucells 2017). Nowadays there is no consensus about the classification of chromatic disorders, but according to recent proposals of chromatic disorders in bats, the category of hypopigmentation in this *V. thyone* represents a case of white spots (Zalapa et al. 2016) or piebaldism (Lucati & López-Baucells 2017). Failures in pigmentation differentiation or deposition, or infectious diseases are possible causes of hypopigmentation (Rose et al. 2017), though the causes and consequences of this phenomenon in bats are still unclear (Zalapa et al. 2016). We agree with authors that suggest that survival is not affected by hypopigmentation, as the specimen we report was an adult when captured, and many other bats with hypopigmentation even show signs of reproduction (e.g. Sánchez-Hernández et al., 2012). Besides, this condition is a taxonomic character in the bat *Diaemus youngi*, and there is no evidence that it can be detrimental to survival and reproduction of the species (Greenhall & Schut 1996).

Vampyressa pusilla was described by Wagner (1843), and *V. thyone* was originally described as a species by Thomas (1909), but the holotype of *V. pusilla* is a sub-adult male. For that reason, Goodwin (1963) recommended that *V. thyone* be considered a subspecies of *V. pusilla*, because he suggested that characters that distinguish both entities were related to age rather than geographic variables (Lim et al. 2003). Later, the work of Lim et al. (2003) re-evaluated the systematic relationship between *V. pusilla* and *V. thyone*. Based on quantitative mensural data, quantitative morphological characters, chromosomal differences, and mitochondrial synapomorphies, they recognized *V. thyone* as an independent and valid species, and here we are increasing the limited knowledge about the natural history of the Northern little yellow-eared bat, *V. thyone*.

Acknowledgments

We are grateful to M. Laura Ruiz-Vega, Martin Y. Cabrera-Garrido and Alfredo A. Gutiérrez-González for support during fieldwork, and with Joaquín Arroyo-Cabrales and three reviewers for his comments and criticisms. We are also grateful to local community authorities of Coacoyulichán for allowing us to conduct fieldwork. The first author is grateful to CONACyT and Posgrado en Ciencias Biológicas – UNAM for scholarships. This work was founded by the project CONACyT 239482.

Authors contribution

Giovani Hernández-Canchola: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Yire A. Gómez-Jiménez: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to manuscript preparation.

Iván Hernández-Chávez: Contribution to data collection; Contribution to manuscript preparation.

Sara Carolina Lucero-Verdugo: Contribution to manuscript preparation.

Livia León-Paniagua: Substantial contribution in the concept and design of the study; Contribution to manuscript preparation.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

BROSSET, A. & CHARLES-DOMINIQUE, P. 1990. The bats from French-Guiana: a taxonomic, faunistic and ecological approach. Mammalia 54(4):509–560.

BURGIN, C.J., COLELLA, J.P., KAHN, P.L. & UPHAM, N.S. 2018. How many species of mammals are there? J. Mammal. 99(1):1–14.

ESPINOSA-MARTÍNEZ, D. V., RÍOS-MUÑOZ, C.A., ROSALES NANDUCA, H., ARROYO-CABRALES, J. & LEÓN-PANIAGUA, L. 2017. Mamíferos de Guerrero. Rev. Mex. Mastozoología Nueva época 7(2):38–67.

GOODWIN, G.G. 1963. American bats of the genus *Vampyressa*, with the description of a new species. Am. Museum Novit. 2125:1–24.

GREENHALL, A.M. & SCHUT JR., W.A. 1996. *Diaemus youngi*. Mamm. Species 533:1–7.

HERNÁNDEZ-CANCHOLA, G. & LEÓN-PANIAGUA, L. 2017. Genetic and ecological processes promoting early diversification in the lowland Mesoamerican bat *Sturnira parvidens* (Chiroptera: Phyllostomidae). Mol. Phylogenet. Evol. 114:334–345.

HOOFER, S.R. & BAKER, R.J. 2006. Molecular systematics of Vampyressine bats (Phyllostomidae: Stenodermatinae) with comparison of direct and indirect surveys of mitochondrial DNA variation. Mol. Phylogenet. Evol. 39(2):424–438.

LANFEAR, R., FRANDSEN, P.B., WRIGHT, A.M., SENFELD, T. & CALCOTT, B. 2017. PartitionFinder 2: new methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Mol. Biol. Evol. 34(3):772–773.

LIM, B.K., PEDRO, W.A. & PASSOS, F.C. 2003. Differentiation and species status of the Neotropical yellow-eared bats *Vampyressa pusilla* and *V. thyone* (Phyllostomidae) with a molecular phylogeny and review of the genus. Acta Chiropterologica 5(1):15–29.

LUCATI, F. & LÓPEZ-BAUCELLS, A. 2017. Chromatic disorders in bats: a review of pigmentation anomalies and the misuse of terms to describe them. Mamm. Rev. 47(2):112–123.

PACHECO R., J. 2014. *Chiroderma villosum* Peters, 1860. In Mammals of Mexico (G. Ceballos, ed.) Johns Hopkins University Press, Baltimore, Maryland, p.730–731.

PORTER, C.A. & BAKER, R.J. 2004. Systematics of *Vampyressa* and related genera of phyllostomid bats as determined by *cytochrome-b* sequences. J. Mammal. 85(1):126–132.

REID, F.A. 2009. A field guide to the mammals of Central America and Southeast Mexico. Oxford University Press, New York, New York.

RONQUIST, F., TESLENKO, M., VAN DER MARK, P., AYRES, D.L., DARLING, A., HÖHNA, S., LARGET, B., LIU, L., SUCHARD, M.A. & HUELSENBECK, J.P. 2012. Mrbayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61(3):539–542.

ROSE, A., BRÄNDEL, S.D., CVECKO, P., ENGLER, S., HILLER, T., KNÖRNSCHILD, M. & TSCHAPKA, M. 2017. New records of hypopigmentation in two neotropical phyllostomid bat species with different roosting habits (*Uroderma bilobatum*, *Glossophaga soricina*). Mammalia 81(6):615–619.

SÁNCHEZ-HERNÁNDEZ, C., ROJAS-MARTÍNEZ, A.E., LÓPEZ-VIDAL, J.C., ELIZALDE-ARELLANO, C., ROMERO-ALMARAZ, M. de L., AGUILLAR-LÓPEZ, M. & TABOADA-SALGADO, A. 2012. Leucism in five species of bats from Mexico. Chiropt. Neotrop. 18(2):1123–1127.

SEMARNAT. 2010. Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. D. Of. la Fed. 30 diciemb1–78.

SIKES, R.S., GANNON, W.L. & THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGIST. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. J. Mammal. 92(1):235–253.

SMN. 2009. Información climatológica por estado: Guerrero, Coacoyulichan, Cuautepec. http://smn.cna.gob.mx/es/informacion-climatologica-ver-estado?estado=gro Acceced on 26 March 2018.

TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A. & KUMAR, S. 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30(12):2725–2729.

TAVARES, V. da C., GARDNER, A.L., RAMÍREZ-CHAVES, H.E. & VELAZCO, P.M. 2014. Systematics of *Vampyressa melissa* Thomas, 1926 (Chiroptera: Phyllostomidae), with descriptions of two new species of *Vampyressa*. Am. Museum Novit. 3813:1–27.

TAVARES, V. da C., MUÑOZ, A. & ARROYO-CABRALES, J. 2015. *Vampyressa thyone*. The IUCN red list of threatened species 2015: e.T136671A21989318. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T136671A21989318.en. Acceced on 04 April 2018.

TELLEZ-GIRON, G. 2014. *Platyrrhinus helleri* (Peters, 1866). In Mammals of Mexico (G. Ceballos, ed.) Johns Hopkins University Press, Baltimore, Maryland, p.736–737.

TÉLLEZ-GIRÓN, G. 2014. *Vampyressa thyone* Thomas, 1909. In Mammals of Mexico (G. Ceballos, ed.) Johns Hopkins University Press, Baltimore, Maryland.

VELAZCO, P.M. & PATTERSON, B.D. 2008. Phylogenetics and biogeography of the broad-nosed bats, genus *Platyrrhinus* (Chiroptera: Phyllostomidae). Mol. Phylogenet. Evol. 49(3):749–759.

ZALAPA, S.S., GUERRERO, S., ROMERO-ALMARAZ, M. de L. & SÁNCHEZ-HERNÁNDEZ, C. 2016. Coloración atípica en murciélagos: frecuencia y fenotipos en Norte y Centroamérica e islas del Caribe y nuevos casos para México y Costa Rica. Rev. Mex. Biodivers. 87:474–482.

Received: 02/08/2018
Revised: 17/01/2019
Accepted: 11/02/2019
Published online: 25/02/2019

Biota Neotropica 19(2): e20180645, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

News from the west: the orchid bees from Parque Nacional do Iguaçu, Paraná, Brazil (Hymenoptera, Apidae, Euglossina)

Luiz R. R. Faria [1,2]*, ***Betânia Cristina Neves*** [1], ***Araceli Judith Arce*** [1], ***Maria Noel Clerici Hirschfeld*** [1,3] **&**

Fernando César Vieira Zanella [1,2]

[1] *Universidade Federal da Integração Latino-Americana, Instituto Latino-Americano de Ciências da Vida e da Natureza, Foz do Iguaçu, PR, Brasil*
[2] *Universidade Federal da Integração Latino-Americana, Programa de Pós-Graduação em Biodiversidade, Foz do Iguaçu, PR, Brasil*
[3] *Universidade Federal de Pernambuco, Programa de Pós-Graduação em Biologia Animal, Recife, PE, Brasil*
**Corresponding author: Luiz R. R. Faria, e-mail: nunofariajr@gmail.com*

FARIA, L.R.R.; NEVES, B.C.; ARCE, A.J.; HIRSCHFELD, M. N. C.; ZANELLA, F. C. V. **News from the west: the orchid bees from Parque Nacional do Iguaçu, Paraná, Brazil (Hymenoptera, Apidae, Euglossina)**. Biota Neotropica. 19(2): e20180645. http://dx.doi.org/10.1590/1676-0611-BN-2018-0645

Abstract: Orchid bees were surveyed at Parque Nacional do Iguaçu, the largest remnant of Seasonal Semideciduous Forest in Brazil. Seven species were collected, and *Eufriesea violacea* (Blanchard) was the most common species, followed by *Euglossa annectans* Dressler. The observed species richness and species composition agree with what is known for the local euglossine faunas in fragments of Seasonal Semideciduous Forest of southern and southeastern Brazil. On the other hand, the abundance of individuals was remarkably low. The occurrence of a severe winter in 2013 and the collection of several males in other fragments suggest that this noteworthy low abundance can be fortuitous.

Keywords: *inventory, species list, Iguazu, Euglossini, Seasonal Semideciduous Forest, Atlantic forest.*

Notícias do oeste: as abelhas-das-orquídeas do Parque Nacional do Iguaçu, Paraná, Brasil (Hymenoptera, Apidae, Euglossina)

Resumo: Abelhas-das-orquídeas foram amostradas no Parque Nacional do Iguaçu, o maior fragmento de Floresta Estacional Semidecidual do Brasil. Sete espécies foram coletadas e *Eufriesea violacea* (Blanchard) foi a espécie mais comum, seguida por *Euglossa annectans* Dressler. A riqueza e composição de espécies observadas concordam com o que se conhece para as faunas locais de abelhas euglossinas em fragmentos de Floresta Estacional Semidecidual. Por outro lado, a abundância de indivíduos foi notavelmente baixa. A ocorrência de um inverno severo em 2013 e a coleta de muitos machos em outros fragmentos sugerem que tal baixa abundância pode ser fortuita.

Palavras-chave: *inventário, lista de espécies, Iguaçu, Euglossini, Floresta Estacional Semidecidual, Mata Atlântica.*

Introduction

Orchid bees are a primarily Neotropical and monophyletic group of bees (e.g Ramírez et al. 2010) distributed from southern United States (Skov & Wiley 2005) to southern Brazil (Wittmann et al. 1988) and northern and central Argentina (Pearson & Dressler 1985; Cordoba, Central Argentina; FCVZ, personal observation). The importance of these bees in the dynamics of forestry ecosystems are fairly recognized for their role as pollinators of several plant clades, mainly Orchidaceae, Gesneriaceae, Araceae, Euphorbiaceae, Apocynaceae and Solanaceae (e.g. Ramírez et al. 2002, Cameron 2004). These bees are closely related to forests (e.g. Moure 1967) and are by far recognized as key elements in Neotropical forests (Dodson et al. 1969). Approximately 60 species are known to occur in the Atlantic Forest domain (Nemésio 2009, Moure et al. 2012). But, despite the considerable number of inventories carried out in this region, most of these studies are concentrated in coastal areas of Brazil and neighboring sites (e.g. Sydney et al. 2010, see Gonçalves et al. 2014). This geographical caveat implies in a more limited appraisal of the patterns of species richness, diversity and composition of orchid bee faunas of interior forests, which seems even more relevant when one considers the existence of a significant distinction between the faunas of interior and coastal Atlantic Forest (Nemésio & Silveira 2007, Nemésio 2009, Sydney et al. 2010). Regarding the Semideciduous Seasonal forests (from now on, SSF) of southern Brazil, efforts in systematic inventorying orchid bees are quite punctual, restricted to studies in small fragments of up to 680 ha, in the region of Londrina (Santos & Sofia 2002, Sofia & Suzuki 2004, Sofia et al. 2004, Ferronato et al. 2018), Telêmaco Borba (Giangarelli et al. 2015) and Palotina (Gonçalves et al. 2014), all in Paraná state.

In this context, the Parque Nacional do Iguaçu (PNI) could be considered a key area, in view of its status as the largest fragment of Atlantic Forest in southern Brazil (see Ribeiro et al. 2009), encompassing ca. 185,000 ha in western Paraná state (Conforti & Azevedo 2003), and its position near the western boundaries of the hotspot (e.g. Paviolo et al. 2016). It is worth to mention that PNI, Iguazú National Park, Urugua-í Provincial Park, and eight other smaller private and provincial reserves in Argentina correspond to a continuous protected area of 340,800 ha (Di Bitetti et al. 2003) separated only by the Iguaçu river. Previous studies in areas of SSF in southern Brazil have suggested that the local orchid bee richness ranges from seven to nine species; see Santos & Sofia 2002, Sofia & Suzuki 2004, Sofia et al. 2004, Giangarelli et al. 2015, Gonçalves et al. 2014, Ferronato et al. 2018). If we consider that fragmentation could be affecting the orchid bee species in the region (Sofia & Suzuki 2004, Giangarelli et al. 2009), it seems reasonable to assume that the chance of collecting the species naturally inhabiting seasonal forests in southern Brazil would be higher at this large-sized forest fragment. Herein, we present data from a survey of orchid bees carried out at the Parque Nacional do Iguaçu, Brazil.

Material and Methods

1. *Study site*

Fieldwork was carried out in the Parque Nacional do Iguaçu (PNI), located in the Triple Frontier (Argentina, Brazil and Paraguay) region. PNI is the largest national conservation unity of Brazil within the Atlantic Forest domain and comprises areas of SSF (most part of the Park) and Mixed Ombrophilous forests (restricted to areas with higher elevational quotas, at the western part of the park) (see Cervi & Borgo 2007).

The climate in the region is the Cfa of Köppen, subtropical, mesothermal, with average temperature in the hottest month >22°C, average temperature in the coldest month <18°C, hot summers, uncommon frosts, tendency of rainfall concentration in the summer months but without a defined dry season (IAPAR 1994). Annual average rainfall is about 1,700 mm and daily relative humidity is around 80% (Salamuni et al. 2002).

2. *Data collection*

Two sample points, separated from each other by ca. 50 m, were established near the Poço Preto trail (25°37’31.9” S, 54°27’12.9” W, inside the forest, and 25°37’28.2” S, 54°27’11.0” near the border of the 2 m wide trail), a 9 km long trail located near the park administration in Foz do Iguaçu. The region of the Poço Preto trail was a farm about 30 years ago (Rodolfo et al. 2008). At each point two monthly samples (one day per sample) were carried out from November/2013 to June/2014. Seven aromatic compounds, known to be attractive to male orchid bees, were employed during fieldwork: 1,8-cineole, β-ionone, benzyl acetate, eugenol, methyl salicylate, trans-methyl cinnamate and vanillin. Two collecting methods were employed, (i) hand nets, where the collector remains on sampling site inspecting the baits and collecting any bee approaching the chemical lures, and (ii) bait traps, where a given aromatic compound is offered to bees inside traps and only the bees that enter and remain in the traps are actually collected. For active collection (hand nets), the seven aromatic baits, made of cotton waddings soaked with one of the presented substances, were placed ca. 1.5 m apart from each other and at ca. 1.5 m above the ground. Regarding scent traps, we used a modified version of traps previously presented (Mattozo et al. 2011, Gonçalves et al. 2014) and, in the same way, a single compound was placed at each trap. Traps were also arranged ca. 1.5 m apart from each other and ca. 1.5 m above the ground. These methods were alternated in the two monthly samplings carried out at each point, so that in the same month each point was assessed one way and the other. In all cases, daily sampling procedure was performed from 08:00 to 15:00 h. This way, a total sampling effort of 112 h was carried out considering both sampling points and methods.

A last relevant methodological detail regards that hourly replenishments have been made on 1,8-cineole baits due to its volatility.

Bees attracted to those lures were killed with ethyl acetate (in the case of traps after being gently removed from them) and kept apart in paper bags. Posteriorly, they were mounted in entomological pins for identification. Bees were identified with help of taxonomic keys (Rebêlo & Moure 1996, Faria & Melo 2007, Nemésio 2009) and by comparison with specimens previously identified by specialists. Taxonomy follows Moure et al. (2012) and the organization of the previous known distribution of species follows Zanella et al. (2000).

In the labels of examined specimens transcribed in the species list, quotations marks indicate distinct labels associated with a given specimen, and one inverted bar (\) indicates distinct lines in a label. When a label was very similar to other associated to a specimen, only the different information (e.g. date, scent, etc.) is presented. All the collected specimens are deposited in the entomological collection of the Universidade Federal da Integração Latino-Americana (UNILA), Foz do Iguaçu, Paraná, Brazil. The study was conducted under ICMBIO permit 41.140-1.

Results

A total of 60 specimens belonging to seven species was sampled in the Parque Nacional do Iguaçu (Table 1). The most common species was *Eufriesea violacea*, followed by *Euglossa annectans* and *E. pleosticta*.

The higher abundance of orchid bees, ca. 88% of the collected specimens, and all the seven species were collected from December to February. Moreover, the tripleton, the doubleton and all the singletons were recorded only in this period. *Eufriesea violacea,* the most common species, presented an even more restrict phenology, since it was only collected in November and December. On the other hand, only one species, *Euglossa annectans*, with only one individual, was collected between April and June (Table 2).

The most attractive scent was cineole, where ca. 53% of the specimens (32 individuals) were collected, followed by vanillin (21 specimens; 35%). All the species, excepting *Eufriesea* cfr. *auriceps*, were registered in cineole baits, and regarding the other employed attractants, two species were found in trans-methyl cynammate and vanillin, and only one species at β-ionona and eugenol. The two remaining scents, benzyl acetate and methyl salicylate, were not visited by any orchid bee (Table 1).

Table 1. Number of individuals of each species at an area of Seasonal Semideciduous Forest, Parque Nacional do Iguaçu, according to attractive baits. BI: β-ionone; C: 1,8-cineole; E: eugenol; MC: trans-methyl cinnamate; and V: vanillin. Benzyl acetate and methyl salicylate are not included as no specimens were collected at these two scents.

Species	**Attractant**					**Total**
	BI	**C**	**E**	**MC**	**V**	
Eufriesea violacea (Blanchard, 1840)	0	5	0	0	19	24
Euglossa (Glossura) annectans Dressler, 1982	4	16	1	0	2	23
Euglossa (Euglossa) pleosticta Dressler, 1982	0	6	0	0	0	6
Euglossa (Euglossa) cordata (Linnaeus, 1758)	0	2	0	1	0	3
Euglossa (Euglossa) fimbriata Moure, 1968	0	2	0	0	0	2
Euglossa (Euglossa) truncata Rebêlo & Moure, 1996	0	1	0	0	0	1
Eufriesea cfr. *auriceps* (Friese, 1899)	0	0	0	1	0	1
Total individuals/attractant	4	32	1	2	21	
Grand total individuals						60

Table 2. Number of individuals of each species at an area of Seasonal Semideciduous Forest, Parque Nacional do Iguaçu, collected by month.

Species	**Month**								**Total**
	Nov	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	
Eufriesea violacea (Blanchard, 1840)	1	23	0	0	0	0	0	0	24
Euglossa (Glossura) annectans Dressler, 1982	2	0	9	8	3	0	1	0	23
Euglossa (Euglossa) pleosticta Dressler, 1982	0	0	1	5	0	0	0	0	6
Euglossa (Euglossa) cordata (Linnaeus, 1758)	0	1	0	2	0	0	0	0	3
Euglossa (Euglossa) fimbriata Moure, 1968	0	1	0	1	0	0	0	0	2
Euglossa (Euglossa) truncata Rebêlo & Moure, 1996	0	1	0	0	0	0	0	0	1
Eufriesea cfr. *auriceps* (Friese, 1899)	0	1	0	0	0	0	0	0	1
Total individuals/attractant	3	27	10	16	3	0	1	0	
Grand total individuals									60

Species list

1. *Eufriesea cfr. auriceps* (Friese, 1899)

Distribution (for *Eufriesea auriceps*, see additional comments below). Brazil: Tocantins, Goiás, Mato Grosso do Sul, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina; Paraguay; Argentina: Misiones (Moure et al. 2012). Sofia et al. (2004) presented a record of a melanic *Eufriesea*, named by them as *E. auriceps,* collected in a fragment of semideciduous forest in northern Paraná state.

Examined material. 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cinam. Met.\ 25º37'28.2" 54º27'11.0"\ 01/XII/2013 207 m\ Arce leg.", "CE-UNILA\ HYEU 10025".

Additional information. As stated by G.A.R. Melo (unpublished, in Moure et al. 2012), "The systematics of the melanic forms in the *mussitans* group is far from being resolved (…) and the entire group needs a thorough revision, since many additional undescribed forms remain". It does not seem too much to say that any black-bodied *Eufriesea* in this group has, at the moment, an uncertain identity. The single male was collected in methyl trans-cynnamate.

2. *Eufriesea violacea* (Blanchard, 1840)

Distribution. Brazil: Goiás, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul; Paraguay; Argentina: Catamarca, Córdoba, Misiones, Tucumán (Moure et al. 2012).

Examined material. 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Vanilina\ 25º37'28.2" 54º27'11.0"\ 15/XI/2013 207 m\ Clerici leg.", "CE-UNILA\ HYEU 10019"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Vanilina\ 25º37'31.9" 54º27'12.9"\ 1/XII/2013 194 m\ Faria & Neves leg.", "CE-UNILA\ HYEU 10001"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Vanilina\ 25º37'28.2" 54º27'11.0"\ 1/XII/2013 207 m\ Arce leg.", "CE-UNILA\ HYEU 10002"; 1 male, idem, except "10003"; 1 male, idem, except "10004"; 1 male, idem, except "10005"; 1 male, idem, except "10006"; 1 male, idem, except "10007"; 1 male, idem, except "10008"; 1 male, idem, except "10009"; 1 male, idem, except "10011"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cineol\ 25º37'28.2" 54º27'11.0"\ 1/XII/2013 207 m\ Arce leg.", "CE-UNILA\ HYEU 10013"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37'31.9" 54º27'12.9"\ 1/XII/2013 194 m\ Faria & Neves leg.”, “CE-UNILA\ HYEU 10010”; 1 male, idem, except “10012”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37’28.2” 54º27’11.0”\ 5/XII/2013 207 m\ Neves leg.”, “CE-UNILA\ HYEU 10014”; 1 male, idem, except “10017”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Vanilina\ 25º37’28.2” 54º27’11.0”\ 5/XII/2013 207 m\ Neves leg.”, “CE-UNILA\ HYEU 10020”; 1 male, idem, except “10021”; 1 male, idem, except “10022”; 1 male, idem, except “10023”; 1 male, idem, except “10024”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Arm Vanilina\ 25º37’31.9” 54º27’12.9”\ 5/XII/2013 194 m\ Faria leg.”, “CE-UNILA\ HYEU 10015”; 1 male, idem, except “10016”; 1 male, idem, except “10018”.

Additional information. *Eufriesea violacea* is regarded as a species typical of the semideciduous forests of the interior of the Atlantic forest (Nemésio 2009). Moreover, in some fragments of semideciduous forests of southern Brazil, this species appears as the dominant element of the local faunas (e.g. Sofia & Suzuki 2002, Gonçalves et al. 2014). A relevant characteristic of species of the genus *Eufriesea* regards the fact that most species has their activities restricted to some few months in the rainy season (e.g. Kimsey 1982). Males were collected in cineole and vanillin.

3. *Euglossa (Glossura) annectans* Dressler, 1982

Distribution. Brazil: Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo Paraná, Santa Catarina, Rio Grande do Sul; Paraguay; Argentina: Misiones (Faria & Melo 2007, Moure et al. 2012). Records in semideciduous forests of Paraná are provided by Giangarelli et al. (2015), Gonçalves et al. (2014) and Ferronato et al. (2018).

Examined material. 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Arm B-ionona\ 25º37’31.9” 54º27’12.9”\ 02/XI/2013 194 m\ Zanella leg.”, “CE-UNILA\ HYEU 10046”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cineol\ 25º37’28.2” 54º27’11.0”\ 15/XI/2013 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10047”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37’31.9” 54º27’12.9”\ 10/I/2014 194 m\ Faria leg.”, “CE-UNILA\ HYEU 10040”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Eugenol\ 25º37’31.9” 54º27’12.9”\ 10/I/2014 194 m\ Faria leg.”, “CE-UNILA\ HYEU 10041”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cineol\ 25º37’28.2” 54º27’11.0”\ 10/I/2014 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10042”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede B-ionona\ 25º37’28.2” 54º27’11.0”\ 21/I/2014 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10037”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37’28.2” 54º27’11.0”\ 21/I/2014 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10038”; 1 male, idem, except “10035”; 1 male, idem, except “10039”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Vanilina\ 25º37’28.2” 54º27’11.0”\ 21/I/2014 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10034”; 1 male, idem, except “10036”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cineol\ 25º37’28.2” 54º27’11.0”\ 18/II/2014 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10031”; 1 male, idem, except “10033”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37’31.9” 54º27’12.9”\ 18/II/2014 194 m\ Arce leg.”, “CE-UNILA\ HYEU 10032”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede B-ionona\ 25º37’28.2” 54º27’11.0”\ 19/II/2014 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10026”; 1 male, idem, except “10030”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Arm Cineol\ 25º37’31.9” 54º27’12.9”\ 19/II/2014 194 m\ Clerici leg.”, “CE-UNILA\ HYEU 10027”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37’28.2” 54º27’11.0”\ 19/II/2014 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10028”; 1 male, idem, except “10029”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37’28.2” 54º27’11.0”\ 16/III/2014 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10043”; 1 male, idem, except “10045”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Arm Cineol\ 25º37’31.9” 54º27’12.9”\ 16/III/2014 194 m\ Zanella leg.”, “CE-UNILA\ HYEU 10044”; 1 male, “PNI Arm\ Cineol\ 18-05-2014\ Zanella\ (annectans)”, “CE-UNILA\ HYEU 10048”.

Additional information. As stated by Faria & Melo (2007), available locality records indicate that *E. annectans* is restricted to the southern portion of the Atlantic forest in Brazil, including the inland forests of the Paraná basin, south to Misiones, in Argentina. The species can be found in coastal areas (from Rio de Janeiro to Santa Catarina states) and, regarding inland areas of southeastern Brazil, *E. annectans* have been found both in semideciduous forests and savannas (see Faria & Melo 2007 for details). Males were collected in β-ionona, cineole, eugenol and vanillin baits.

4. *Euglossa (Euglossa) cordata* (Linnaeus, 1758)

Distribution. Mexico; Guatemala; Honduras; Costa Rica; Panama; Jamaica; Trinidad and Tobago; Colombia; Venezuela; Guyana; Suriname; French Guiana; Ecuador; Brazil: Amapá, Pará, Amazonas, Maranhão, Ceará, Paraíba, Alagoas, Sergipe, Bahia, Goiás, Minas Gerais, Espírito Santo, Pernambuco, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul; Paraguay (Moure et al. 2012). Besides these countries presented by Moure et al. (2012), Bembé (2007) also provided a record of the species in Bolivia. Records regarding specifically the semideciduous forests of Paraná are presented by Sofia & Suzuki (2004), Sofia et al. (2004), Giangarelli et al. (2015), Gonçalves et al. (2014) and Ferronato et al. (2018).

Examined material. 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37’31.9” 54º27’12.9”\ 1/XII/2013 194 m\ Faria & Neves leg.”, “CE-UNILA\ HYEU 10057”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37’28.2” 54º27’11.0”\ 19/II/2014 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10060”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cinam. Met.\ 25º37’28.2” 54º27’11.0”\ 19/II/2014 207 m\ Clerici leg.”, “CE-UNILA\ HYEU 10053”.

Additional information. Males were collected in cineole and trans-methyl cinnamate.

5. *Euglossa (Euglossa) fimbriata* Moure, 1968

Distribution. Brazil: Maranhão, Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo; Bolivia: Santa Cruz (Moure et al. 2012). Nemésio (2009) stated that the species also reaches Paraíba and Pernambuco states, northwards in the Brazilian Atlantic forest, and the Maranhão state. Records in the semideciduous forests of Paraná were provided by Sofia & Suzuki (2004), Sofia et al. (2004), Giangarelli et al. (2015), Gonçalves et al. (2014) and Ferronato et al. (2018).

Examined material. 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1A Arm Cineol\ 25º37’28.2” 54º27’11.0”\ 01/XII/2013 207 m\ Arce leg.”, “CE-UNILA\ HYEU 10058”; 1 male “Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37’31.9” 54º27’12.9”\ 18/II/2014 194 m\ Arce leg.”, “CE-UNILA\ HYEU 10059”.

Additional information. The two males were collected in cineole.

6. *Euglossa (Euglossa) pleosticta* Dressler, 1982

Distribution. Brazil: Bahia, Minas Gerais, Espírito Santo, Rio de Janeiro, São Paulo (Moure et al., 2012). Nemésio (2012) have also presented records for the states of Maranhão, Alagoas, Sergipe and Paraná. Regarding specifically the Paraná state, the species was recorded both in semideciduous forests (Sofia & Suzuki 2004; Sofia et al. 2004; Giangarelli et al. 2015, Gonçalves et al. 2014 and Ferronato et al. 2018) and in coastal ombrophilous forests (Mattozo et al. 2011; Giangarelli et al. 2015).

Examined material. 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37'31.9" 54º27'12.9"\ 10/I/2014 194 m\ Faria leg.", "CE-UNILA\ HYEU 10056"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1B Rede Cineol\ 25º37'31.9" 54º27'12.9"\ 18/II/2014 194 m\ Arce leg.", "CE-UNILA\ HYEU 10054"; 1 male, idem, except "10055"; 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37'28.2" 54º27'11.0"\ 19/II/2014 207 m\ Clerici leg.", "CE-UNILA\ HYEU 10051"; 1 male, idem, except "10050"; 1 male, idem, except "10052".

Additional information. *Euglossa pleosticta* seems to be more common in areas in the drier portions of the interior of the Atlantic Forest (e.g. Rebêlo & Garófalo 1997). *Euglossa pleosticta* appears as the most common species in fragments of semideciduous forests in the interior of São Paulo state (Rebêlo & Garófalo 1997). The species is also very common in semideciduous forests of Paraná (Sofia & Suzuki 2004, Sofia et al. 2004). Nemésio (2007, 2009, 2012) have considered this species inhabiting the Amazon Basin (records for the Brazilian states of Amazonas and Acre). However, we follow here the interpretation of Moure et al. (2012) considering *E. pleosticta* as a species endemic to the Atlantic forest. Males were collected in cineole baits.

7. *Euglossa (Euglossa) truncata* Rebêlo & Moure, 1996

Distribution. Brazil: Maranhão, Bahia, Minas Gerais, São Paulo, Paraná (Moure et al. 2012). Nemésio (2009, 2012) have also considered the occurrence of this species in the states of Espírito Santo and Rio de Janeiro, in Brazil, and have presented records of the species in Argentina (province of Misiones). *Euglossa truncata* is also a very common element in the local faunas of orchid bees in inland areas of the Atlantic forest. In the Paraná state, *Euglossa truncata* was recorded by Sofia & Suzuki (2004), Sofia et al. (2004), Giangarelli et al. (2015) and Ferronato et al. (2018). It is noteworthy that Gonçalves et al. (2014) did not collect the species in nearby Palotina.

Examined material. 1 male "Brasil, PR, Foz do Iguaçu\ PNI 1A Rede Cineol\ 25º37'28.2" 54º27'11.0"\ 5/XII/2014 207 m\ Arce leg.", "CE-UNILA\ HYEU 10049".

Additional information. The single male was collected in a cineole bait.

Discussion

Data collected at the Parque Nacional do Iguaçu revealed an orchid bee species richness comparable to what have been found elsewhere in seasonal semideciduous areas in southern Brazil and, with regard to species composition, results also suggested that the local orchid bee fauna of PNI comprises elements that are very common in these areas (Sofia & Suzuki 2004, Sofia et al. 2004, Giangarelli et al. 2015, Gonçalves et al. 2014, Ferronato et al. 2018). On the other hand, the remarkably low abundance of orchid bees collected at PNI draws most attention.

An additional orchid bee species, *Euglossa* (*Euglossella*) *mandibularis* Friese, 1899 is known to inhabit Parque Nacional do Iguaçu (Faria & Zanella 2015), bringing the number of species found in PNI to eight. This value is comparable to the orchid bee richness assessed in other SSF fragments in southern Brazil (Paraná state), e.g. in the region of Londrina, where species richness ranged from five to nine (Sofia & Suzuki 2004, Sofia et al. 2004, Ferronato et al. 2018), Telêmaco Borba (seven species; Giangarelli et al. 2015) and Palotina (seven species; Gonçalves et al. 2014) (Table 3). When comparing these values to the species richness found in lower latitude sites of SSF in southeastern Brazil, where species richness ranges from ten up to 14 species (Rebêlo & Garófalo 1997, Silveira et al. 2011, Mateus et al. 2015) (Table 3), it is possible to perceive the alleged impoverishment of orchid bee faunas southwards in the SSF (see e.g. Sydney et al. 2010), which seems even more noticeable when considering the poor local faunas of areas farther south in Brazil (Wittmann et al. 1988).

Regarding species composition, it is possible to realize that the species found at PNI are, for the most part, the species commonly collected in assessments carried out in SSF areas in southern Brazil. The only species that was collected in PNI but is rare in other surveys employing aromatic attractants in the Atlantic forest is *Euglossa mandibularis* Friese. As far as we know, besides Faria and Zanella (2015), only Costa and Francoy (2017) report the attraction of this species to aromatic baits (even if the scent attracting the species was not identified in this latter case). However, *E. mandibularis* is widely distributed in southeastern and southern Brazil, southern Paraguay and in the provinces of Corrientes and Misiones in Argentina (see Faria & Zanella 2015). The occurrence of *E. mandibularis* at PNI seems to regard much more a spatial variation concerning the attractiveness of some aromatic compounds to males than any geographical restriction.

Some species that have commonly been found in the surveys carried out in SSF areas in Southern Brazil was not collected in PNI. The most noticeable case regards *Eulaema nigrita* Lepeletier, 1841 that was surveyed in all the other studies in SSF areas in Paraná state (Sofia & Suzuki 2004, Sofia et al. 2004, Giangarelli et al. 2015, Gonçalves et al. 2014, Ferronato et al. 2018) and is one of the most widespread euglossine species in the Neotropical region, whose distribution stands from Costa Rica to northern Argentina (e.g. Nemésio 2009). It is important to emphasize that this species is one of the most common elements in some local faunas in SSF areas in southern Brazil, the dominant species in a fragment in Telêmaco Borba (Giangarelli et al. 2015), and the second most common species in a 680 ha fragment in Londrina (Sofia et al. 2004).

Other three species recorded in these studies were also not collected in PNI, *Exaerete smaragdina* (Guérin, 1844), *Euglossa melanotricha* Moure, 1967 and *Euglossa townsendi* Cockerell, 1904 (see Sofia & Suzuki 2004, Sofia et al. 2004, Gonçalves et al. 2014 and Ferronato et al. 2018). But, unlike *E. nigrita*, these species have always been sampled with a very low number of individuals. Regarding *E. smaragdina*, nine and five individuals were assessed in two fragments in northern Paraná state, in the region of Londrina by Ferronato et al. (2018). In the other two studies where the species was recorded, it appears as a singleton in fragments in Londrina (Sofia & Suzuki 2004) and Palotina (Gonçalves et al. 2014). The other two species, *E. melanotricha* and *E. townsendi*, seem to be a common element in the local faunas of northern Paraná, although infrequent in the sites they occur (Sofia et al. 2004, Ferronato et al. 2018).

Table 3. Surveys of orchid bee assemblages carried out in Seasonal Semideciduous Forest areas in the states of Paraná and São Paulo.

Locality	Latitude (Degrees and minutes)	Area description	Sampling effort (h)/number of compounds employed	Species richness	References
Cajuru, SP	21°20' - 21°27' S	A fragment of ca. 99 ha	108/3	14	Rebêlo & Garófalo 1997
Patrocínio Paulista, SP	20°46' S	A fragment of 49 ha	48/3	13	Silveira et al. 2011
Pedregulho, SP	20°14' S	Parque Estadual Furnas do Bom Jesus, 2,070 ha	36/3	12	Mateus et al. 2015
Londrina, PR	23°27' S*	Parque Estadual Mata dos Godoy, ca. 680 ha*, a fragment of 86 ha, a fragment of 8.5 ha and a reforested area of 20 ha	276/8**	11	Sofia et al. 2004, Sofia & Suzuki 2004 and Ferronato et al. 2018
Sertãozinho, SP	21°8 S'	A fragment of ca. 75 ha	108/3	10	Rebêlo & Garófalo 1997
Alvorada do Sul, PR	22°49' S	A forest fragment of 128.1 ha and a reforested area of 11.3 ha	72/8	9	Ferronato et al. 2018
Primeiro de Maio, PR	22°46' S	A forest fragment of 32.1 ha and a reforested area with 33.3 ha	72/8	8	Ferronato et al. 2018
Rancho Alegre, PR	22°59' S	A forest fragment of 108.0 ha and a reforested area of 11.8 ha	72/8	8	Ferronato et al. 2018
Telêmaco Borba, PR	24°12' S	A fragment of ca. 217 ha	76/8	7	Giangarelli et al. 2015
Palotina, PR	24°18' S	Parque Estadual São Camilo, ca. 386 ha	54/3	7	Gonçalves et al. 2014
Foz do Iguaçu, PR	25°37' S	Parque Nacional do Iguaçu, ca. 185,000 ha; see methods	112/7	7	This study

* Latitude and total area of Mata dos Godoy according to Sofia et al. 2004; ** Combined sampling effort and number of scents.

Considering that in PNI most of species was also represented by few individuals, it seems reasonable to state that most of the difference regarding the local faunas in the SSF of southern Brazil regards the presence of these locally rare species. It seems also possible to hypothesize that many of these species have naturally low populations in southern Brazil, since the region is likely to represent the limit (or at least close to it) of their distributions (for a discussion on the population size towards species range limits see Gaston 2009). If this is the case, we could expect that a long-term survey in PNI could also reveal the occurrence of these nowadays unrecorded species in this large remnant.

With respect to species abundance, results found in PNI are noteworthy. A rapid comparison among the number of individuals collected per sampling hour in assessments in SSF areas in southern Brazil (Sofia & Suzuki 2004: 12.66, 3.75 and 4.00 [sampling was carried out in three fragments]; Sofia et al. 2004: 2.58; Giangarelli et al. 2015: 3.50; Gonçalves et al. 2014: 3.44; Ferronatto et al. 2018: 1.25, 0.75, 0.85, 0.61, 0.98, 0.11, 0.70 and 0.78 [sampling was carried out in six fragments; as they also have used both hand nets and traps simultaneously, we consider duplicate number of sampling hours] shows how few specimens were collected in PNI (this work: 0.53). In comparison to our results, the only lower number of individuals per sampling hour was found by Ferronato et al. (2018) in a 33 ha reforested area in Primeiro de Maio, northern Paraná.

But it seems to us that this remarkably low abundance in PNI is fortuitous, at least regarding *Eufriesea violacea* a common species in semideciduous forests. There are two main reasons leading us to this interpretation: (i) the occurrence in 2013 of a winter with more severe cold events than usual in southern Brazil (see Dolif Neto et al. 2016), could lead to higher mortality of bees (e.g. Bosch & Kemp 2003). This could be particularly relevant for the most common species at PNI, *Eufriesea violacea*, a univoltine species whose adults emerge in September-October (Wittmann et al. 1989, Peruquetti & Campos 1997); (ii) surveys carried out in western Paraná in subsequent years, even in small fragments, have resulted in the collection of several individuals of *Eufriesea violacea* inclusive along a single day (F.C.V. Zanella, pers. obs.).

Results found in PNI reinforces some observations on what seems to be the main patterns regarding the local faunas of orchid bees in SSF of southern Brazil: (i) species richness with values commonly ranging from seven to nine species, with decreasing values along the latitudinal gradient (mainly when compared to assessments carried out in southeastern Brazil); (ii) local faunas consisting of widespread species (e.g. *Eulaema nigrita* and *Euglossa cordata*) and species “typical of the semideciduous forests of the interior” (*sensu* Nemésio 2009) (e.g. *Euglossa pleosticta* and *Eufriesea violacea*); (iii) marked seasonality; (iv) most species presenting low populations and possibly with local extinction-colonization dynamics.

Acknowledgements

We thank Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) for providing the collection permit. We are indebted to Raphael Xavier and all the staff in Parque Nacional do Iguaçu for all the support before and during field work. We acknowledge UNILA for all the facilities and for scientific initiation scholarship for BCN, AJA and MNCH.

Author Contributions

Luiz Roberto Ribeiro Faria: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; contribution to manuscript preparation.

Betânia Cristina Neves: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content.

Araceli Judith Arce: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content.

Maria Noel Clerici Hirschfeld: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content.

Fernando Fernando César Vieira Zanella: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content; contribution to manuscript preparation.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

BEMBÉ, B. 2007. Revision der *Euglossa cordata*-Gruppe und Untersuchungen zur Funktionsmorphologie und Faunistik der Euglossini (Hymenoptera, Apidae). Entomofauna, Supplement, 14:1-146.

BOSCH J. & KEMP, W.P. 2003. Effect of wintering duration and temperature on survival and emergence time in males of the orchard pollinator *Osmia lignaria* (Hymenoptera: Megachilidae). Environ. Entomol. 32(4):711-716.

CAMERON, S.A. 2004. Phylogeny and biology of neotropical orchid bees (Euglossini). Annu. Rev. Entomol. 49:377-404.

CERVI, A.C. & BORGO, M. 2007. Epífitos vasculares no Parque Nacional do Iguaçu, Paraná (Brasil). Levantamento preliminar. Fontqueria 55(51):415-422.

CONFORTI, V.A. & AZEVEDO, F.C.C. 2003. Local perceptions of jaguars (*Panthera onca*) and pumas (*Puma concolor*) in the Iguaçu National Park area, south Brazil. Biol. Conserv. 111(2):215-221.

COSTA, C.P. & FRANCOY, T.M. 2017. The Impact of Different Phytophysiognomies on the Composition of Orchid Bee Communities (Hymenoptera: Apidae: Euglossini) in the Atlantic Forest in Brazil. Ann. Entomol. Soc. Am. 110(3): 255-262.

DI BITETTI, M.S., PLACCI, G. & DIETZ, L.A. 2003. A biodiversity vision for the Upper Paraná Atlantic Forest Ecoregion: designing a biodiversity conservation landscape and setting priorities for conservation action. World Wildlife Fund, Washington, D.C.

DODSON, C.H., DRESSLER, R.L., HILLS, H.G., ADAMS, R.M. & WILLIAMS, N.H. 1969. Biologically active compounds in orchid fragrances. Science 164(3885):1243-1249.

DOLIF NETO, G., MARKET, P.S., PEZZA, A.B., MORALES RODRIGUEZ, C.A., CALVETTI, L., DA SILVA DIAS, P.L. & ESCOBAR, G.C.J. 2016. Thundersnow in Brazil: a case study of 22 July 2013. Atmos. Sci. Lett. 17(1):26-32.

FARIA, L.R.R. & MELO, G.A.R. 2007. Species of *Euglossa* (*Glossura*) in the Brazilian Atlantic forest, with taxonomic notes on *Euglossa stellfeldi* Moure (Hymenoptera, Apidae, Euglossina). Rev. Bras. Entomol. 51(3):275-284.

FARIA, L.R.R. & ZANELLA, F.C.V. 2015. Beta-ionone attracts *Euglossa mandibularis* (Hymenoptera, Apidae) males in western Paraná forests. Rev. Bras. Entomol. 59(3):260-264.

FERRONATO, M.C.F., GIANGARELLI, D.C., MAZZARO, D., UEMURA, N. & SOFIA, S.H. 2018. Orchid bee (Apidae: Euglossini) communities in Atlantic forest remnants and restored areas in Paraná state, Brazil. Neotrop. Entomol. 47(3):352-361.

GIANGARELLI, D.C., FREIRIA, G.A., COLATRELI, O.P., SUZUKI, K.M. & SOFIA, S.H. 2009. *Eufriesea violacea* (Blanchard) (Hymenoptera: Apidae): an orchid bee apparently sensitive to size reduction in forest patches. Neotrop. Entomol. 38(5):610-615.

GIANGARELLI, D.G., AGUIAR, W.M. & SOFIA, S.H. 2015. Orchid bee (Hymenoptera: Apidae: Euglossini) assemblages from three different threatened phytophysiognomies of the subtropical Brazilian Atlantic forest. Apidologie 46(1):71-83.

GONÇALVES, R.B., SCHERER, V.L. & OLIVEIRA, P.S. 2014. The orchid bees (Hymenoptera, Apidae, Euglossina) in a forest fragment from western Paraná state, Brazil. Pap. Avulsos Zool. 54(6):63-68.

IAPAR (Instituto Agronômico do estado do Paraná). 1994. Cartas climáticas do Estado do Paraná. IAPAR, Londrina.

KIMSEY, L.S. 1982. Systematics of bees of the genus *Eufriesea*. Univ. Calif. Publ. Entomol. 95:1-125.

MATEUS, S., ANDRADE-SILVA, A.C.R. & GARÓFALO, C.A. 2015. Diversity and temporal variation in the orchid bee community (Hymenoptera: Apidae) of a remnant of a Neotropical seasonal semi-deciduous forest. Sociobiology 62(4):571-577.

MATTOZO, V.C., FARIA, L.R.R. & MELO, G.A.R. 2011. Orchid bees (Hymenoptera: Apidae) in the coastal forests of southern Brazil: diversity, efficiency of sampling methods and comparison with other Atlantic forest surveys. Pap. Avulsos Zool. 51(33):505-515.

MOURE, J.S. 1967. A checklist of the known euglossine bees. Atas Simp. Biota Amaz. 5:395-415.

MOURE, J.S., MELO, G.A.R. & FARIA, L.R.R. 2012. Euglossini Latreille, 1802. In Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical region - online version (J.S. Moure, D. Urban & G.A.R. Melo, eds.). Available at <http://www.moure.cria.org.br/catalogue> last access in June, 2018.

NEMÉSIO, A. 2007. Three new species of *Euglossa* Latreille (Hymenoptera: Apidae) from Brazil. Zootaxa 1547:21-31.

NEMÉSIO, A. 2009. Orchid bees (Hymenoptera: Apidae) of the Brazilian Atlantic Forest. Zootaxa 2041:1-242.

NEMÉSIO, A. 2012. Species of *Euglossa* Latreille, 1802 (Hymenoptera: Apidae: Euglossina) belonging to the *purpurea* species group occurring in eastern Brazil, with description of *Euglossa monnei* sp. n. Zootaxa 3151:35-52.

NEMÉSIO, A. & SILVEIRA, F.A. 2007. Diversity and distribution of orchid bees (Hymenoptera: Apidae) with a revised checklist of species. Neotrop. Entomol. 36(6):874-888.

PAVIOLO, A., DE ANGELO, C., FERRAZ, K.M.P.M.B., MORATO, R.G., PARDO, J.M., SRBEK-ARAUJO, A.C., BEISIEGEL, B.M., LIMA, F., SANA, D., DA SILVA, M.X., VELÁZQUEZ, M.C., CULLEN, L., CRAWSHAW Jr., P., JORGE, M.L.S.P., GALETTI, P.M., DI BITETTI, M.S., DE PAULA, R.C., EIZIRIK, E., AIDE, T.M., CRUZ, P., PERILLI, M.L.L., SOUZA, A.S.M.C., QUIROGA, V., NAKANO, E., PINTO, F.R., FERNÁNDEZ, S., COSTA, S., MORAES JR, E.A. & AZEVEDO, F. 2016. A biodiversity hotspot losing its top predator: the challenge of jaguar conservation in the Atlantic Forest of South America. Sci. Rep. 6:37147.

PEARSON, D.L. & DRESSLER, R.L. 1985. Two-year study of male orchid bee (Hymenoptera: Apidae: Euglossini) attraction to chemical baits in lowland south-eastern Perú. J. Trop. Ecol. 1(1):37-54.

PERUQUETTI, R.C. & CAMPOS, L.A.O. 1997. Aspectos da biologia de *Euplusia violacea* (Blanchard) (Hymenoptera, Apidae, Euglossini). Rev. Bras. Zool. 14(1):91-97.

RAMÍREZ, S., DRESSLER, R.L. & OSPINA, M. 2002. Abejas Euglosina (Hymenoptera: Apidae) de la Región Neotropical: listado de espécies con notas sobre su biología. Biota Colomb. 3:7-118.

RAMÍREZ, S.R., ROUBIK, D.W., SKOV, C. & PIERCE, N.E. 2010. Phylogeny, diversification patterns and historical biogeography of euglossine orchid bees (Hymenoptera: Apidae). Biol. J. Linn. Soc. 100(3):552-572.

REBÊLO, J.M.M. & MOURE, J.S. 1996 [1995]. As espécies de *Euglossa* Latreille do nordeste de São Paulo (Apidae, Euglossinae). Rev. Bras. Zool. 12(3):445-466.

REBÊLO, J.M.M. & GARÓFALO, C.A. 1997. Comunidades de machos de Euglossinae (Hymenoptera, Apidae) em matas semidecíduas do nordeste do estado de São Paulo. An. Soc. Entomol. Bras. 26(2):243-256.

RIBEIRO, M.C., METZGER, J.P., MARTENSEN, A.C., PONZONI, F.J. & HIROTA, M.M. 2009. The Brazilian Atlantic Forest: how much is left, and how is the remaining forest distributed? Implications for conservation. Biol. Conserv. 142(6):1141-1153.

RODOLFO, A.M., CÂNDIDO JR., J.F., TEMPONI, L.G. & GREGORINI, M.Z. 2008. *Citrus aurantium* L. (laranja-apepu) e *Hovenia dulcis* Thunb. (uva-do-japão): espécies exóticas invasoras da trilha do Poço Preto no Parque Nacional do Iguaçu, Paraná, Brasil. Rev. Bras. Bioci. 6:16-18.

SALAMUNI, R., SALAMUNI, E., ROCHA, L.A. & ROCHA, A.L. 2002. Parque Nacional do Iguaçu, PR. Cataratas de fama mundial. In Sítios Geológicos e Paleontológicos do Brasil (C. Schobbenhaus, D.A. Campos, E.T. Queiroz, M. Winge & M.L.C. Berbert-Born, eds). DNPM/CPRM, Brasília, p. 313-321.

SANTOS, A.M. & SOFIA, S.H. 2002. Horário de atividade de machos de Euglossinae (Hymenoptera, Apidae) em um fragmento de floresta semidecídua no norte do Estado do Paraná. Acta Sci. 24(2):375-381.

SILVEIRA, G.C., NASCIMENTO, A.M., SOFIA, S.H. & AUGUSTO, S.C. 2011. Diversity of the euglossine bee community (Hymenoptera, Apidae) of an Atlantic Forest remnant in southeastern Brazil. Rev. Bras. Entomol. 55(1):109-115.

SKOV, C. & WILEY, J. 2005. Establishment of the Neotropical orchid bee *Euglossa viridissima* (Hymenoptera: Apidae) in Florida. Fla. Entomol. 88(2):225-227.

SOFIA, S.H. & SUZUKI, K.M. 2004. Comunidades de machos de abelhas Euglossina (Hymenoptera: Apidae) em fragmentos florestais no sul do Brasil. Neotrop. Entomol. 33(6):693-702.

SOFIA, S.H., SANTOS, A.M. & SILVA, C.R.M. 2004. Euglossine bees (Hymenoptera, Apidae) in a remnant of Atlantic Forest in Paraná state, Brazil. Iheringia, Sér. Zool. 94(2):217-222.

SYDNEY, N.V., GONÇALVES, R.B. & FARIA, L.R.R. 2010. Padrões espaciais na distribuição de abelhas Euglossina (Hymenoptera, Apidae) da região Neotropical. Pap. Avulsos Zool. 50(43):667-679.

WITTMANN, D., HOFFMANN, M. & SCHOLZ, E. 1988. Southern distributional limits of euglossine bees in Brazil linked to habitats of the Atlantic and subtropical rain forest (Hymenoptera, Apidae, Euglossini). Entomol. Gen. 14(1):53-60.

WITTMANN, D., RADTKE, R., HOFFMANN, M. & BLOCHSTEIN, B. 1989. Seasonality and seasonal changes in preferences for scent baits in *Euplusia violacea* in Rio Grande do Sul/Brazil (Hymenoptera, Apidae, Euglossini). Entomol. Gen. 14(3-4):217-221.

ZANELLA, F.C.V., OLIVEIRA, M.L., & GAGLIANONE, M.C. 2000. Standardizing lists of locality data for examined specimens in systematic and biogeography studies of new world taxa. Biogeographica 76(4):145-160.

Received: 29/08/2018
Revised: 21/01/2019
Accepted: 25/02/2019

Biota Neotropica 19(2): e20180673, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Arctiini Leach, [1815] (Lepidoptera, Erebidae, Arctiinae) of the Brazilian Amazon. III – Subtribe Ctenuchina Kirby, 1837

José A. Teston[1]**, *Débora S. de Abreu*[1] & *Viviane G. Ferro*[2]

[1]*Universidade Federal do Oeste do Pará, Instituto de Ciências da Educação, Laboratório de Estudos de Lepidópteros Neotropicais, Rua Vera Paz s/n, CEP 68040-255, Santarém, PA, Brasil*
[2]*Universidade Federal de Goiás, Instituto de Ciências Biológicas, Departamento de Ecologia, Caixa Postal 131, CEP 74001-970, Goiânia, GO, Brasil*

**Corresponding author: José A. Teston, e-mail: jateston@gmail.com*

TESTON, J. A., ABREU, D.S.; FERRO, V.G. **Arctiini Leach, [1815] (Lepidoptera, Erebidae, Arctiinae) of the Brazilian Amazon. III – Subtribe Ctenuchina Kirby, 1837**. Biota Neotropica. 19(2): e20180673. http://dx.doi.org/10.1590/1676-0611-BN-2018-0673

Abstract: The Ctenuchina moths comprise 874 New World species. Here we provide a list of Ctenuchina species from the Brazilian Amazon. The list was produced from specimens deposited in the most important Brazilian collections and from literature data. Nearly 31% (273) of the New World Ctenuchina species were recorded, including 28 new occurrences for the Brazilian Amazon. Santarém and Belém were the municipalities with the highest number of species records, with 139 and 88, respectively. The high number of Ctenuchina records in the Amazon is underestimated because this subtribe has never been sampled in the vast majority of the biome. This is a worrying scenario because the Amazon has the world highest absolute rate of forest reduction. To suggest efficient conservation policies for the Arctiinae fauna of Amazon, it is urgent to intensify the sampling effort in this biome.

Keywords: *Amazon, Tiger moths, Inventory, Noctuoidea, Wasp Moths.*

Arctiini Leach, [1815] (Lepidoptera, Erebidae, Arctiinae) da Amazônia Brasileira. III – Subtribo Ctenuchina Kirby, 1837

Resumo: As mariposas Ctenuchina compreendem 874 espécies no Novo Mundo. Neste trabalho, nós apresentamos uma lista das espécies de Ctenuchina que ocorrem na Amazônia brasileira. A lista foi produzida através de observação de espécimes depositados nas mais importantes coleções brasileiras e também através de dados da literatura. Aproximadamente 31% (273) das espécies de Ctenuchina que ocorrem no Novo Mundo foram registradas, incluindo 28 novas ocorrências para a Amazônia brasileira. Santarém e Belém foram os municípios com maior número de espécies registradas, com 139 e 88, respectivamente. Embora o número de registros de Ctenuchina seja considerado alto na Amazônia, este valor é subestimado visto que a fauna nunca foi amostrada na imensa maioria do bioma. Este cenário é preocupante, pois a Amazônia possui a maior taxa absoluta de redução florestal do mundo. A fim de podermos sugerir políticas de conservação eficientes para a fauna de Arctiinae da Amazônia, é urgente que se intensifique o esforço de coleta neste bioma.

Palavras-chave: *Amazônia, mariposas-tigre, Inventário, Noctuoidea, mariposas-vespa.*

Introduction

Arctiinae is distributed worldwide (Heppner 1991). With approximately 11,000 species, the Arctiinae moths are divided in four tribes (Zahiri et al. 2012), but only Arctiini and Lithosiini occurr in the Neotropics. Arctiini is classified in seven subtribes: Arctiina, Callimorphina, Ctenuchina, Euchromiina, Phaegopterina, Pericopina, and Spilosomina (Weller et al. 2009, Vincent & Laguerre 2014).

The Ctenuchina moths are found in the "New World" and comprise 874 species (Weller et al. 2009). Several species have nocturnal activity, but some species are exclusively diurnal (Hagmann 1938). Many species of Ctenuchina form mimicry rings with several wasp species (Simmons, 2009). They have a pair of tympanic organs located in the metathorax, above the spiracles, covered by an operculum. The hind wings are small or reduced, with absence of $S_c + R_1$ veins and separated C_uA branches (Kitching & Rawlins 1999, Jacobson & Weller 2002). The larvae feed on Apocynaceae, Euphorbiaceae, Ebenaceaa, Moraceae, Fabaceae, Ulmaceae, Poaceae, Loganiaceae, Urticaceae, Malpighiaceae, Vochysiaceae, Malvaceae, Ochnaceae and Cyperaceae (Janzen & Hallwachs 2005). The adults of several species are pharmacophagous and feed on pyrrolizidine alkaloids (Conner & Jordan 2009).

We here report a list of Ctenuchina moths occurring in the Brazilian Amazon based on the specimens deposited in the most important Brazilian collections and also from literature data. This study is a continuation of Teston & Ferro (2016a, b) and aims to increase the knowledge on Arctiinae in the Amazon region.

Materials and methods

We intensively searched the literature and examined specimens from entomological collections of the Instituto Nacional de Pesquisas na Amazônia (INPA; Manaus), Museu Paraense Emilio Goeldi (MPEG; Belém), Coleção Becker (VOB; Camacan), Coleção Entomológica Padre Jesus Santiago Moure of the Universidade Federal do Paraná (DZUP; Curitiba), Fundação Instituto Oswaldo Cruz (FIOC; Rio de Janeiro), Museu de Zoologia of the Universidade de São Paulo (MZUSP; São Paulo), Museu Nacional of the Universidade Federal do Rio de Janeiro (MNRJ; Rio de Janeiro), and Laboratório de Estudos de Lepidópteros Neotropicais (LELN) of the Universidade Federal do Oeste do Pará (UFOPA; Santarém). To identify the species, we used literature (Hampson 1898, 1914, Seitz 1919-1925) and specimens deposited in the visited collections. The systematic organization to generic level follows Hampson (1898) and Weller et al. (2000), with updates of the generic names according Watson et al. (1995) and corrections of Pinheiro & Duarte (2013), Pinheiro & Gaal-Haszler (2015) and Pinheiro (2016).

The geographical coordinates of the localities in the Brazilian Amazon with Arctiinae records were obtained from the Geo Loc tool of "Species Link date & tools" (http://splink.cria.org.br/geoloc) and Google Earth (https://earth.google.com/web/). The list is organized alphabetically. Species and records without precise location data, and those from locations that belong to more than one biome (e.g., Cerrado and Amazon) were not included in the list.

Results

Our research generated a list of 273 Ctenuchina species (Table 1), including 28 new occurrences for the Brazilian Amazon (indicated by "NEW"). Twenty-three species appear as new records for the municipalities and their respective States (indicated by "AMZ"). In total, 75 (nearly 10%) Amazonian municipalities had Ctenuchina species records (Table 2 and Figure 1). Santarém (PA), Belém (PA), and Cacaulândia (RO) were the municipalities with the highest number of species, with 139, 88, and 60, respectively.

We recorded 61 genera, of which 20 were monospecific. The genera *Eucereon* Hübner, 1819 had the highest number of species (37), followed by *Delphyre* Walker, 1854 (20), and *Heliura* Butler, 1876 (17). The species with the highest number of locality records were *Eucereon varia* (Walker, 1854) (with 23), followed by *Telioneura glaucopis* R. Felder, 1869 (22), *Correbidia calopteridia* (Butler, 1878) (21) and *Epidesma ursula* (Cramer, 1782) (20). Eighty-nine species (32.6%) occurred in only one locality (Table 1).

Discussion

The number of Ctenuchina species recorded for the Brazilian Amazon reported on this study is high. It corresponds to 31.2% of "New World" Ctenuchina fauna (Weller et al. 2009) and is more than two times higher than what has been reported for Mexico (Hernández-Baz et al. 2013) and the Brazilian Cerrado (Ferro et al. 2010). The fact that Santarém and Belém are the Amazon municipalities with the highest number of Ctenuchina records is probably related to the proximity and access to the collection sites. Belém is the capital of the state of Pará and has an important museum (Museu Paraense Emílio Goeldi, founded in 1866) and other research institutions, as well as easy access to sampling sites. Santarém has been widely sampled by H. Zerny and G. Hagmann in the 1930s (Zerny 1931, Hagmann 1938). Hagmann resided in Santarém and Zerny spent a season collecting in forests and other natural areas nearby Santarém. Belém is also the second municipality with the highest number of Phaegopterina and Pericopina records (Teston & Ferro 2016a, b).

The Ctenuchina richness of Santarém and Belém is the largest recorded in Brazil. For example, in Salesópolis it has been recorded 42 Ctenuchina species (Ferro & Diniz 2007) and in Joinville 57 species (Ferro et al. 2012). These two last sites are located in the Atlantic Forest biome and were intensively sampled in the recent past. In the Cerrado sites, a much lower number of Ctenuchina species has been recorded, ranging from 3 (Scherrer et al. 2013) to 26 (Moreno & Ferro 2016). Moreover, the number of Ctenuchina species in a single Amazon site (Santarém, 139) is higher than that recorded for the whole Cerrado (133 species, Ferro et al. 2010). It may indicate that this taxon is more diverse in rain-forest than in xeric environments.

Until now Teston and Ferro (2016b) have reported 819 Arctiinae species in the Brazilian Amazon. Due to the new records reported in this study (28) there has been an increase in richness for the biome, and now a total of 847 tiger moth species are recorded for the Amazon. Similarly to the the subtribes Phaegopterina (Teston & Ferro 2016a) and Pericopina (Teston & Ferro 2016b), the number of Amazonian species of Ctenuchina is underestimated because it has never been sampled in the vast majority of the biome (less than 10% of the municipalities has been sampled until now). In addition, the sampling points are poorly distributed and usually restricted to more populated cities and along major rivers. This is a worrying scenario because the Amazon has the world highest absolute rate of forest reduction (Silva et al. 2005). This biome has suffered from logging, fires, fragmentation, mining, wildlife extinction, invasion of alien species, and wildlife trafficking (Fearnside 2003). To suggest efficient conservation policies for the Arctiinae moths in the Amazon, it is urgent to intensify the collection effort in this biome.

http://dx.doi.org/10.1590/1676-0611-BN-2018-0673

Table 1. Ctenuchina (Erebidae, Arctiinae, Arctiini) species of the Brazilian Amazon. The column with the records shows the Brazilian state in abbreviated form followed by municipality. The name of the locality is enclosed in braces and the author of the first record is in parentheses. * New record. States abbreviations: AC = Acre, AM= Amazonas, AP = Amapá, MA = Maranhão, MT = Mato Grosso, PA = Pará, RO = Rondônia and RR = Roraima.

	Species	**Record**
1.	*Abrochia aequalis* (Walker, [1865])	AM, Tefé {Ega} (Walker [1865]); AP, Serra do Navio*; MA, Açailândia*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
2.	*Abrochia atridorsata* (Hampson, 1909)	AM, São Gabriel [da Cachoeira] {Taracuá} (Bryk 1953)
3.	*Abrochia aurantivena* (Hampson, 1918)	PA, [Belém] (Zerny 1931)
4.	*Abrochia consobrina* (Walker, 1856)	[AM], Valley of Amazonas [river] (Zerny 1931); PA, [Belém] (Walker 1856), Tapajós [river] (Hampson 1898)
5.	*Abrochia discoplaga* (Schaus, 1905)	MA, Açailândia*; MT, Sinop*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
6.	*Abrochia eumenoides* (Gaede, 1926)	AM, [Boa Vista do Ramos] {Massauari} (Gaede 1926)
7.	*Abrochia fulvisphex* (Druce, 1898)	AM, Manaus (Bryk, 1953); MT, Sinop*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
8.	*Abrochia mellina* (Herrich-Schäffer, 1855)	PA, Cametá (Hampson 1898)
9.	*Abrochia munda* (Walker, 1856)	PA, [Belém] (Walker 1854a), Santarém (Hampson 1898)
10.	*Abrochia postica* (Walker, 1854)	PA, [Belém] (Walker 1854a), Santarém (Hampson 1898)
11.	*Abrochia singularis* (Walker, 1854)	PA, [Belém] (Hampson 1898)
12.	*Abrochia zethus* (Hübner, 1827)	PA, [Belém] (Walker 1854a), Santarém (Hampson 1898), Tapajós [river] (Walker 1854a)
13.	*Aclytia albistriga* Schaus, 1910^{AMZ}	PA, Belém*, Capitão Poço*
14.	*Aclytia apicalis* (Walker, 1854)	PA, [Belém] (Walker 1854a)
15.	*Aclytia gynamorpha* Hampson, 1898	AM, [Jutaí] {Jutaí river} (Hampson 1898), Juruá river (Hampson 1898), Santa Isabel do Rio Negro*; PA, Belém {Miramar}*, Belterra {National Forest of Tapajós} (Freitas 2014), Breves*, Marabá* Santarém (Hampson 1898), Santarém {Taperinha} (Zerny 1931)
16.	*Aclytia heber* (Cramer, [1780])	AM, [Carauari] {Juruá river, [Lake of] Pupunha} (Butler 1878), [Jutaí] {Jutaí river, Boa Vista} (Butler 1878), [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953); MA, Açailândia*; MT, Sinop*; [PA], lower Amazonas [river] (Druce 1884), PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Santarém (Butler 1876), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
17.	*Aclytia hoffmannsi* Rothschild, 1912	AM, Santa Isabel do Rio Negro*; PA (Hagmann 1938), Belém {Miramar}*, Santarém*; RO, Porto Velho*
18.	*Aclytia petra* Schaus, 1892^{AMZ}	PA, Santarém*; RO, Ariquemes*, Cacaulândia*
19.	*Aclytia punctata* Butler, 1876	AM, Manicoré*; MA, Açailândia*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Belterra {National Forest of Tapajós} (Freitas 2014), Santarém (Hampson 1898), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Porto Velho*
20.	*Aclytia reducta* Rothschild, 1912	AM, Manicoré {Madeira river} (Zerny 1931), São Gabriel da Cachoeira*; PA, Santarém (Hampson 1914)
21.	*Aclytia terra* Schaus, 1896^{AMZ}	MT, Sinop*; RO, Cacaulândia*
22.	*Aethria analis* Schaus, 1901^{NEW}	RO, Vila Rondônia*
23.	*Aethria haemorrhoidalis* (Stoll, [1790])^{AMZ}	AM, Borba*; AP, Mazagão*; PA, Benevides*, Curralinho*, Parauapebas {Serra Norte, Carajás}*, Santarém*; RO, Porto Velho*, RR, Alto Alegre*
24.	*Aethria paula* Schaus, 1894^{NEW}	AM, Manaus*

Continuation Table 1.

	Species	Record
25.	*Agyrta auxo* Hübner, 1817	AM, São Paulo de Olivença*; AP, Serra do Navio*; PA, [Belém] (Zerny 1931), Belém {Miramar}*, Ourém*, Santarém*
26.	*Agyrta bifasciata* Rothschild, 1912	PA, Santarém {Mojú} (Zerny 1931)
27.	*Agyrta chena* Druce, 1893	AM, Manaus (Bryk 1953), [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953); PA, [Belém] (Druce 1893)
28.	*Agyrta dux* (Walker, 1854)	AM, [Eirunepé] {Juruá river, São Felipe} (Zerny 1931), Manicoré*, Maués*; AP, Serra do Navio*; PA, Anajás*, Belém*, Parauapebas {Serra Norte, Carajás}*, Óbidos*, Santarém {Taperinha} (Zerny 1931)
29.	*Agyrta klagesi* Rothschild, 1912$^{\text{NEW}}$	PA, Santarém*
30.	*Agyrta micilia* (Cramer, 1780)	AM, Manicoré*, Santa Isabel do Rio Negro*; PA, [Belém] (Hampson 1898); RO, Cacaulândia*, Candeias do Jamari*
31.	*Agyrta pandemia* Druce, 1893	PA, [Belém] (Druce 1893)
32.	*Agyrta porphyria* (Cramer, 1782)	AM, [Eirunepé] {Juruá river, São Felipe} (Zerny 1931), Manaus (Zerny 1931), Manicoré*, [Maués] {Rio Parauary}*, São Paulo de Olivença*, Tefé*; AP, Santana*, Serra do Navio*; PA, [Belém] (Hampson 1898), Itaituba*, Santarém {Taperinha} (Zerny 1931); RO, Vila Rondônia*
33.	*Agyrtidia olivensis* Machado Filho e Rêgo Barros, 1970	AM, São Paulo de Olivença (Machado Filho & Rêgo Barros 1970)
34.	*Agyrtidia uranophila* (Walker, 1866)	[AC], Alto Juruá (Machado Filho & Rêgo Barros 1970); AM, [Autazes] {Autaz river, Campinhas} (Bryk 1953), Benjamin Constant*, Borba (Machado Filho & Rêgo Barros 1970), [Eirunepé] {Rio Juruá, São Felipe} (Machado Filho & Rêgo Barros 1970), [São Gabriel da Cachoeira] {Rio Negro, Jauaretê} (Machado Filho & Rêgo Barros 1970), São Paulo [de Olivença] (Walker 1866); PA, Itaituba*, Santarém (Machado Filho & Rêgo Barros 1970); RO, Cacaulândia*, Jarú*
35.	*Agyrtiola niepelti* Gaede 1926	AM, São Paulo de Olivença (Gaede 1926)
36.	*Anaphlebia caudatula* R. Felder, 1869	AM, Amazonas [river] (R. Felder 1869)
37.	*Androcharta diversipennis* (Walker, 1854)	AM, Benjamin Constant*, [Carauari] {Juruá river, [Lake of] Pupunha} (Butler 1878), [Eirunepé] {Juruá river, São Felipe} (Zerny 1931), [Jutaí] {Barreiras de Jutaí} (Butler 1878), Manaus*, São Paulo [de Olivença] (Butler 1876), Tabatinga (Butler 1876), Tefé {Ega} (Butler 1876); AP, Serra do Navio*; PA, Itaituba*, Santarém {Mojú and Taperinha} (Zerny 1931), Tapajós [river] (Walker 1854a)
38.	*Androcharta hoffmannsi* (Rothschild, 1912)	AM, Benjamin Constant*, Humaitá (Hampson 1914), São Paulo de Olivença*; RO, Cacaulândia*
39.	*Androcharta meones* (Cramer, 1780)	AM, Negro river (Zerny 1931), São Paulo de Olivença (Santos 1939); PA, Altamira {Monte Santo} (Delfina & Teston 2013), Santarém {Mojú and Taperinha} (Zerny 1931)
40.	*Androcharta stretchii* Butler, 1876	AM, Benjamin Constant*, Maués*, São Paulo [de Olivença] (Butler 1876); AP, Serra do Navio*; PA, Itaituba*, Santarém*, Tucuruí*
41.	*Antichloris affinis* (Rothschild, 1912)	AM, Tefé (Rothschild 1912); PA (Hagmann 1938)
42.	*Antichoris caca* Hübner, 1818	AM, São Paulo de Olivença*, Tefé (Field 1975); AP, Serra do Navio*; PA, [Belém] (Hampson 1898), Soure*
43.	*Antichloris chloroplegia* (Druce, 1905)$^{\text{NEW}}$	AM, São Paulo de Olivença*; AP, Serra do Navio*
44.	*Antichloris eriphia* (Fabricius, 1776)	AC, Feijó*; AM, Benjamin Constant*, Manaus*, Manicoré*, São Paulo de Olivença*, Tefé*; PA, [Belém] (Butler 1876), Altamira {Monte Santo} (Delfina & Teston 2013), Breves*, Santarém {Taperinha} (Zerny 1931), Tapajós [river] (Walker 1854a); RO, Ariquemes*, Cacaulândia*, Porto Velho*
45.	*Antichloris scudderii* Butler, 1876	AM, Itacoatiara*, Manaus (Zerny 1931), Manicoré*, São Paulo de Olivença*; PA, Oriximiná {Rio Cuminá}*, Santarém (Butler 1876), Santarém {Taperinha} (Zerny 1931)
46.	*Argyroeides flavicornis* Rothschild, 1911	AM, Tefé (Rothschild 1911)

Continuation Table 1.

	Species	Record
47.	*Argyroeides ophion* (Walker, 1854)AMZ	AM, Manicoré*
48.	*Argyroeides strigula* Druce, 1896NEW	MA, Santa Luzia*
49.	*Atyphopsis modesta* Butler, 1878	AM, [Jutaí] {Rio Jutaí, Boaventura} (Butler 1878), Manicoré {Rio Madeira} (Zerny 1931); AP, Serra do Navio*; MT, Sinop*; PA, Belém*, Santarém (Butler 1878), Santarém {Taperinha} (Zerny 1931); RO, Porto Velho*
50.	*Cacostatia buchwaldi* (Rothschild, 1912)	RO, [Porto Velho] {Madeira river, Aliança} (Rothschild 1912)
51.	*Cacostatia discalis* (Walker, 1856)	[AM], Valley on the Amazon [river] (Walker 1856)
52.	*Cacostatia flaviventralis* Dognin, 1909	PA, Almeirim {Jari} (Hawes et al. 2009)
53.	*Cacostatia germana* (Rothschild, 1912)NEW	AM, Manaus*; PA, Belém*
54.	*Ceramidia fumipennis* (Walker, 1854)	AM, Fonte Boa (Field 1975), Humaitá (Field 1975), Manaus (Field 1975), Manicoré {Madeira river} (Field 1975), [Novo Airão] {Igarapé Preto} (Field 1975), Solimões [river] (Bryk 1953), São Paulo de Olivença (Field 1975) Tefé {Ega} (Walker 1854a), Tonantins (Field 1975); PA, Tapajós river (Field 1975); RO, [Porto Velho] {Rio Madeira, Aliança} (Field 1975)
55.	*Ceramidia phemonoides* (Möschler, 1854)	AM, Manaus (Zerny 1931); PA, Tapajós [river] (Butler 1876)
56.	*Ceramidiodes obscurus* (Butler, 1877)	AM, Benjamin Constant*, Manaus*, Manicoré (Hampson 1898), [Tapauá] {Rio Purus, [Lake] Sobral} (Butler 1877), Tefé (Rothschild 1912)
57.	*Cercopimorpha dolens* Schaus, 1905	MT, Sinop*; PA, Santarém {Taperinha} (Zerny 1931)
58.	*Cercopimorpha homopteridia* Butler, 1876	AM, Manicoré*; MT, Sinop*; PA, [Belém] (Walker 1854a), Santarém {Taperinha} (Zerny 1931)
59.	*Cercopimorpha postflavida* (Rothschild, 1912)NEW	PA, Santarém*; RO, Cacaulândia*
60.	*Clystea andromacha* (Fabricius, 1775)	PA, [Belém] (Walker 1854a), Altamira {Monte Santo} (Delfina & Teston 2013); RR, Alto Alegre*
61.	*Clystea aner* (Hampson, 1905)	PA (Hagmann 1938)
62.	*Clystea carnicauda* (Butler, 1876)	PA, [Belém] (Hampson 1898), Altamira{51°BIS} (Teston et al. 2012)
63.	*Clystea daltha* (Druce, 1895)	AM, Benjamin Constant*, [Eirunepé] {Rio Juruá, Matto Pyri} (Zerny 1931), Manaus*, Manicoré, São Paulo de Olivença*; PA, [Belém] (Druce 1895), Parauapebas {Serra Norte, Carajás}*, Santarém*; RO, Cacaulândia*
64.	*Clystea eburneifera* (R. Felder, 1869)	AM, Amazonas [river] (R. Felder 1869), Tefé*
65.	*Clystea gracilis* (Möschler, 1878)AMZ	PA, Benevides*, Parauapebas {Serra Norte, Carajás}*
66.	*Clystea leucaspis* (Cramer, 1775)	PA (Hagmann 1938)
67.	*Clystea stipata* (Walker, 1854)	AM, Santa Isabel do Rio Negro*; MA, Santa Luzia*; PA, [Belém] (Walker 1854a), [Igarapé-Miri] {Estuary of Tocantins [river]} (Zerny 1931), Parauapebas {Serra Norte, Carajás}*
68.	*Corematura chrysogastra* (Perty, 1834)	AM, Benjamin Constant*, Borba*, Fonte Boa*, Rio Madeira (Butler 1878), [Santa Isabel do Rio Negro] {Rio Preto}*, São Paulo [de Olivença] (Butler 1876), São Paulo de Olivença {Rio Solimões, S. Jerônimo} (Travassos Filho 1952) and {Rio Preto} (Travassos Filho 1938), Tefé*
69.	*Correbia elongata* Rothschild, 1912AMZ	AM, Manaus*; MA, Açailândia*; PA, Novo Progresso {Cachimbo}*, Santarém*
70.	*Correbia felderi* Rothschild, 1912	[AM], Amazonas (Rothschild 1912); PA, Santarém {Taperinha} (Zerny 1931)
71.	*Correbia lycoides* (Walker, 1854)	AM, Barcelos {Moura}*, Benjamin Constant*, [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), São Gabriel da Cachoeira {Querari}*; AP, Serra do Navio*;MT, Sinop*; PA, Altamira {Monte Santo} (Teston & Delfina 2010), [Belém] (Walker 1854a), Belterra {National Forest of Tapajós} (Freitas 2014), Capitão Poço*, Igarapé-Açu*, Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Porto Velho*; RR, Alto Alegre*
72.	*Correbia obscura* Schaus, 1905NEW	RO, Porto Velho*
73.	*Correbia tristitia* Kaye, 1911	AM, Codajás (Rothschild 1912), Fonte Boa (Rothschild 1912), PA, Santarém {Taperinha} (Zerny 1931)

Continuation Table 1.

	Species	Record
74.	*Correbidia assimilis* (Rothschild, 1912)	AM, [Atalaia do Norte] {Santo Antonio do Javari} (Rothschild 1912), PA, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém (Valente et al.2018)
75.	*Correbidia calopteridia* (Butler, 1878)	AM, Barcelos {Moura}*, Benjamin Constant*, Fonte Boa*, [Manaus] {Rio Urubu}*, Santa Isabel do Rio Negro*, São Gabriel da Cachoeira {Querari}*, São Paulo de Olivença*,Tefé (Zerny 1931); AP, Serra do Navio*; MA, Açailândia*; MT, Aripuanã*, Sinop*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), [Belém] (Butler 1878), Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Porto Velho*
76.	*Correbidia germana* (Rothschild, 1912)	PA, Santarém {Taperinha} (Zerny 1931)
77.	*Correbidia notata* (Butler, 1878)	AM, Barcelos {Moura}*, [Jutaí] {Rio Jutaí} (Butler 1878); PA, Altamira{51°BIS} (Teston et al. 2012), Marabá*, Santarém {Taperinha} (Zerny 1931)
78.	*Correbidia simonsi* (Rothschild, 1912)	AM, Fonte Boa (Hampson 1914)
79.	*Correbidia steinbachi* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912)
80.	*Ctenucha circe* (Cramer, 1780)	PA, [Belém] (Walker 1854b)
81.	*Ctenucha mortia* Schaus, 1901^{AMZ}	RO, Candeias do Jamari*
82.	*Ctenucha tapajoza* Dognin, 1923	PA, Tapajós river (Dognin 1923)
83.	*Cyanopepla glaucopoides* (Walker, 1854)	AM, [Eirunepé] {Juruá river, São Felipe} (Zerny 1931), Manaus*, Maués*; PA, [Belém] (Walker 1854b), Santarém*
84.	*Cyanopepla hurama* (Butler, 1876)	PA, Itaituba (Staudinger 1894)
85.	*Delphyre aclytioides* (Hampson, 1901)	PA, Belterra {National Forest of Tapajós} (Freitas 2014)
86.	*Delphyre albiventus* (Druce, 1898)	AM (Zerny 1912); PA, Paragominas*
87.	*Delphyre discalis* (Druce, 1905)	MT, Sinop*; PA, Altamira {51°BIS} (Teston et al. 2012), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
88.	*Delphyre distincta* (Rothschild, 1912)	RO, [Porto Velho] {Madeira river, Aliança} (Hampson 1914)
89.	*Delphyre dizona* (Druce, 1898)	AM, Manicoré*; MA, Açailândia*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Porto Velho*
90.	*Delphyre fenestrata* Bryk, 1953	AM, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953)
91.	*Delphyre flaviceps* (Druce, 1905)	AM, Manicoré (Zerny 1931); PA, Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, [Porto Velho] {Madeira river, Aliança and Calama} (Rothschild 1912)
92.	*Delphyre flaviventralis* Hampson, 1901	PA, Santarém {Taperinha} (Zerny 1931)
93.	*Delphyre hampsoni* (Rothschild, 1912)	MA, Açailândia*; PA, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, [Porto Velho] {Madeira river, Aliança} (Rothschild 1912)
94.	*Delphyre hebes* Walker, 1854^{AMZ}	RO, Cacaulândia*
95.	*Delphyre macella* (Dognin, 1911)^{NEW}	PA, Marabá*
96.	*Delphyre maculosa* (Hampson, 1898)	AP, Oiapóque*; PA, [Belém] (Hampson 1898)
97.	*Delphyre meridionalis* (Rothschild, 1912)	RO, [Porto Velho] {Madeira river, Calama} (Rothschild 1912)
98.	*Delphyre minuta* (Möeschler, 1877)	PA, Santarém {Taperinha} (Zerny 1931)
99.	*Delphyre parcipuncta* Hampson, 1914	PA, Altamira {51°BIS} (Teston et al. 2012), [Breves] {Furos de Breves} (Zerny 1931); RO, [Porto Velho] {Madeira river, Aliança} (Rothschild 1912)

Continuation Table 1.

	Species	Record
100.	*Delphyre pieroides* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912)
101.	*Delphyre pumila* (Rothschild, 1912)	RO, [Porto Velho] {Madeira river, Aliança} (Rothschild 1912)
102.	*Delphyre pusilla* (Butler, 1878)	AM, [Atalaia do Norte] {Rio Javary, Braga} (Butler 1878), São Paulo de Olivença*; AP, Serra do Navio*; PA, Marabá*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*, Porto Velho*
103.	*Delphyre roseiceps* Dognin, 1909	AM, Fonte Boa (Rothschild 1912); PA, Santarém {Taperinha} (Zerny 1931); RO, Ariquemes*, Porto Velho*
104.	*Delphyre varians* Hampson, 1901	[AM] (Hampson 1901); PA (Hagmann 1938)
105.	*Dinia aeagrus* (Cramer, 1779)	AC, Feijó {Rio Embira} (Travassos Filho & Urban 1967); AM, Benjamin Constant*, Fonte Boa*, Humaitá*, [Manaus] {Rio Urubu}*, Manicoré*, São Paulo de Olivença {Rio Preto} (Travassos Filho 1957), Tabatinga*; MA, Açailândia*; MT, Aripuanã*; PA, Altamira {Monte Santo} (Teston & Delfina 2010), Belém*, Capitão Poço*, Parauapebas {Serra Norte, Carajás}*, Tucuruí*, Viseu*; RO, Porto Velho {Rio Madeira} (Travassos Filho & Urban 1967)
106.	*Dinia mena* (Hübner, [1827])AMZ	AM, Benjamin Constant*; MA, Açailândia*
107.	*Diptilon aurantiipes* Rothschild, 1911	[AM], Amazonas river (Rothschild 1911); RO, [Porto Velho] {Madeira river, Calama} (Rothschild 1911)
108.	*Ecdemus carmania* (Druce, 1883)	PA, Santarém {Taperinha} (Zerny 1931)
109.	*Ecdemus fuliginosa* (Rothschild, 1912)NEW	PA, Santarém*
110.	*Ecdemus hypoleucus* Herrich-Schäffer, [1855]	AM, [Jutaí] {Rio Jutaí} (Butler 1878), Manaus*, Santa Isabel do Rio Negro*; AP, Serra do Navio*; MA, Açailândia*; PA, Cametá (Herrich-Schäffer [1855]), Altamira {51°BIS} (Teston et al. 2012), Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931)
111.	*Ecdemus imbecillus* (Zerny, 1931)	PA, Santarém {Taperinha} (Zerny 1931)
112.	*Ecdemus pereirai* Travassos, 1940AMZ	PA, Santarém*
113.	*Ecdemus rubrothorax* (Rothschild, 1911)	AM, Humaitá {Madeira river} (Rothschild 1911)
114.	*Epanycles imperialis* (Walker, 1854)	AM, [Carauari] {Rio Juruá, Pupunhazinho} (Butler 1878), Manicoré {Madeira river} (Zerny 1931), Tefé {Ega} (Butler 1876); MA, Açailândia*, PA, Marabá*, Novo Progresso {Cachimbo}*, Oriximiná {Rio Cuminá}*, Rio Tapajós (Butler 1876), Santarém (Walker 1854a), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Porto Velho*
115.	*Epidesma albicincta* (Hampson, 1905)	PA (Hagmann 1938), Altamira {Monte Santo} (Delfina & Teston 2013), Santarém*
116.	*Epidesma aurimacula* (Schaus, 1905)	AM, São Paulo de Olivença*; PA (Hagmann 1938), Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Marabá*, Santarém*, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
117.	*Epidesma hoffmannsi* (Rothschild, 1912)AMZ	RO, Cacaulândia*
118.	*Epidesma klagesi* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912); PA, Santarém {Taperinha} (Zerny 1931); RO, [Porto Velho] {Madeira river, Calama} (Rothschild 1912)
119.	*Epidesma lenaeus* (Cramer, 1780)	AM, Tefé (Zerny 1931); MA, Açailândia*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Belém*, Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
120.	*Epidesma metapolia* (Dognin, 1912)AMZ	PA, Marabá*; RO, Cacaulândia*, Jarú*, Porto Velho*
121.	*Epidesma parva* (Rothschild, 1912)	PA, Altamira {Monte Santo} (Delfina & Teston 2013), Marabá*, Santarém*, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Porto Velho*

Continuation Table 1.

	Species	Record
122.	*Epidesma perplexa* (Rothschild, 1912)	AM, [Atalaia do Norte] {Santo Antonio do Javari} (Rothschild 1912), Fonte Boa (Rothschild 1912), Tefé (Rothschild 1912)
123.	*Epidesma similis* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912)
124.	*Epidesma ursula* (Cramer, 1782)	AM, [Jutaí] {Rio Jutaí} (Butler 1878), [Lábrea] {Rio Purus, Man[aua]} (Butler 1878), Manaus*, Rio Sapo (Butler 1878), [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953), São Paulo de Olivença*, Tefé {Ega} (Hampson 1898); MA, Açailândia*; PA, Almeirim {Jari} (Hawes et al. 2009), Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Belterra {National Forest of Tapajós} (Freitas 2014), Cametá*, Marabá*, Novo Progresso {Cachimbo}*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015), Tapajós [river] (Hampson 1898); RO, Cacaulândia*, Porto Velho*; [RR], [Caracaraí] {Branco river} (Bryk 1953)
125.	*Episcepsis capyscoides* Dognin, 1911^NEW^	RO, Cacaulândia*
126.	*Episcepsis endodasia* (Hampson, 1898)	AM, Borba*; PA, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
127.	*Episcepsis frances* Dyar, 1910	PA, Altamira {51°BIS} (Teston et al. 2012), Breves*, Santarém {Taperinha} (Zerny 1931)
128.	*Episcepsis gnoma* (Butler, 1877)	AM, [Barcelos] {Rio Pada[ua]r[i]} (Butler 1877), Santa Isabel do Rio Negro*, São Paulo de Olivença*; PA, Belém*, Breves (Zerny 1931), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
129.	*Episcepsis gnomoides* Schaus, 1910	PA, Santarém {Taperinha} (Zerny 1931)
130.	*Episcepsis klagesi* Rothschild, 1911	AM, São Paulo de Olivença*; PA, Belterra {National Forest of Tapajós} (Freitas 2014), Breves*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*
131.	*Episcepsis lamia* (Butler, 1877)	AM, Benjamin Constant*, Borba*, [Maués] {Rio Maués} (Butler 1877), [Tapauá] {Rio Purus, Ta[u]aria} (Butler 1877); MA, Açailândia*; PA, Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Breves*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Ariquemes*
132.	*Episcepsis littoralis* Rothschild, 1911	AM, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953)
133.	*Episcepsis melanitis* (Hübner, 1827)	AM, Manaus*, Manicoré*, Santa Isabel do Rio Negro*, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953), Tefé*; MT, Sinop*; PA, [Belém] (Hagmann 1938)
134.	*Episcepsis nereus* Zerny, 1931	AM, São Paulo de Olivença*; PA, Belém*, Santarém {Taperinha} (Zerny 1931)
135.	*Episcepsis luctuosa* (Möschler, 1877)	AM, Fonte Boa (Rothschild 1911); PA, Breves*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, [Porto Velho] {Madeira river, Aliança and Calama} (Rothschild 1911)
136.	*Episcepsis thetis* (Linnaeus, 1771)	MT, Sinop*; PA, Santarém*, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
137.	*Episcepsis venata* Butler, 1877	AM, [Jutaí] {Rio Jutaí near Rio Cur[uena]} (Butler 1877); PA, Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Prainha to Monte Alegre (Zerny 1931), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Ariquemes*, Cacaulândia*
138.	*Eriphioides tractipennis* (Butler, 1876)^NEW^	PA, Santarém*
139.	*Euagra coelestina* (Cramer, 1782)	AM, Santa Isabel do Rio Negro*; PA, [Belém] (Walker 1854b), Santarém*
140.	*Euagra intercisa* Butler, 1876^AMZ^	PA, Anajás*, Belém*
141.	*Eucereon aeolum* Hampson, 1898^AMZ^	MA, Açailândia*

Continuation Table 1.

	Species	Record
142.	*Eucereon amazonum* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912), São Paulo de Olivença*; PA, Breves*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
143.	*Eucereon aoris* Möschler, 1877	AM, Manicoré*; AP, Serra do Navio*; MT, Sinop*; PA, Altamira {Monte Santo} (Teston & Delfina 2010), Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931)
144.	*Eucereon archias* (Stoll, 1790)	AM, Manicoré {Rio Madeira} (Travassos 1959), Rio Javari (Butler 1878), Rio Japura (Pinheiro 2016), Tefé (Zerny 1931), Purus river (Bryk 1953); PA, Ale[n] quer (Pinheiro 2016), Gurupá to Almeirim (Zerny 1931), [Breves] {Furos de Breves} (Zerny 1931), Santarém {Taperinha} (Zerny 1931)
145.	*Eucereon arenosa* Butler, 1877	AM, São Paulo de Olivença*; PA, Santarém*; RO, [Porto Velho] {Rio Madeira, Jam[a]ry} (Butler 1877)
146.	*Eucereon atriguttum* Druce, 1905	AP, Serra do Navio*; PA, Marabá*, Santarém*, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015), São Miguel do Guamá*; RO, Cacaulândia*
147.	*Eucereon brunnea* (Hampson, 1903)	PA, Santarém {Taperinha} (Zerny 1931); RO, [Porto Velho] {Madeira river, Aliança} (Rothschild 1912)
148.	*Eucereon complicatum* Butler, 1877	AM, Manicoré {Rio Madeira} (Zerny 1931); Rio Juruá (Butler 1877); PA, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
149.	*Eucereon confinis* Herrich-Schäffer, 1855	[AM], Vale do [Rio] Amazonas (Travassos 1952)
150.	*Eucereon dorsipuncta* Hampson, 1905^{AMZ}	RO, Cacaulândia*
151.	*Eucereon exprata* Dognin, 1921	PA, [Igarapé Açu] {Prata 100 km of Pará [Belém]} (Dognin 1921)
152.	*Eucereon fuscoirroratum* (Rothschild, 1912)	PA, Santarém {Taperinha} (Zerny 1931)
153.	*Eucereon hagmanni* (Travassos, 1952)	AM, Borba {Lago Acará} (Travassos 1952), São Paulo de Olivença (Travassos 1952); PA, Belém {Bosque} (Travassos 1952), Santarém*
154.	*Eucereon hoffmansi* (Rothschild, 1912)	RO, [Porto Velho] {Aliança} (Rothschild 1912)
155.	*Eucereon latifascia* (Walker, 1856)	AM, [Autazes] {Autaz river} (Bryk 1953); MA, Açailândia*; PA, [Belém] (Walker 1856), Altamira {Monte Santo} (Teston & Delfina 2010), Marabá*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015), [Novo Progresso] {Cachimbo} (Travassos 1964a), Óbidos (Zerny 1931); RO, Cacaulândia*
156.	*Eucereon maia* Druce, 1884	AM, Tefé*; PA, Altamira {Monte Santo} (Teston & Delfina 2010), Belém*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*
157.	*Eucereon marmoratum* Butler, 1877	AM, [Carauari] {Rio Juruá, Curimatá and [Lake of] Pupunha) (Butler 1877), Beruri {Rio Purus, Aruman} (Butler 1877), [Lábrea] {Rio Purus, Mabid[e]r[i]} (Butler 1877), [Novo Airão] {Rio Negro, Ayrão} (Butler 1877), [Santa Isabel do Rio Negro] {Rio Preto}*, São Paulo de Olivença*; PA, Breves*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015), [Oriximiná] {Rio Trombetas, Cachoeira [da] Porteira} (Butler 1877); RO, Porto Velho*
158.	*Eucereon melanoperas* Hampson, 1898	AM, São Paulo de Olivença*, Purus river (Hampson 1898), Tefé {Ega} (Hampson 1898); PA, Santarém {Taperinha} (Zerny 1931)
159.	*Eucereon metoidesis* Hampson, 1905	AM, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953); AP, Serra do Navio*; PA, [Belém] (Butler 1876), Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931)
160.	*Eucereon mitigatum* Walker, 1857	AM, Benjamin Constant {Rio Javary, Alto Amazonas} (Pinheiro 2016), Fonte Boa (Pinheiro 2016), [Jutaí] {Rio Jutaí, Boa Vista} (Butler 1877), [Lábrea] {Rio Purus, Huitanaã} (Butler 1878), [Novo Airão] {Igarapé Preto, Alto Amazonas} (Pinheiro 2016), São Paulo de Olivença (Pinheiro 2016); PA, [Belém] (Pinheiro 2016) Chaves {Marajó Island} (Hampson 1898), Santarém {Taperinha} (Pinheiro 2016)

Continuation Table 1.

	Species	Record
161.	*Eucereon obscura* (Möschler, 1872)	AM, Benjamin Constant*, Fonte Boa*, [Jutaí] {Rio Jutaí} (Butler 1877), Manicoré*, São Paulo de Olivença*; AP, Serra do Navio*; MA, Açailândia*; PA, Altamira {51°BIS} (Teston et al. 2012), Belém*, Belterra {National Forest of Tapajós} (Freitas 2014), Breves*, Santarém {Taperinha} (Zerny 1931), Viseu*; RO, Porto Velho*
162.	*Eucereon parascyton* Hampson, 1914	AM, São Paulo de Olivença*; PA, Santarém {Taperinha} (Zerny 1931)
163.	*Eucereon patrona* Schaus, 1896$^{\text{AMZ}}$	RO, Cacaulândia*
164.	*Eucereon plumbicollum* Hampson, 1898$^{\text{NEW}}$	AP, Serra do Navio*
165.	*Eucereon pseudarchias* Hampson, 1898	AM, Juruá river (Hampson 1898); PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), [Belém] (Travassos 1964a), Belém {Miramar}*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
166.	*Eucereon punctatum* (Guérin-Méneville, [1844])	AM, Benjamin Constant*, [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), São Paulo de Olivença*, Tefé*; PA, Breves*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia (Pinheiro 2016)
167.	*Eucereon reniferum* Hampson, 1898$^{\text{NEW}}$	RO, Cacaulândia*
168.	*Eucereon rosa* (Walker, 1854)	AM, Beruri {Rio Purus, Paricatuba} (Butler 1878)
169.	*Eucereon rosina* (Walker, 1854)$^{\text{NEW}}$	MA, Açailândia*; RO, Cacaulândia*
170.	*Eucereon scyton* (Cramer, 1777)	AM, Benjamin Constant*; PA, Marabá*, Santarém {Taperinha} (Zerny 1931); RO, Ariquemes*, Cacaulândia*, Jarú*, Porto Velho*
171.	*Eucereon setosa* (Sepp, [1830])$^{\text{AMZ}}$	MA, Açailândia*; PA, Novo Progresso {Cachimbo}*
172.	*Eucereon simile* (Draudt, 1915)	AM, Manicoré (Zerny 1931); PA, Santarém {Taperinha} (Zerny 1931); RO, [Porto Velho] {Aliança} (Rothschild 1912)
173.	*Eucereon sylvius* (Stoll, 1790)	[AC], Upper Juruá river (Zerny 1931); AM, [Jutaí] {Rio Jutaí, Barreira Branca} (Butler 1878), [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), Rio Juruá {Pupunhazinho} (Butler 1878), Rio Negro (Travassos 1959), São Paulo de Olivença {Rio Preto} (Travassos 1959), Tefé (Zerny 1931); PA, [Belém] "Amazonas inferior" (Travassos 1959), Breves*, Santarém (Hampson 1898), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
174.	*Eucereon taperinhae* Dognin, 1923	PA, Santarém {Taperinha} (Dognin 1923)
175.	*Eucereon tarona* Hampson, 1898	PA, Marabá*, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
176.	*Eucereon varia* (Walker, 1854)	AM, [Atalaia do Norte] {Rio Quichito} (Travassos 1964a), Benjamin Constant*, Borba {Rio Madeira e Lago Acar[á]} (Travassos 1964a), [Canutama] {Rio Purus, Urucuri} (Butler 1878), [Carauari] {Rio Juruá, [Lake of] Pupunha} (Butler 1878), [Eirunepé] {Juruá river, São Felipe} (Zerny 1931), Manaus (Travassos 1964a), Manicoré {Madeira river} (Zerny 1931), [Maués] {Rio Maués} (Travassos 1964a), São Paulo de Olivença*, Rio Preto (Travassos 1964a), Rio Pauary (Travassos 1964a); MT, Sinop*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), [Belém] (Walker 1854a), Breves*, Gurupá-Almeirim (Zerny 1931), Marabá*, [Novo Progresso] {Cachimbo} (Travassos 1964a), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
177.	*Eucereon velutina* Schaus, 1896$^{\text{NEW}}$	RO, Cacaulândia*
178.	*Euceriodes wernickei* (Draudt, 1917)	PA, [Novo Progresso] {Cachimbo} (Travassos 1964a)
179.	*Eumenogaster affinis* Rothschild, 1911$^{\text{NEW}}$	PA, Belém*
180.	*Eumenogaster haemacera* Hampson, 1898$^{\text{NEW}}$	AM, Manicoré*
181.	*Eumenogaster nigricauda* Dognin, 1911$^{\text{NEW}}$	PA, Belém*
182.	*Eumenogaster notabilis* (Walker, [1865])	AM, Manaus {Pensador} (Bryk 1953); PA, Belém*, Santarém {Taperinha} (Zerny 1931), Tapajós [river] (Walker [1865])

Continuation Table 1.

	Species	Record
183.	*Eumenogaster psedosphecia* Hampson, 1898	AM, Manaus (Bryk 1953), Tefé (Zerny 1931), Tonantins (Hampson 1898); PA, Belém*, Santarém {Taperinha} (Zerny 1931)
184.	*Heliura amazonicum* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912)
185.	*Heliura balia* (Hampson, 1898)	PA, Altamira {Monte Santo} (Teston & Delfina 2010), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
186.	*Heliura cadroe* Schaus, 1924NEW	MA, Açailândia*; PA, Belém*, Capitão Poço*; RO, Cacaulândia*
187.	*Heliura excavata* (Dognin, 1910)	AM, Benjamin Constant*; PA, Belterra {National Forest of Tapajós} (Freitas 2014), Igarapé Açú*, Santarém (Valente et al. 2018)
188.	*Heliura hagmanni* Zerny, 1931	AM, Manicoré*; PA, Santarém {Taperinha} (Zerny 1931)
189.	*Heliura marica* (Cramer, 1775)	AM, Borba*, [Lábrea] {Purus river, [S]epatiny} (Hampson 1898), São Gabriel da Cachoeira {Querari}*, São Paulo de Olivença*, Tefé*; AP, Serra do Navio*; MT Sinop*; PA, [Belém] (Walker 1854a), Breves*, Santarém {Taperinha} (Zerny 1931)
190.	*Heliura perexcavatum* (Rothschild, 1912)	AM, Borba*, Fonte Boa (Rothschild 1912); PA, Belterra {National Forest of Tapajós} (Freitas 2014)
191.	*Heliura phaeosoma* Druce, 1905	AM, Manicoré*, Santa Isabel do Rio Negro*; PA, Belém {Miramar}*, Breves*, Marabá*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*
192.	*Heliura pierus* (Cramer, 1782)	AM, Fonte Boa*, [Jutaí] {Rio Jutaí} (Butler 1878); AP, Porto Grande*, Serra do Navio*; PA, Belém*; RO, Cacaulândia*, Porto Velho*
193.	*Heliura postcoeruleum* (Rothschild, 1912)	AM, Manicoré (Zerny 1931), Tefé (Zerny 1931); PA, [Belém] (Hampson 1914), Santarém {Taperinha} (Zerny 1931); RO, [Porto Velho] {Rio Madeira, Aliança and Calama} (Rothschild 1912)
194.	*Heliura rhodophila* (Walker, 1856)	AM, Manicoré*; MA, Açailândia*; PA, [Belém] (Butler 1876), Altamira {Monte Santo} (Teston & Delfina 2010), Marabá*, Santarém {Taperinha} (Zerny 1931); RO, Ariquemes*
195.	*Heliura semihyalina* (Rothschild, 1912)	AM, Fonte Boa (Rothschild 1912)
196.	*Heliura stolli* Rothschild, 1912	AM, Benjamin Constant*, Fonte Boa (Rothschild 1912), Tefé (Rothschild 1912)
197.	*Heliura suffusa* (Lathy, 1899)	PA, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
198.	*Heliura tetragramma* (Walker, 1854)	MA, Açailândia*; MT, Sinop*; PA, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém (Walker 1854a), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
199.	*Heliura thysbodes* Dognin, 1914	AM, São Paulo de Olivença (Dognin 1923)
200.	*Heliura zonata* Druce, 1905	AM, Benjamin Constant*, Fonte Boa*, Manaus*; MA, Açailândia*; MT, Sinop*; PA, Óbidos*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
201.	*Herea metaxanthus* (Walker, 1854)	PA, [Belém] (Walker 1854a)
202.	*Herea ruficeps* (Walker, 1854)	PA, [Belém] (Walker 1854a), Santarém {Taperinha} (Zerny 1931), Tapajós [river] (Hampson 1898)
203.	*Hyaleucerea erythotela* (Walker, 1854)	PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), [Belém] (Walker 1854a), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
204.	*Hyaleucerea fusiformis* (Walker, 1856)	AM, Valley of Amazonas [river] (Walker 1856), São Gabriel [da Cachoeira] {Taracuá} (Bryk 1953); PA, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
205.	*Hyaleucerea lemoulti* Schaus, 1905	PA, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931)
206.	*Hyaleucerea leucosticta* Druce, 1905	PA, Belterra {National Forest of Tapajós} (Freitas 2014), Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)

Continuation Table 1.

	Species	Record
207.	*Hyaleucerea manicorensis* Rêgo Barros & Machado Filho, 1971	AM, Manicoré (Rêgo Barros & Machado Filho 1971)
208.	*Hyaleucerea trifasciata* (Butler, 1877)	AM, [Lábrea] {Rio Purus, Jurucuá} (Butler 1877), Solimões [river] (Hampson 1898)
209.	*Hyaleucerea vulnerata* Butler, 1875	AM, São Paulo de Olivença (Rêgo Barros & Machado Filho 1971); AP, Serra do Navio (Rêgo Barros & Machado Filho 1971); MT, Sinop*; PA (Travassos Filho 1940), Marabá*, Novo Progresso {Cachimbo}*, Parauapebas {Serra Norte, Carajás}*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*
210.	*Hypocladia elongata* Druce, 1905	AM, Benjamin Constant*, Manaus (Zerny 1931), Tefé*; AP, Serra do Navio*; PA, [Igarapé-Miri] {estuary of Tocantins [river]} (Zerny 1931), Santarém {Taperinha} (Zerny 1931)
211.	*Hypocladia militaris* (Butler, 1877)	AM, Silves (Butler 1877); RO, Cacaulândia*
212.	*Hypocladia parcipuncta* Hampson, 1909	PA, [Breves] {Furos de Breves} (Zerny 1931)
213.	*Loxozona lanceolata* (Walker, 1854)	PA, [Belém] (Walker 1854a), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
214.	*Lymire fulvicollis* Dognin, 1914	AM, São Gabriel [da Cachoeira] (Bryk 1953)
215.	*Lymire metamelas* (Walker, 1854)	AM, Santa Isabel do Rio Negro*; AP, Serra do Navio*; PA, [Belém] (Walker 1854a), Breves*, Santarém {Taperinha} (Zerny 1931); RO, Porto Velho*
216.	*Lymire nitens* (Rothschild, 1912)NEW	PA, Belém*
217.	*Lymire strigivenia* Druce, 1905	PA, Belterra {National Forest of Tapajós} (Freitas 2014)
218.	*Metastatia pyrrhorhoea* (Hübner, 1827)	AM, Manicoré*, [Maués] {Rio Parauary}*, Santa Isabel do Rio Negro*, Tefé*; PA, Anajás*, [Belém] (Hampson 1898), Capitão Poço*, Óbidos*, Ourém*, Santarém {Taperinha} (Zerny 1931)
219.	*Paraethria angustipennis* (Rothschild, 1911)	PA, Itaituba to Óbidos (Rothschild 1911)
220.	*Paraethria mapiria* Draudt, 1915	PA, [Belém](Hagmann 1938), Belterra {National Forest of Tapajós} (Freitas 2014)
221.	*Patreliura capys* (Cramer, 1775)	AM, Beruri {Rio Purus, Aruman} (Zerny 1931), Borba*, [Lábrea] {Rio Purus, Jurucuá} (Zerny 1931), Rio Juruá (Hampson 1898), [Santa Isabel do Rio Negro] {Rio Preto}*, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953), São Paulo de Olivença*, Tefé {Ega} (Butler 1876); PA, Marabá*, Santarém {Taperinha} (Zerny 1931), [Rio] Tapajós (Butler 1876); RO, Porto Velho*; [RR], [Caracaraí] {Rio Branc[o]} (Bryk 1953)
222.	*Philoros rubriceps* (Walker, 1854)	[AM], (Zerny 1912); PA, Belém*
223.	*Pseudaclytia opponens* (Walker, [1865])	AM, [Autazes] {Rio Autaz} (Bryk 1953), Barcelos {Rio Negro , Carvoeiro} (Butler 1878), Borba*, Manicoré*, [Maués] {Rio Maués} (Butler 1878), Rio Juruá (Butler 1878), Rio Negro (Butler 1878), Rio Purus (Bryk 1953), São Gabriel [da Cachoeira] (Bryk 1953), Tefé {Ega}(Walker [1865]), Tonantins (Butler 1878); AP, Serra do Navio*; PA, Breves*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*, Porto Velho*; [RR], [Caracaraí] {Rio Branco} (Bryk 1953)
224.	*Pseudaclytia umbrica* Druce, 1898AMZ	AM, Fonte Boa*
225.	*Pseudaclytia unimacula* (Schaus, 1905)	PA, Belterra {National Forest of Tapajós} (Freitas 2014)
226.	*Pseudohyaleucerea romani* (Bryk, 1953)	AM, Manaus (Bryk 1953)
227.	*Pseudopompilia mimica* Druce, 1898	[AM], Amazonas [river] (Druce 1898); AM, Fonte Boa*, Manicoré*, Tefé*; PA, Belém*, Novo Progresso {Cachimbo}*, Parauapebas {Serra Norte, Carajás}*, Santarém (Valente et al. 2018), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*, Jarú*
228.	*Pseudosphenoptera basalis* (Walker, 1854)	AM, Tefé {Ega} (Walker 1854a)

Continuation Table 1.

	Species	Record
229.	*Pseudosphenoptera boyi* Zerny, 1931	PA, Santarém {Mojú} (Zerny 1931)
230.	*Pseudosphex aracia* D. Jones, 1914NEW	PA, Belém*, Breves*, Santarém*
231.	*Pseudosphex caurensis* Klages, 1906	PA, [Belém] (Rothschild 1931)
232.	*Pseudosphex ichneumonea* Herrich-Schäffer, 1854AMZ	AM, Santa Isabel do Rio Negro*; PA, Novo Progresso {Cachimbo}*
233.	*Pseudosphex klagesi* Rothschild, 1911	PA [Belém] (Hagmann 1938), Santarém*
234.	*Pseudosphex polistes* Hübner, 1827	AM, Tefé {Ega} (Walker 1854a); PA, Belém*, Capitão Poço*, Santarém {Taperinha} (Zerny 1931); RO, [Porto Velho] {Rio Machados} (Zerny 1931)
235.	*Pseudosphex rubripalpus* Hampson, 1901AMZ	AM, Manicoré*; MT, Sinop*
236.	*Ptychotricos fenestrifer* Zerny, 1931	PA, Santarém {Taperinha} (Zerny 1931)
237.	*Ptychotricos zeus* Schaus, 1894	MA, Açailândia*; MT, Sinop*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Novo Progresso {Cachimbo}*, Parauapebas {Serra Norte, Carajás}*, Santarém {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
238.	*Riccia aliaria* (Druce, 1890)	AM, Borba*, São Paulo de Olivença (Travassos Filho 1953)
239.	*Schasiura mimica* Butler, 1877	AM, Rio Solimões {Barreira das Araras} (Butler 1877); MT, Sinop*; PA, Novo Progresso {Cachimbo}*; RO, Cacaulândia*
240.	*Sciopsyche tropica* (Walker, 1854)	AM, Tefé (Butler 1876); PA, Santarém (Butler 1876); RO, Cacaulândia*, Jarú*
241.	*Sesiura smaragdina* (Walker, [1865])	AM, [Autazes] {Rio Autaz} (Bryk 1953), Manicoré*, Tefé {Ega} (Walker [1865]); PA, Belém*, Santarém*
242.	*Syntrichura sphecomorpha* Bryk, 1953	AM, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953)
243.	*Syntrichura virens* Butler, 1876NEW	AM, São Paulo de Olivença*; PA, Belém*, Santarém*
244.	*Telioneura albapese* (Druce, 1898)	AM, Manicoré*; MT, Sinop*; PA, Belém*, Santarém (Valente et al. 2018)
245.	*Telioneura brevipennis* (Butler, 1877)	AM, Manicoré*, [Tapauá] {Rio Purus, Guajaratuba} (Butler 1877); MA, Açailândia*; PA, Altamira {Monte Santo} (Delfina & Teston 2013), Belém*; RO, Porto Velho*
246.	*Telioneura fuliginosa* (Rothschild, 1910)	AM, Fonte Boa (Rothschild 1910)
247.	*Telioneura glaucopis* R. Felder, 1869	AM, Barcelos {Moura}*, Beruri {Rio Purus, Aruman} (Butler 1878), [Canutama] {Rio Purus, Urucuri} (Butler 1878), [Carauari] {Rio Juruá, Curimatá} (Butler 1878), [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), Fonte Boa*, [Lábrea] {Rio Purus, Jurucuá e Man[aua]} (Butler 1878), Manicoré*, [Maués] {Rio Parauary}*, Amazonas [river] (R. Felder 1869), Rio Negro (Butler 1878), São Paulo de Olivença*, Tefé*; AP, Serra do Navio*; PA, Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Parauapebas {Serra Norte, Carajás}*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*, Candeias do Jamari*, Jarú*, Porto Velho*
248.	*Telioneura hypophaeus* (Hampson, 1905)	PA, Santarém {Taperinha} (Zerny 1931)
249.	*Theages albidias* (Rothschild, 1912)	AM, Fonte Boa (Hampson 1914), Manaus (Hampson 1914); PA, Marabá*, [Novo Progresso] {Cachimbo} (Travassos 1964b), Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*, Porto Velho*
250.	*Theages leucophaea* Walker, 1855	PA, São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015)
251.	*Theages xanthura* (Schaus, 1910)AMZ	AM, Benjamin Constant*; MT, Sinop*; PA, Santarém*; RO, Cacaulândia*, Candeias do Jamari*
252.	*Timalus leucomela* (Walker, 1856)	AM, Manicoré*; MT, Sinop*; PA, [Belém] (Walker 1856), Novo Progresso {Cachimbo}*, Parauapebas {Serra Norte, Carajás}*, Santarém {Mojú} and {Taperinha} (Zerny 1931), São Félix do Xingu {Serra do Pardo National Park} (Teston & Correa 2015); RO, Cacaulândia*

Continuation Table 1.

	Species	Record
253.	*Trichura aurifera* Butler, 1876	AM, Santa Isabel do Rio Negro*; AP, Serra do Navio*; MA, Santa Luzia*; PA, [Belém] (Butler 1876), Altamira {Serra do Pardo National Park} (Teston & Correa 2015), Santarém {Taperinha} (Zerny 1931)
254.	*Trichura cerberus* (Pallas, 1772)	AM, Manaus*, Upper Amazon (Zerny 1912), São Paulo de Olivença*, Santa Isabel do Rio Negro*; PA, Belém*, Breves*, Capitão Poço*, Óbidos*, Santarém {Taperinha} (Zerny 1931); RO, Porto Velho*
255.	*Trichura coarctata* (Drury, 1773)	AM, Manaus*, [São Gabriel da Cachoeira] {Taracuá} (Bryk 1953); PA, [Belém] (Butler 1876), Santarém*
256.	*Trichura dixanthia* Hampson, 1898NEW	AM, Manaus*
257.	*Trichura esmeralda* (Walker, 1854)	AM, Anamã*; PA, [Belém] (Draudt 1915)
258.	*Trichura latifascia* (Walker, 1854)	PA, [Belém] (Walker 1854a), Santarém*
259.	*Trichura mathina* Druce, 1898	AM, [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), São Paulo de Olivença*; PA, [Belém] (Druce 1898), Santarém*
260.	*Uranophora albiplaga* (Walker, 1854)NEW	AM, Benjamin Constant*; MA, Açailândia*; PA, Parauapebas {Serra Norte, Carajás}*; RO, Cacaulândia*
261.	*Uranophora alterata* (Walker, [1865])	AM, Tefé (Walker [1865]); PA (Hagmann 1938); RO, Cacaulândia*
262.	*Uranophora atalanta* (Druce, 1899)	[AM], Amazonas [river] (Druce 1899)
263.	*Uranophora felderi* (Zerny, 1912)NEW	AM, Manicoré*
264.	*Uranophora jynx* (Hübner-Geyer, 1832)	AM, Amazonas [river] (Zerny 1912), Fonte Boa*, São Paulo de Olivença*
265.	*Uranophora leucotela* (Butler, 1876)	AM, Manaus*; PA, Altamira {Monte Santo} (Teston & Delfina 2010), Itaituba*, Santarém {Taperinha} (Zerny 1931)
266.	*Uranophora metamela* (Dognin, 1911)	PA, [Belém] (Gaede 1926)
267.	*Uranophora quadrimaculata* (Möschler, 1872) NEW	AM, São Gabriel da Cachoeira*
268.	*Uranophora splendida* (Herrich-Schäffer, [1854])AMZ	AM, Tefé*; RO, Candeias do Jamari*
269.	*Uranophora superba* (Druce, 1906)NEW	MA, Santa Luzia*
270.	*Uranophora terminalis romani* (Bryk, 1953)	AM, Manaus (Bryk 1953); RO, Porto Velho*
271.	*Uranophora walkeri* (Druce, 1889)	AP, Serra do Navio*; MA, Açailândia*; PA, Belterra {National Forest of Tapajós} (Freitas 2014), Marabá*, Parauapebas {Serra Norte, Carajás}*, Santarém {Taperinha} (Zerny 1931); RO, Cacaulândia*
272.	*Urolosia brodea* (Schaus, 1896)	PA, Santarém {Taperinha} (Zerny 1931)
273.	*Xanthopleura perspicua* (Walker, 1856)	AM, [Eirunepé] {Rio Juruá, São Felipe} (Zerny 1931), Manicoré*; PA, Altamira {Monte Santo} (Delfina & Teston 2013), [Belém] (Walker 1856), Belterra {National Forest of Tapajós} (Freitas 2014), Novo Progresso {Cachimbo}*, Parauapebas {Serra Norte, Carajás}*, Santarém*; RO, Cacaulândia*

AMZ Species recorded for states within the Amazon biome by Ferro and Diniz (2010), but without precise location and biome information + species recorded in Ferro and Diniz (2007). So these species are new records for the municipalities. NEW New record for the Brazilian Amazon.

Table 2. Geographic coordinates of municipalities and richness of the Ctenuchina species (Erebidae, Arctiinae, Arctini) in the Brazillian Amazon.

Nº	State	Municipality	Richness	Latitude	Longitude
1	AC	Feijó	2	08°09'52"S	70°21'13"W
2	AP	Mazagão	1	00°06'55"S	51°17'21"W
3	AP	Oiapóque	1	03°50'35"N	51°50'05"W
4	AP	Porto Grande	1	00°37'01"N	51°38'60"W
5	AP	Santana	1	00°03'30"S	51°10'54"W
6	AP	Serra do Navio	27	00°53'45"N	52°00'07"W
7	AM	Anamã	1	03°34'47"S	61°24'15"W
8	AM	Autazes	4	03°24'02"S	58°57'00"W
9	AM	Atalaia do Norte	4	04°25'46"S	70°15'41"W
10	AM	Barcelos	6	01°27'01"S	61°37'59"W
11	AM	Benjamin Constant	24	04°22'60"S	70°01'52"W
12	AM	Beruri	4	04°44'09"S	62°09'01"W
13	AM	Boa Vista do Ramos	1	03°03'19"S	57°38'20"W
14	AM	Borba	12	04°23'17"S	59°35'37"W
15	AM	Canutama	2	06°32'02"S	64°22'59"W
16	AM	Carauari	6	05°03'01"S	66°56'59"W
17	AM	Codajás	1	03°50'13"S	62°03'25"W
18	AM	Eirunepé	13	06°40'01"S	69°52'00"W
19	AM	Fonte Boa	28	02°30'51"S	66°05'30"W
20	AM	Humaitá	4	07°30'23"S	63°01'14"W
21	AM	Itacoatiara	1	03°08'36"S	58°26'39"W
22	AM	Jutaí	12	04°18'57"S	67°20'42"W
23	AM	Lábrea	7	07°18'51"S	65°08'40"W
24	AM	Manaus	31	03°06'07"S	60°01'30"W
25	AM	Manicoré	41	05°48'34"S	61°18'00"W
26	AM	Maués	9	03°23'01"S	57°43'07"W
27	AM	Novo Airão	3	02°37'37"S	60°57'00"W
28	AM	Santa Isabel do Rio Negro	18	00°24'51"S	65°01'08"W
29A	AM	São Gabriel da Cachoeira	18	00°07'50"S	67°05'20"W
29B	AM	São Gabriel da Cachoeira {Querari}	3	01º04'60"N	69°50'59"W
30	AM	São Paulo de Olivença	46	03°22'42"S	68°52'20"W
31	AM	Silves	1	02°50'20"S	58°12'33"W
32	AM	Tabatinga	2	04°15'10"S	69°56'17"W
33	AM	Tapauá	3	05°37'42"S	63°11'00"W
34	AM	Tefé	38	03°21'16"S	64°42'40"W
35	AM	Tonantins	3	02°52'24"S	67°48'08"W
36	MA	Açailândia	28	04°56'49"S	47°30'17"W
37	MA	Santa Luzia	4	03°57'48"S	45°39'29"W
38	MT	Aripuanã	2	10°10'01"S	59°27'33"W
39	MT	Sinop	24	11°52'51"S	55°30'08"W
40	PA	Almeirim	2	01º31'24"S	52º34'54"W

Continuation Table 2.

41	PA	Alenquer	1	01º56'31"S	54°44'18"W
42A	PA	Altamira[†]	22	03º11'55"S	52º10'15"W
42B	PA	Altamira {Serra do Pardo National Park}[†]	16	05°38'21"S	52º41'52"W
43	PA	Anajás	3	00°59'13"S	49°56'23"W
44	PA	Belém	87	01°27'21"S	48°30'15"W
45	PA	Belterra {National Forest of Tapajós}[†]	28	03°01'05"S	54°58'10"W
46	PA	Benevides	2	01°21'42"S	48°14'40"W
47	PA	Breves	21	01°40'56"S	50°28'49"W
48	PA	Cametá	3	02°14'40"S	49°29'45"W
49	PA	Capitão Poço	7	01°44'48"S	47°03'33"W
50	PA	Chaves	1	00°09'36"S	49°59'18"W
51	PA	Curralinho	1	01°48'49"S	49°47'42"W
52	PA	Igarapé Açú	1	01°07'45"S	47°37'11"W
53	PA	Igarapé-Miri	2	01°58'31"S	48°57'34"W
54	PA	Itaituba	6	04°16'35"S	55°59'01"W
55	PA	Marabá	27	05°22'07"S	49°07'04"W
56	PA	Novo Progresso {Cachimbo}	15	09°19'60"S	54°52'59"W
57	PA	Óbidos	5	01°55'04"S	55°31'04"W
58	PA	Oriximiná	3	01°21'60"S	56°04'44"W
59	PA	Ourém	2	01°33'07"S	47°06'52"W
60	PA	Paragominas	1	02°59'45"S	47°21'10"W
61	PA	Parauapebas	14	06°00'56"S	50°17'51"W
62	PA	Santarém	139	02°26'36"S	54°42'29"W
63	PA	São Félix do Xingu {Serra do Pardo National Park}[†]	44	05º46'26"S	52°37'13"W
64	PA	São Miguel do Guamá	1	01°37'37"S	47°29'00"W
65	PA	Soure	1	00°43'01"S	48°31'24"W
66	PA	Tucuruí	2	03°42'01"S	49°42'00"W
67	PA	Viseu	2	01°11'49"S	46°08'23"W
68	RO	Ariquemes	7	09°54'48"S	63°02'26"W
69	RO	Cacaulândia	60	10°20'21"S	62°53'43"W
70	RO	Candeias do Jamari	5	08°48'35"S	63°41'44"W
71	RO	Jarú	6	10°26'20"S	62°27'58"W
72	RO	Porto Velho	45	08°45'43"S	63°54'13"W
73	RO	Vila Rondônia	2	10°52'01"S	61°57'00"W
74	RR	Alto Alegre	3	02°53'45"N	61°29'51"W
75	RR	Caracaraí	3	01°47'60"N	61°07'50"W

Geographic coordinates of municipality marked with [†] are the citations referred, other obtained by Google Earth or Geo Loc tool (see Materials and Methods).

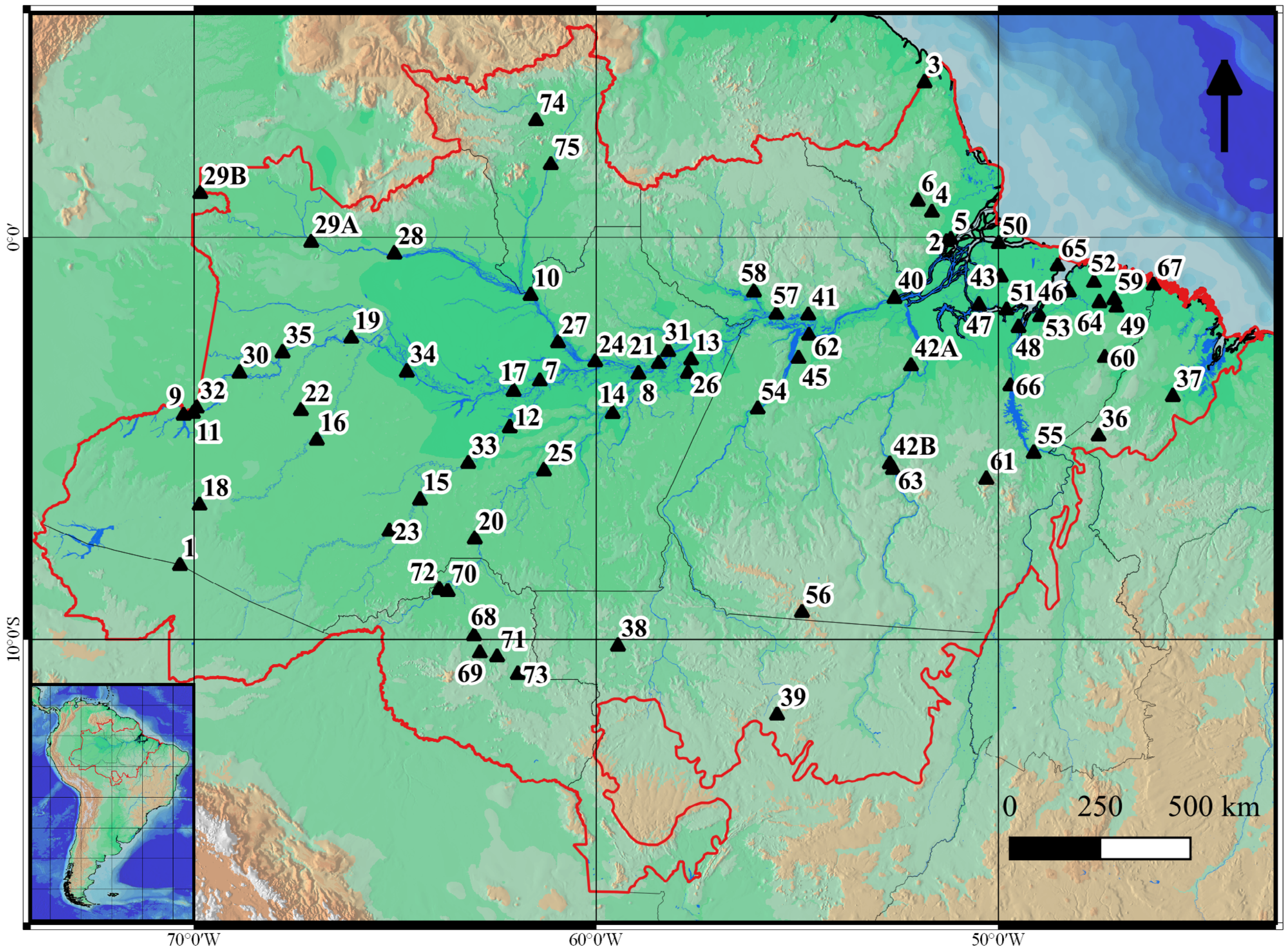


Figure 1. Geographic distribution of Ctenuchiina species records in Brazillian Amazon. The numbers refer to the municipalities of Table 2.

Acknowledgements

We are indebted to the museum curators for allowing VGF's access to the collections under their care and for logistical support. This publication is part of the RedeLep "Rede Nacional de Pesquisa e Conservação de Lepidópteros", SISBIOTA-Brasil, CNPq (563332/2010-7).

Author contributions

José A. Teston: Substantial contribution in the concept and design of the study.

Viviane G. Ferr: Substantial contribution in the concept and design of the study.

Débora S. de Abreu: Contribution to data collection.

Conflicts of interest

The authors declares that they have no conflict of interest related to the publication of this manuscript.

References

BRYK, F. 1953. Lepidoptera aus dem amazonasgebiete und aus Peru gesammelt von Dr. Douglas Melin und Dr. Abraham Roman. Ark. zool. 5(1): 1–268.

BUTLER, A.G. 1876. Notes on the Lepidoptera of the family Zygaenidae, whit descriptions of new genera and species. J. Linn. Soc. Lond., Zool. 12(60-62): 342–407.

BUTLER, A.G. 1877. Illustrations of typical specimens of Lepidoptera Heterocera in the British Museum part I. Taylor & Francis, London.

BUTLER, A.G. 1878. On the Lepidoptera of the Amazons, collected by Dr James W. H. Trail during the years 1873 to 1875. Trans. Entomol. Soc. Lond. 26(1): 39–84.

CONNER, W.E. & JORDAN, A.T. 2009. From armaments to ornaments: the relationship between chemical defense and sex in tiger moths. In Tiger moths and wooly bears. Behavior, ecology, and evolution of the Arctiidae (W.E. Conner, ed.). Oxford University Press, New York, p.155–172.

DELFINA, M.C. & TESTON, J.A. 2013. Arctiinae (Lepidoptera, Arctiidae) ocorrentes em uma área de pastagem na Amazônia Oriental em Altamira, Pará, Brasil. Acta Amaz. 43(1): 81–90.

DOGNIN, P. 1921. Hétérocères nouveaux de l'Amérique du Sud Fascicule XIX. Imprimerie Oberthür, Rennes.

DOGNIN, P. 1923. Hétérocères nouveaux de l'Amérique du Sud Fascicule XXIII. Imprimerie Oberthür, Rennes.

DRAUDT, M. 1914-1919. Familie: Syntomidae. In Die Gross-Schmetterlinge der Erde. II. Abteilung: Die Gross-Schmetterlinge des Amerikanischen Faunengebietes. 6. Band. Die Amerikanischen Spinner und Schwärmer (A. Seitz, ed.). Alfred Kernen, Stuttgart, p.33–230.

DRUCE, H. 1884 (1881-1900). Insecta. Lepidoptera-Heterocera. Vol. I. In Biologia Centrali-Americana (F.D. Godman & O. Salvin, eds.). H. R. Porter, London, p.1-490.

DRUCE, H. 1893. Descriptions of new species of Lepidoptera Heterocera from Central and South America. Proc. Zool. Soc. Lond. 1893: 280–311.

DRUCE, H. 1895. Descriptions of some new Species of Heterocera from the Eastern Islands and Tropical America. Ann. Mag. nat. Hist. 15(6): 41–50.

DRUCE, H. 1898. Descriptions of some new species of Syntomidae, chiefly in the Oxford Museum. Ann. Mag. nat. Hist. 7(1): 401–408.

DRUCE, H. 1899. Descriptions of some new species of Heterocera from Tropical America. Ann. Mag. nat. Hist. 7(3): 293–302.

FELDER, C. & FELDER, R. 1864–1875. Pl 75–120 (1874). In Reise der österreichischen fregatte novara um die Erde in den Jahren 1857, 1858, 1859 unter den Befehlen des Commodore B. Von Wüllestorf-Urbair. Zoologischer Theil, Zweiter Band, Zweite Abtheilung: Lepidoptera (C. Felder, R. Felder & A.F. Rogenhofer, eds.). Staatsdruckerei, Vienna.

FERRO, V.G. & DINIZ, I.R. 2007. Arctiidae (Insecta: Lepidoptera) da Estação Biológica de Boracéia (Salesópolis, São Paulo, Brasil). Biota Neotrop. 7(3): 331–338. http://dx.doi.org/10.1590/S1676-06032007000300033 (last access on 15/07/2018)

FERRO, V.G. & DINIZ, I.R. 2010. Riqueza e composição das mariposas Arctiidae (Lepidoptera) no Cerrado. In Cerrado: conhecimento quantitativo como subsídio para as ações de conservação (I.R. Diniz, J. Marinho-Filho, R.B. Machado & R. Cavalcanti, eds.). Editora Thesaurus, Brasília, p.255–313.

FERRO, V.G., MELO, A.S. & DINIZ, I.R. 2010. Richness of tiger moths (Lepidoptera: Arctiidae) in the Brazilian Cerrado: how much do we know? Zoologia 27(5): 725–731.

FERRO, V.G., RESENDE, I.M.H. & DUARTE, M. 2012. Arctiinae (Lepidoptera: Erebidae) do estado de Santa Catarina, Brasil. Biota Neotrop. 12(4): 166–180. http://dx.doi.org/10.1590/S1676-06032012000400018 (last access on 15/07/2018)

FIELD, W.D. 1975. Ctenuchid moths of *Ceramidia* Butler, *Ceramidiodes* Hampson, and the Caca Species Group of *Antichloris* Hubner. Washington. Smithson. contrib. zool. 198(1): 1–44.

FREITAS, M.P. de. 2014. Estratificação vertical de Arctiini (Lepidoptera, Erebidae, Arctiinae) na Floresta Nacional do Tapajós, Amazônia Oriental, Pará, Brasil. Dissertação de Mestrado, Universidade Federal do Oeste do Pará, Santarém.

GAEDE, M. 1926. Amatiden des Berliner Zoologischen Museums (Lep.). Dtsch. entomol. z. 1926(2): 113–136.

HAGMANN, G. 1938. Syntomideos (Amatideos=Euchromideos) do Estado do Pará. In Livro Jubilar do Professor Lauro Travassos. Typographia do Instituto Oswaldo Cruz, Rio de Janeiro, p.185–194.

HAMPSON, G.F. 1898. Catalogue of the Lepidoptera Phalaenae in the British Museum. Catalogue of the Syntomidae in the collection of the British Museum. Taylor & Francis, London.

HAMPSON, G.F. 1901. Catalogue of the Lepidoptera Phalaenae in the British Museum. Catalogue of the Arctiadae (Arctianae) and Agaristidae in the collection of the British Museum. Taylor & Francis, London.

HAMPSON, G.F. 1914. Catalogue of the Lepidoptera Phalaenae in the British Museum. Supplement. Volume I. Catalogue of the Amatidae and Arctiadae (Nolinae and Lithosianae) in the collection of the British Museum. Taylor & Francis, London.

HAWES, J., MOTTA, C. da S., OVERAL, W.L., BARLOW, J., GARDNER, T.A. & PERES, C.A. 2009. Diversity and composition of Amazonian moths in primary, secondary and plantation forest. J. Trop. Ecol. 25(3): 281–300.

HEPPNER, J.B. 1991. Faunal regions and the diversity of Lepidoptera. Trop. Lep. 2(suppl. 1): 1–85.

HERNÁNDEZ-BAZ, F., MORON, M.A., CHAMÉ-VAZQUEZ, E. & GONZÁLEZ, J.M. 2013. Ctenuchina and Euchromiina (Lepidoptera: Erebidae: Arctiinae) of Three Biological Research Stations of México's Ecosur. J. Lepid. Soc. 67(3): 145-155.

HERRICH-SCHÄFFER, G.A.W. [1855]. Sammlung neuer oder bekannter ausseuropaischer Schmetterlinge 1. G.J. Manz, Regensburg.

JACOBSON, N.L. & WELLER, S.J. 2002. A cladistics study of the Arctiidae (Lepidoptera) by using characters of immatures and adults. Thomas Say Publications in Entomology, Lanham.

JANZEN, D. & HALLWACHS, W. 2005. Philosophy, navigation and use of a dynamic database (ACG Caterpillars SRNP) for an inventory of the macrocaterpillar fauna, and its foodplants and parasitoids of the Area de Conservación Guanacaste (ACG), north-western Costa Rica. http://janzen.sas.upenn.edu (last access in 30/07/2018).

KITCHING, I.J. & RAWLINS, J.E. 1999. The Noctuoidea. In Lepidoptera, moths and butterflies. Volume 1: evolution, systematics, and biogeography (N.P. Kristensen, ed. vol.). In Handbook of zoology. Volume IV Arthropoda: insect (M. Fischer, ed.). de Gruyter, Berlin, p.355–401.

MACHADO FILHO, J. & RÊGO BARROS, A. 1970. Ctenuchidae Neotropicais V. Redescrição do gênero *Argyrtidia* Hampson, 1898 e descrição de nova espécie (Lepidoptera - Heterocera). Bol. Mus. Nac. 278(1): 1–17.

MORENO, C. & FERRO, V.G. 2016. Arctiinae moths (Lepidoptera, Erebidae) of the Emas National Park, Goiás, Brazil. Biota Neotropica 16, p. e20150037. doi: http://dx.doi.org/10.1590/1676-0611-BN-2015-0037 (last access on 31/07/2018).

PINHEIRO, L.R. 2016. Re-evaluation of the identities of *Eucereon punctatum* (Guérin-Méneville, [1844]) and *E. archias* (Stoll, 1790), with a discussion on *E. mitigatum* Walker, 1857, rev. stat. (Lepidoptera, Erebidae, Arctiinae, Arctiini, Ctenuchina). Zoosystema 38(1): 127–140.

PINHEIRO, L.R. & DUARTE, M. 2013. Taxonomic notes on Ctenuchina, Euchromiina, and Phaegopterina (Lepidoptera, Erebidae, Arctiinae, Arctiini). Fla. Entomol. 96(2): 351–359.

PINHEIRO L.R, & GAAL-HASZLER, S. 2015. Illustrated catalogue of Neotropical Ctenuchina, Euchromiina and Pericopina types (Lepidoptera, Erebidae, Arctiinae, Arctiini) described by Hans Zerny, with discussion on their taxonomic status. Zootaxa. 3925(4): 505–535.

PINHEIRO, L.R. & MAY, W. 2015. Revision of the Genus *Pseudaethria* Schaus (Lepidoptera, Erebidae). Neotrop. Entomol. 44(6): 588–595.

RÊGO BARROS, A. & MACHADO FILHO, J. 1971. Estudo dos Ctenuchidae Neotropicais - I Sobre o gênero *Hyaleucerea* Butler, 1875 (Lepidoptera – Heterecera). Arq. Mus. Nac. 54(1): 171–176.

ROTHSCHILD, L.W. 1910. Descriptions of new species of Arctiinae in the Tring Museum. Novit. zool. 17(1): 172–188.

ROTHSCHILD, L.W. 1911. New Syntomidae and Arctianae. Novit. zool. 18(1): 154–158.

ROTHSCHILD, L.W. 1912. New Syntomidae. Novit. zool. 19(1): 151–186.

SANTOS, N. 1939. Contribuição ao conhecimento dos Euchromiidae IV. Gênero *Androcharta* Felder, 1862. (Lepidoptera). Boletim Biológico 4(1): 87–97.

SCHERRER, S., FERRO, V.G., RAMOS, M.N. & DINIZ, I.R. 2013. Species composition and temporal activity of Arctiinae (Lepidoptera: Erebidae) in two cerrado vegetation types. Zoologia 30(2): 200–210.

SEITZ, A. 1919-1925. 4. Familie: Arctiidae, Bärenspinner. In Die Gross-Schmetterlinge der Erde. II. Abteilung: Die Gross-Schmetterlinge des Amerikanischen Faunengebietes. 6. Band. Die Amerikanischen Spinner und Schwärmer (A. Seitz, ed.). Alfred Kernen, Stuttgart, p.231–497.

SIMONS, R.B. 2009. Adaptative coloration and mimicry. In Tiger moths and wooly bears. Behavior, ecology, and evolution of the Arctiidae (W.E. Conner, ed.). Oxford University Press, New York, p.115–126.

STAUDINGER, O. 1894 Hochandine Lepidopteren. Dtsch. entomol. z. IRIS. 7(1): 43–100.

TESTON, J.A. & CORREA, D. do C.V. 2015. The Arctiini (Lepidoptera, Erebidae, Arctiinae) fauna of the Serra do Pardo National Park, Pará, Brazil. Check List 11(2): 1-9.

TESTON, J.A. & DELFINA, M.C. 2010. Diversidade de Arctiinae (Lepidoptera, Arctiidae) em área alterada em Altamira, Amazônia Oriental, Pará, Brasil. Acta Amaz. 40(2): 387–396.

TESTON, J.A., NOVAES, J.B. & ALMEIDA JUNIOR, J.O.B. 2012. Abundância, Composição e Diversidade de Arctinae (Lepidoptera, Arctiidae) em um fragmento de floresta na Amazônia Oriental em Altamira, PA, Brasil. Acta Amaz. 42(1): 105–114.

TESTON, J.A. & FERRO, V.G. 2016a. Arctiini Leach, [1815] (Lepidoptera, Erebidae, Arctiinae) of the Brazilian Amazon. I – Subtribe Phaegopterina Kirby, 1892. Check List 12(2): 1-16.

TESTON, J.A. & FERRO, V.G. 2016b. Arctiini Leach, [1815] (Lepidoptera, Erebidae, Arctiinae) of the Brazilian Amazon. II – Subtribe Pericopina Walker, [1865]. Check List 12(6): 1-7.

TRAVASSOS, L. 1952. Contribuição ao conhecimento dos "Arctiidae". XXX, Espécies do gênero "*Nelphe*" H. Schaeffer, 1855. Rev. Bras. Biol. 12(4): 369–384.

TRAVASSOS, L. 1959. Contribuição ao conhecimento dos "Arctiidae". XLII. Gênero *Eucereon* Huebner, 1819 (Lepidoptera, Heterocera). Mem. Inst. Oswaldo Cruz 57(2): 172–184.

TRAVASSOS, L. 1964a. Contribuição ao conhecimento dos Arctiidae. XLIII. Descrição da genitália de cinco espécies dos gêneros *Eucereon* Hubner, 1819 e *Eucereoides* Travassos, 1961. Rev. Bras. Entomol. 11(1): 9–29.

TRAVASSOS, L. 1964b. Cobtribuição ao conhecimento dos Arctiidae (Lepidoptera-Heterocera) gênero *Theages* Walker 1855. An. Acad. Bras. Cienc. 36(2): 173–188.

TRAVASSOS FILHO, L. 1938. Contribuição ao conhecimento dos Euchromiidae. Gênero *Corematura* Butler, 1876. Mem. Inst. Oswaldo Cruz 33(2): 259–262.

TRAVASSOS FILHO, L. 1940. Contribuição à Zoogeografia dos Euchromidae Brasileiros. Arq. Zool. Est. São Paulo. 2(10): 281–297.

TRAVASSOS FILHO, L. 1952. Redescrição de *Corematura* Butler, 1876 e de suas duas espécies. (Lepidoptera Ctenuchidae). Arq. Zool. Est. São Paulo. 8(3): 89–108.

TRAVASSOS FILHO, L. 1953. *Riccia*, novo gênero para *Corematura aliaria* (Druce 1890) e descrição do Alótipo dessa espécie. (Lep. Ctenuchidae). Pap. Avulsos Dep. Zool./Secr. Agric. 11(17): 280–287.

TRAVASSOS FILHO, L. 1957. Contribuição ao conhecimento dos Ctenuchidae. (Lep.) VII- Gênero *Dinia* Walker, 1854. Arq. Zool. Est. São Paulo. 10(2): 186–207.

TRAVASSOS FILHO, L. & URBAN, H. 1967. Bionomia de *Dinia eaegrus* (Cramer, 1779). (Lepidoptera – Ctenuchidae). Pap. Avulsos Zool. 20(18): 217–222.

VALENTE, D.M.P., ZENKER, M.M. & TESTON, J.A. 2018. Tiger-Moths in Savannas in Eastern Amazon: First Assessment of Diversity and Seasonal Aspects. Neotrop. Entomol. 47 (6): doi:10.1007/s13744-017-0579-y.

VINCENT, B. & LAGUERRE, M. 2014. Catalogue of the Neotropical Arctiini Leach, [1815] (except Ctenuchina Kirby, 1837 and Euchromiina Butler, 1876) (Insecta, Lepidoptera, Erebidae, Arctiinae). Zoosystema 36(2): 137–533.

WALKER, F. 1854a. List of the specimens of lepidopterous insects in the collection of the British Museum. Part I. — Lepidoptera Heterocera. Edward Newman, London.

WALKER, F. 1854b. List of the specimens of lepidopterous insects in the collection of the British Museum. Part II. — Lepidoptera Heterocera. Edward Newman, London.

WALKER, F. 1856. List of the specimens of lepidopterous insects in the collection of the British Museum. Part VII. — Lepidoptera Heterocera. Edward Newman, London.

WALKER, F. [1865]. List of the specimens of lepidopterous insects in the collection of the British Museum. Part XXXI. — Supplement. Edward Newman, London.

WALKER, F. 1866. List of the specimens of lepidopterous insects in the collection of the British Museum. Part XXXV. — Supplement Part 5. Edward Newman, London.

WATSON, A., FLETCHER, D.S. & NYE, I.W.B. 1995. Noctuoidea: Arctiidae, Cocytiidae, Ctenuchidae, dilobidae, Dioptidae, lymantriidae, Notodontidae, Strepsimanidae, Thaumetopoeidae & Thyretidae. In the Generic names of moths of the world (I.W.B. Nye, ed.). The Natural Histori Museum, London, v.2, p.1-228.

WELLER, S.J., DACOSTA, M., SIMMONS, R.B., DITTMAR, K. & WHITING, M. 2009. Evolution and taxonomic confusion in Arctiidae. In Tiger Moths and Wolly Bears, Behavior, Ecology and Evolution of the Arctiidae (W.E. Conner, ed.). Oxford University Press, New York, p.11–30.

WELLER, S.J., SIMMONS, R.B., BOADA, R. & CONNER, W.E. 2000. Abdominal Modifications Occurring in Wasp Mimics of the Ctenuchine-Euchromiine Clade (Lepidoptera: Arctiidae). Ann. Entomol. Soc. Am. 93(4): 920–928.

ZAHIRI, R., HOLLOWAY, J.D., KITCHING, I.J., LAFONTAINE, J.D., MUTANEN, M. & WAHLBERG, N. 2012. Molecular phylogenetics of Erebidae (Lepidoptera, Noctuiodea). Syst. Entomol. 37(1): 102–124.

ZERNY, H. 1912. Syntomidae. In Lepidopterorum Catalogus (H. Wagner, ed.). W. Junk, Berlin, p.1–179.

ZERNY, H. 1931. Ergebnisse einer zoologischen Sammerlreise nach Brasilien, insbesondere in das Amazonasgebiet, ausgeführt von Dr. H. Zerny. VII. Teil. Lepidoptera III: Die Syntomiden des Staates Pará. Ann. Nat. hist. Mus. Wien 45(1): 225–263.

Received: 04/10/2018
Revised: 11/12/2018
Accepted: 17/01/2019

Biota Neotropica 19(2): e20180619, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Diversity of soil spiders in land use and management systems in Santa Catarina, Brazil

Marcio Gonçalves da Rosa[1,*], *Antonio Domingos Brescovit*[2], *Carolina Riviera Duarte Maluche Baretta*[3],
Júlio Cesar Pires Santos[1], *Luís Carlos Iuñes de Oliveira Filho*[1] & *Dilmar Baretta*[4]

[1]*Universidade do Estado de Santa Catarina, Centro de Ciências Agroveterinárias, Departamento de Solos e Recursos Naturais, Av. Luiz de Camões, 2090, 88520-000, Lages, SC, Brasil*
[2]*Instituto Butantan, Laboratório Especial de Coleções Zoológicas, Av. Vital Brasil, 1500, 05503-900, São Paulo, SP, Brasil*
[3]*Universidade Comunitária da Região de Chapecó, Av. Servidão Anjo da Guarda, 295-D, 89809-900, Chapecó, SC, Brasil*
[4]*Universidade do Estado de Santa Catarina, Centro de Educação Superior do Oeste, Departamento de Zootecnia, Rua Beloni Trombeta Zanin, 680E, 89815-630, Chapecó, SC, Brasil*

**Corresponding author: Marcio Gonçalves da Rosa, e-mail: mgrmarcio@hotmail.com*

ROSA, M.G.; BRESCOVIT, A.D.; BARETTA, C.R.D.M.; SANTOS, J.C.P.; OLIVEIRA FILHO, L.C.I.; BARETTA, D. **Diversity of soil spiders in land use and management systems in Santa Catarina, Brazil**. Biota Neotropica. 19(2): e20180619. http://dx.doi.org/10.1590/1676-0611-BN-2018-0619

Abstract: The ability of spiders to spread over contiguous areas (Arachnida: Araneae) is directly related to soil management conditions. The objective of this work was to study the effect of land use system (LUS) on the abundance and diversity of soil spider families and their relationship with soil physical and chemical properties. The evaluated LUS were: native forest, eucalyptus reforestation, pasture, crop-livestock integration, and no-tillage crop. Samples were collected in three municipalities of Southern Plateau of Santa Catarina, considered as true replicates, during winter and summer. A total of 270 samples was taken in each area and season. The sampling points were arranged in a grid of 3 × 3 m, spaced by 30 m. We evaluated soil physical, chemical, and microbiological attributes and the abundance and diversity of spider families, collected by soil monolith and soil traps. A total of 448 spiders were captured, 152 in winter and 296 in summer, distributed in 24 families and 52 species/morphospecies. There was a seasonality effect related to the land use systems and the highest Shannon-Wiener diversity index was recorded in the native forest in both sampling periods. Most families of spiders have a direct dependence on soil physical and chemical properties, such as microporosity, exchangeable aluminum, calcium, magnesium, and potassium during the winter. Organic matter, nitrogen, pH in water, weighted average diameter, soil density, and microbial biomass carbon exhibited dependence during the summer. Vegetation type and soil management are the factors that seem to affect most the occurrence of spiders. The families Theridiidae and Nemesiidae are dependent on sites with low human intervention.

Keywords: *Agriculture, Biodiversity, Soil Biology, Soil Ecology.*

Diversidade de aranhas edáficas em sistemas de uso e manejo do solo em Santa Catarina, Brasil

Resumo: A capacidade de dispersão das aranhas em áreas contíguas (Arachnida, Araneae) está ligada diretamente às condições de manejo do solo. Objetivou-se com o presente trabalho estudar o efeito do sistema de uso do solo (SUS) sobre a abundância e diversidade das famílias de aranhas edáficas, bem como a relação dessas com os atributos físicos e químicos do solo. Os SUS avaliados foram: floresta nativa, reflorestamento de eucalipto, pastagem, integração lavoura-pecuária e lavoura com sistema plantio direto. As amostras foram coletadas em três municípios do Planalto Sul-Catarinense, considerados réplicas verdadeiras, durante o inverno e o verão. Um total de 270 amostras foi coletado em cada área e estação do ano. Os pontos de amostragem foram dispostos em uma grade de amostragem de 3 × 3 distanciados 30 m entre si. Foram avaliados atributos físicos, químicos e microbiológicos do solo e abundância e diversidade de famílias de aranhas, coletadas pelo método de monólitos e armadilhas de solo. Ao todo foram capturados 448 indivíduos, sendo 152 no inverno e 296 no verão, distribuídos em 24 famílias, 52 espécies/morfoespécies. Houve efeito da sazonalidade entre os sistemas de uso e a maior diversidade de Shannon-Wiener foi registrada em floresta nativa em ambas as épocas de amostragem. A maior parte das famílias de aranhas possui dependência direta dos atributos físicos e químicos do solo, como: microporosidade e alumínio trocável, cálcio, magnésio e potássio durante o inverno. Matéria orgânica, nitrogênio, pH em água, diâmetro médio ponderado, densidade do solo e carbono da biomassa microbiana apresentaram dependência durante o verão. O tipo de vegetação e o manejo são fatores que mais afetam a ocorrência de aranhas. As famílias Theridiidae e Nemesiidae são dependentes de locais com baixa intervenção antrópica.

Palavras-chave: *Agricultura, Biodiversidade, Biologia do Solo, Ecologia do Solo.*

Introduction

Among the arthropods, spiders are one of the most well-known and diversified groups, occupying different niches and with a cosmopolitan distribution, a characteristic that is basically given by the capacity of exploration and adaptation to various ecological conditions (Mader *et al*. 2016). Spiders are active predators and directly help to control populations, maintaining the balance of the ecosystems. However, the effect of human action on the landscapes has led to increasing losses in biodiversity on many scales, both vegetal and animal (Kovács-Hostyánszki *et al*. 2013, Sas-Kovács *et al*. 2015).

Conventional agriculture is one of the main causes of decline of biodiversity, basically for reducing the vegetal cover and structural complexity of plant communities that are often associated with the low biodiversity of other taxonomic groups (Chen *et al*. 2011, Barsoum *et al*. 2014), because they are predators and dependent on the organizational heterogeneity of the soil fauna. Monoculture and the suppression of forest remnants represent a challenge in terms of management of agroecosystems, especially for reducing the diversity of the soil fauna, particularly of spider communities (Camara *et al*. 2012).

On the other hand, more-diversified managements such as no-tillage and crop-livestock integration can contribute to the sustainable production of food, which generates diverse plant biomass in amount and quality, attracting other organisms that serve as prey for spiders (Liu *et al*. 2015). The fragments of forests are pointed by some authors as buffer zones in areas impacted by the agricultural activity since they work as a temporary refuge for soil organisms (Moraes *et al*. 2015, Jesus *et al*. 2015).

Soil management leads to disturbances, irreversible in many cases, for the soil fauna, and the patches of native forest act as a conditional refuge for the establishment of spider populations (Mineo *et al*. 2010). Many studies have demonstrated that spiders can be an effective indicator of modified environments (Borchard *et al*. 2014, Haase & Balkenhol 2015, Mumme *et al*. 2015, Balkenhol *et al*. 2016). The presence of spiders is frequently associated with the structural quality of the ecosystems, due to their effect of biological control over soil animals. Despite this importance, little is known about the spider fauna in the state of Santa Catarina, especially in agricultural areas.

The present study aimed to evaluate the diversity of spider families in the southern Plateau of the state of Santa Catarina, under different land use and management systems, identifying variations in richness and abundance of these organisms related to an increasing gradient of land use.

Material and Methods

1. *Characterization of the study site*

The study was carried out in the Southern Plateau of Santa Catarina, encompassing land use systems (LUS) with increasing intensity of human intervention, namely: native forest (NF), eucalyptus reforestation (ER), pasture (PA), crop-livestock integration (CLI), and no-tillage crop (NT). The areas were selected according to the previously geographic characteristics, relief, altitude, and soils representative of the region in three municipalities: Campo Belo do Sul (50°39’W and 27°52’S; 1.016 m a.s.l.) and Lages (50°35’W and 27°47’S; 895 m a.s.l.), in Humic Kandiudox, and in Otacílio Costa (49°50’W and 27°35’S; 919 m a.s.l.), in Humic Dystrudept. Soil classification follows USDA Soil Taxonomy (Soil Survey Staff 2014). Further information on the history of use of the areas, sampling scheme, coordinates and altitude by LUS can be obtained in Bartz *et al.* (2014) and Rosa *et al*. (2015).

2. *Sampling methods*

For the collection of the spider, a 1-ha area was delimited in each LUS, constituted of a 3 × 3 m sampling grid, spaced by 30 m with a border of 20 m, in a total of 270 points (winter + summer) for each method, totaling 540 sampling points. The sampling sites in each municipality were considered as true replicates of the LUS. The spiders were collected through the methods of soil monolith (Anderson & Ingram 1993) and soil traps, according to the methodology described by Baretta *et al*. (2007); with sampling during the winter of June and July of 2011 and during the summer, in Dec. 2011 and Jan. 2012, periods in which the conditions of temperature and rainfall are representative of the regional environment. The sampling area for the soil monolith method in each point was 0.25 × 0.25 m, excavating the soil up to 0.20 m depth. The collected soil was placed in plastic bags and taken to the laboratory for hand sorting, under artificial light. Soil traps with a diameter of 0.07 m were installed at the soil level, 0.3 m away from the collection points of the soil monolith method and left at the field for three days and then collected and taken to the laboratory, where they were separated using 0.125-mm-mesh sieves. All organisms found were fixed in 80% alcohol and sent for identification at the Instituto Butantan. All the material is deposited in the Arachnological Collection of the Laboratório Especial de Coleções Zoológicas in the Instituto Butantan (Curator: A.D. Brescovit).

3. *Evaluation of the explicative environmental variables*

Around the spider collection points, 15 soil subsamples were collected in the of 0.0-0.2 m layer and homogenized to form a composite sample. This sample was used for the evaluation of organic matter (OM), nitrogen (N), pH in water (pH), exchangeable aluminum (Al^{3+}), calcium (Ca^{3+}), magnesium (Mg^{2+}), and potassium (K^{+}), and sulfur (S), according to the methodologies of Tedesco *et al*. (1995).

Soil granulometry was determined through the pipette method in the same sample of the chemical analysis (Gee & Bauder 1986). Undisturbed soil samples were also collected beside the points where the spiders were collected. A portion of clods was used to determine weighted average diameter (WAD), according to the wet sieving method of Kemper & Chepil (1965). Volumetric rings collected in these points were used to determine bulk density (Bd) and microporosity (Micro), as described in Embrapa (2011).

For microbiological analysis, samples were collected from a depth of 0.10 m, sieved (<0.002 m), and kept refrigerated (4 °C) for analyses. Determination of microbial biomass carbon (MBC) was performed with the fumigation-extraction method (Vance *et al*., 1987).

4. *Metrics of diversity*

The Shannon-Wiener index (H’) was calculated according to Odum (1988), in order to verify how the environmental pressures (intensification of land use) might interfere with the distribution of spider families. Estimations of Shannon-Wiener index were performed using Vegan Community Ecology Package in R Core Team software, v. 2.12.2. The mean values of H’ were compared point by point (n = 27) by Tukey test at 0.05 probability level, using Agricolae Package in R Core Team software, v. 2.12.2.

5. *Statistical analyses*

Since the objective of the present study was not to compare the collecting methods, the data of the total abundance of both methods was summed to minimize the limitations of the methods. The obtained values were used to determine the length of the gradient (DCA). The total abundance of the spider families and studied types of LUS were compared through the principal component analysis (PCA). Physical, chemical, and microbiological attributes were considered as explicative environmental variables, in which the collinear parameters were removed from the statistical model and later used to establish the correlation between them and the spider families in the redundancy analysis (RDA). Multivariate analyses were performed using CANOCO software, v. 4.5.

Results

In total, considering the collection methods and LUS, 448 spiders were captured, 152 in the winter and 296 in the summer, distributed into 24 families and 52 genus/species (Table 1). There were differences ($p < 0.05$) between the evaluated LUS. The highest diversity (H') was recorded in the NF, followed by PA > ER = CLI > NT during the winter. In the summer, the diversities followed the anthropization gradient NF > ER > PA > CLI > NT (Table 1).

The richness of the families obeyed the following order: NF, ER, and PA with 11 families, CLI with 8 families and NT with 6 families in the summer. During the winter, according to the use of intensity, the richness of the families increased for NF (12) and decreased in all remaining LUS, ER (9), PA (06), CLI (05), and NT (04), respectively, evidencing that, in general, the modification in the vegetal complexity reduces the biodiversity of soil spiders.

The principal component analysis explained 43% of the total variation, 37% by the principal component 1 (PC1) and 7% by the PC2 during the winter and 56% during the summer, in which the PC1 explained 37% and PC2 explained 19% (Figure 1). Among the land use systems, Nesticidae, Mecicobothriidae, Pholcidae, Amaurobiidae, Xenoctenidae, Lycosidae, and Nemesiidae were more associated with the NF. Theridiidae, Zodariidae, Hahniidae, Gnaphosidae, and Araneidae were associated with ER, while systems with a higher level of human intervention (PA, CLI, and NT) grouped the families Ctenidae, Palpimanidae, Tetragnathidae, Corinnidae, and Linyphiidae during the winter (Figure 1A).

During the summer, Oxyopidae, Linyphiidae, Mysmenidae, Lycosidae, Ctenidae, Prodidomidae, Theridiidae, Pholcidae, Salticidae, and Nesticidae were more associated with the NF. Filistatidae, Titanoecidae, and Amphinectidae were related to the PA system. Palpimanidae, Hahniidae, and Gnaphosidae were more associated with the systems NT, ER, and CLI (Figure 1B).

Among the soil physical and chemical attributes that showed correlation with the spider families during the winter, the following ones stood out: Micro, Al^{3+}, Ca^{3+}, Mg^{2+}, and K^{+}. Among them, the contents of Ca^{2+}, Mg^{2+}, and K^{+} were correlated with the families Palpimanidae, Tetragnathidae, Araneidae, Hahniidae, Lycosidae, Gnaphosidae, and Salticidae, while the families Nesticidae, Amaurobiidae, Mecicobothriidae, Zodariidae, Ctenidae, Xenoctenidae, Pholcidae, Corinnidae, Nemesiidae, Theridiidae, and Linyphiidae showed correlation with Al^{3+} and microporosity (Figure 2A).

For the samplings made in the summer, the physical, chemical, and microbiological attributes that showed correlation with the spider families were: OM, N, pH, WAD, Bd, and MBC. From these variables, pH and Bd were correlated with the families Corinnidae and Palpimanidae, while WAD was strongly correlated with the abundance of Salticidae, Prodidomidae, Gnaphosidae, and Hahniidae. On the other hand, the N and OM favored the presence of the Mysmenidae, Linyphiidae, Theridiidae, Nesticidae, Amaurobiidae, Lycosidae, and Ctenidae, while MBC showed a direct correlation with Amphinectidae, Titanoecidae, Oxyopidae, and Filistatidae (Figure 2B).

Discussion

The richness of the spider families responded to the seasonality, especially because of the modifications in the vegetation structure of the evaluated land use and management systems. Similar results were obtained by Marín *et al*. (2015), who found differences between the sampling periods in the structure of spider communities in coffee plantations in Mexico. In the present study, the most abundant families were Linyphiidae (40%), Lycosidae (28%), and Theridiidae (8%), regardless of the LUS, corroborating the findings of Marín *et al*. (2015) and Lopes *et al*. (2009), who also found higher proportions of Linyphiidae and Lycosidae in areas with different types of vegetal cover.

The type of vegetation acts as a filter for spider families and this was also reported by Buchholz (2016), who studied spider families in peat areas with different flower compositions. He found that the occurrence of larger spiders is correlated with denser vegetation and of smaller spiders with areas where the predominant vegetation was formed by mosses. According to this author, Linyphiidae and Theridiidae occurred more frequently in the upper layers of the vegetation, corroborating the results of the present study, in which the native forest concentrated most individuals of from these families.

The changes in the distribution pattern between the sampling periods are probably due to the characteristic of each one of the land use types and to the complexity of the vegetal structure in each one of the ecosystems, which regulates the microclimate and soil moisture. Attention should be paid to this latter, because the positive correlation between the volume of micropores and certain families of spiders (Pholcidae, Theridiidae, Xenoctenidae, Zodariidae, and Nemesiidae) during the winter (Figure 2A) indicates the dependence of these families on soil moisture. It should be pointed out that this physical attribute (micropores) is responsible for water retention in the soil.

However, during the winter, the areas of native forest (NF) showed differences in terms of the composition of the spider families, compared with the anthropized systems (ER, PA, CLI, and NT). During the summer, this behavior was similar, but most families showed a positive correlation with NF, which sheltered a large portion of the individuals. In ER, NT, and CLI, only Gnaphosidae and Palpimanidae were more abundant, which suggests that these two families easily adapt to areas with high levels of human intervention. However, the lack of previous studies in this region of the state does not allow any type of comparison and discussion on this subject.

Table 1. Total abundance of spiders observed using two collection methods (soil monolith and soil traps) in systems of native forest (NF), eucalyptus reforestation (ER), pasture (PA), crop-livestock integration (CLI), and no-tillage (NT) in the winter and summer in the Southern Plateau of the state of Santa Catarina.

Family/Genus/Species	**Winter**					**Summer**				
	NF	**ER**	**PA**	**CLI**	**NT**	**NF**	**ER**	**PA**	**CLI**	**NT**
Amaurobiidae*	5	2	0	0	0	1	0	0	0	0
Amphinectidae**	0	0	0	0	0	0	0	1	0	0
Metaltella sp. 1	0	0	0	0	0	0	0	1	0	0
Araneidae*	0	1	0	0	0	0	0	0	0	0
Corinnidae**	1	2	1	0	0	0	4	2	1	0
NI***	1	2	0	0	0	0	3	2	1	0
Castianeira sp. 1	0	0	0	0	0	0	1	0	0	0
Corinna sp. 1	0	0	1	0	0	0	0	0	0	0
Ctenidae**	0	0	0	1	0	2	4	0	1	0
NI	0	0	0	0	0	1	4	0	1	0
Isoctenus ordinario Polotow & Brescovit, 2009	0	0	0	0	0	1	0	0	0	0
Isoctenus sp. 1	0	0	0	1	0	0	0	0	0	0
Filistatidae**	0	0	0	0	0	0	0	1	0	0
Misionella mendensis (Mello-Leitão 1920)	0	0	0	0	0	0	0	1	0	0
Gnaphosidae**	0	1	2	1	0	1	4	1	1	1
NI	0	1	2	1	0	0	3	0	0	0
Apopyllus sp. 1	0	0	0	0	0	1	0	1	0	0
Zimiromus sp. 1	0	0	0	0	0	0	1	0	1	1
Hahniidae**	0	2	1	0	1	4	4	3	4	9
NI	0	0	1	0	0	4	1	0	0	1
Neohahnia sp. 1	0	2	0	0	1	0	3	2	3	1
Neohahnia sp. 2	0	0	0	0	0	0	0	1	1	7
Linyphiidae**	11	7	11	25	4	51	18	12	19	18
NI	6	4	1	6	1	28	7	4	6	6
Agyneta sp. 1	1	0	1	2	2	3	7	3	2	2
Agyneta sp. 2	0	0	0	0	0	1	0	0	3	0
Erigone sp. 1	3	0	2	4	0	0	0	3	4	3
Erigone sp. 2	0	0	1	2	0	0	2	0	3	3
Exocora ribeiroi Lemos & Brescovit, 2013	0	0	0	0	0	0	1	0	0	0
Laminacauda sp. 1	0	0	0	1	0	0	0	0	0	0
Meioneta sp. 1	0	1	1	0	0	4	0	0	0	0
Mermessus sp. 1	1	0	4	2	0	6	0	0	1	0
Moyosi sp. 1	0	0	0	0	0	2	0	1	0	0
Neomaso sp. 1	0	1	0	0	0	0	0	1	0	0
Neomaso sp. 2	0	1	0	0	0	0	0	0	0	0
Ostearius sp. 1	0	0	1	8	1	0	1	0	0	4
Sphecozone novaeteutoniae (Baert, 1987)	0	0	0	0	0	7	0	0	0	0
Lycosidae**	12	3	4	10	7	27	9	16	23	12
NI	9	1	2	10	5	16	7	13	11	10
Allocosa sp. 1	0	0	0	0	0	0	0	1	9	0
Lobizon humilis (Mello-Leitão, 1944)	3	1	0	0	2	11	1	1	0	0

continued...

...continuation

Lycosa erythrognatha Lucas, 1836	0	0	0	0	0	0	0	0	1	0
Schizocosa sp. 1	0	0	0	0	0	0	0	1	0	0
Trochosa sp. 1	0	1	1	0	0	0	1	0	2	2
Trochosa sp. 2	0	0	1	0	0	0	0	0	0	0
Mecicobothriidae**	1	0	0	0	0	0	0	0	0	0
Mecicobothrium baccai Lucas, Indicatti, Brescovit & Francisco, 2006	1	0	0	0	0	0	0	0	0	0
Mysmenidae**	0	0	0	0	0	5	0	0	0	0
NI	0	0	0	0	0	1	0	0	0	0
Microdipoena sp. 1	0	0	0	0	0	4	0	0	0	0
Nemesiidae*	2	0	0	0	0	0	0	0	0	0
Nesticidae**	4	0	0	0	0	2	0	0	0	0
Nesticella sp. 1	4	0	0	0	0	2	0	0	0	0
Oxyopidae*	0	0	0	0	0	0	0	1	0	0
Palpimanidae*	0	1	0	0	0	0	1	0	0	0
Pholcidae**	3	0	0	0	0	1	1	1	0	0
NI	2	0	0	0	0	1	1	1	0	0
Mesabolivar aff. *difficilis*	1	0	0	0	0	0	0	0	0	0
Prodidomidae*	0	0	0	0	0	0	2	0	0	0
Salticidae**	1	0	0	0	0	1	1	0	1	1
Corythalia sp. 1	1	0	0	0	0	1	1	0	0	1
Corythalia sp. 2	0	0	0	0	0	0	0	0	1	0
Tetragnathidae**	0	0	0	1	2	0	0	0	0	0
Glenognatha australis (Keyserling, 1883)	0	0	0	1	2	0	0	0	0	0
Theridiidae**	6	4	4	0	0	12	4	4	2	2
NI	1	3	0	0	0	1	0	0	0	2
Dipoena pumicata (Keyserling, 1886)	0	0	2	0	0	0	0	1	0	0
Euryopis sp. 1	0	0	1	0	0	4	0	3	1	0
Guaraniella sp. 1	3	1	1	0	0	3	3	0	1	0
Stemmops sp. 1	0	0	0	0	0	1	0	0	0	0
Styposis selis Levi, 1964	1	0	0	0	0	2	0	0	0	0
Styposis sp. 1	1	0	0	0	0	1	0	0	0	0
Thymoites sp. 1	0	0	0	0	0	0	1	0	0	0
Titanoecidae**	0	0	0	0	0	0	0	1	0	0
Goeldia sp. 1	0	0	0	0	0	0	0	1	0	0
Xenoctenidae**	6	0	0	0	0	0	0	0	0	0
Odo aff. *blumenauensis*	1	0	0	0	0	0	0	0	0	0
Odo sp. 1	5	0	0	0	0	0	0	0	0	0
Zodariidae*	1	0	0	0	0	0	0	0	0	0
Total	53	23	23	38	14	107	52	43	52	43
Diversity index (H')	0.44a	0.17b	0.18b	0.17b	0.03b	0.63a	0.41ab	0.31ab	0.30ab	0.22ab

* Unidentified specimens on any level below family. ** The number of individuals in families corresponds to the sum of the number of individuals of genus and/or species.
*** NI: Unidentified specimens on any level below family.

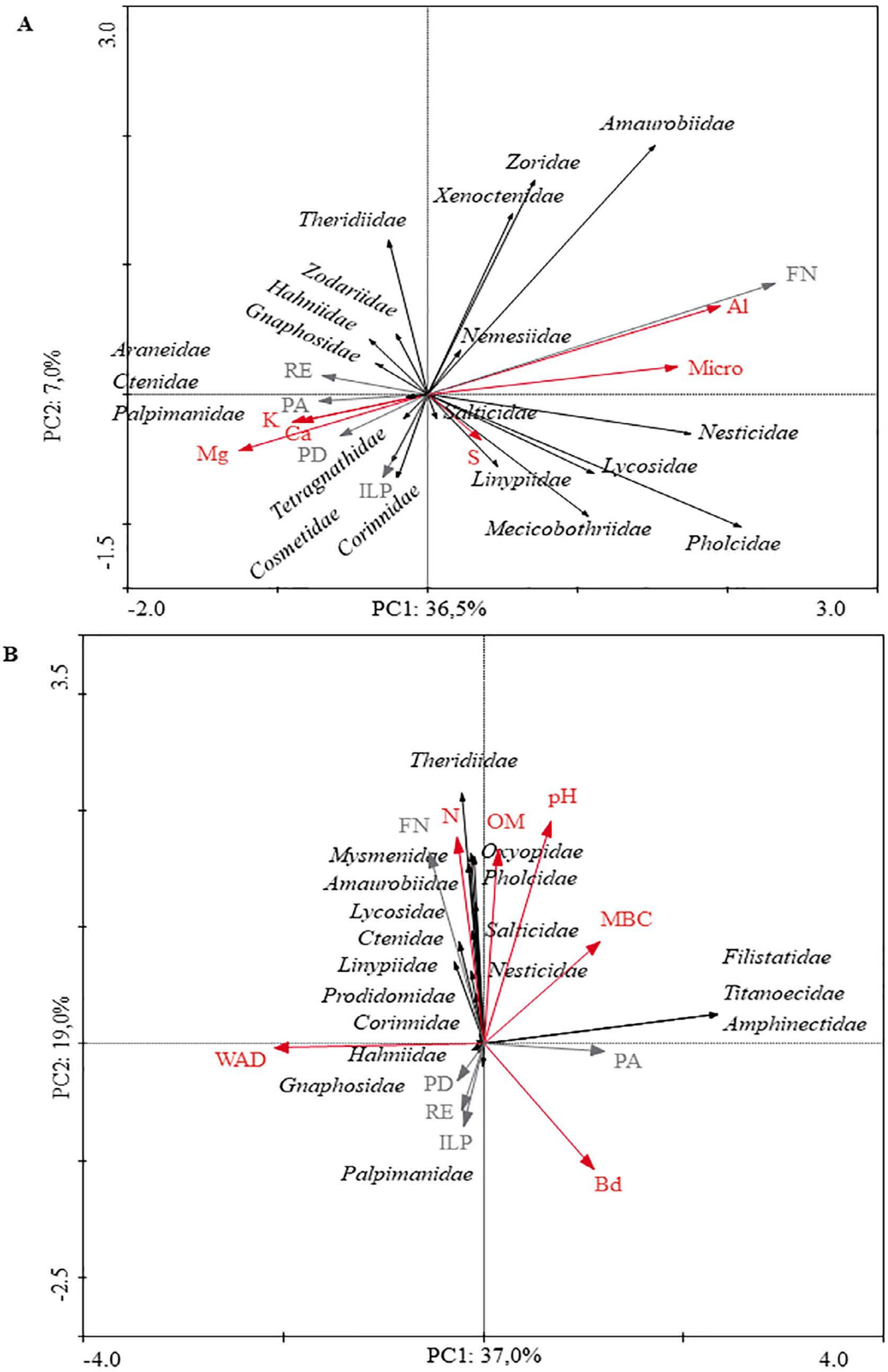


Figure 1. Principal component analysis (PCA) (a = winter, b = summer), for soil spider families in the Southern Plateau of the state of Santa Catarina (n = 27) in each land use system: native forest (NF), eucalyptus reforestation (ER), pasture (PA), crop-livestock integration (CLI), and no-tillage (NT). Environmental properties: Organic matter (OM), Nitrogen (N), Microbial biomass carbon (MBC), Bulk density (Bd), pH in water (pH), Weighted average diameter (WAD), Magnesium (Mg), Calcium (Ca), Potassium (K), Aluminum (Al), Microporosity (Micro), and Sulfur (S).

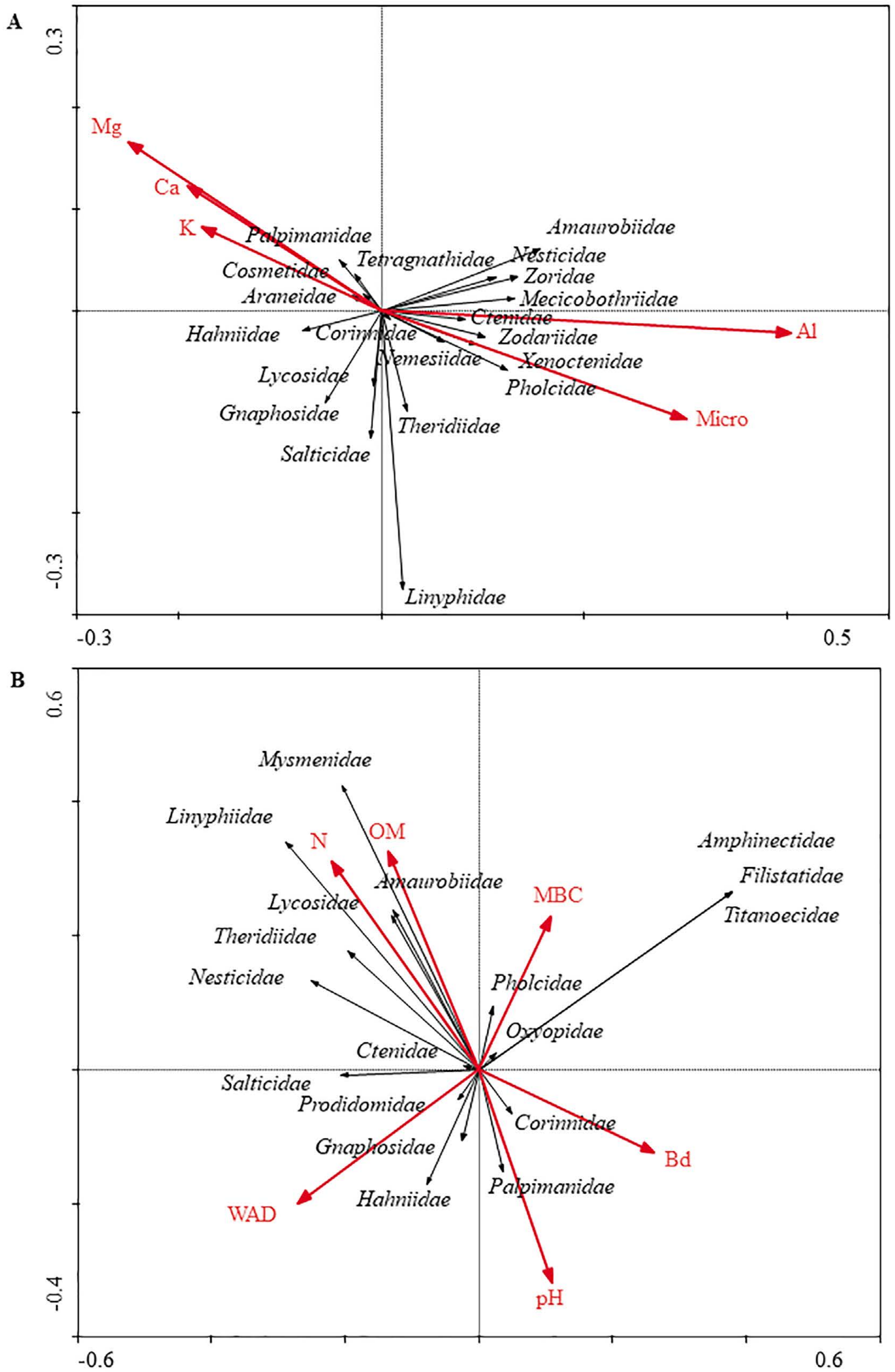


Figure 2. Redundancy analysis (RDA) (a = winter, b = summer), for soil spider families in the Southern Plateau of the state of Santa Catarina (n = 27) in each land use system: native forest (NF), eucalyptus reforestation (ER), pasture (PA), crop-livestock integration (CLI), and no-tillage (NT). Environmental properties: Organic matter (OM), Nitrogen (N), Microbial biomass carbon (MBC), Bulk density (Bd), pH in water (pH), Weighted average diameter (WAD), Magnesium (Mg), Calcium (Ca), Potassium (K), Aluminum (Al), Microporosity (Micro), and Sulfur (S).

In a study conducted by Richman *et al*. (2011) in the Chihuahan desert, in Mexico, Gnaphosidae and Salticidae were more abundant in at least one period of the year. Although the desert is an inhospitable environment for many species, for these two families the reduction of resources does not seem to substantially affect their distribution, because they can forage in many environments and cover long distances searching for food and shelter.

The low relationship between the spider families and the agricultural areas may be explained by the use of agrochemicals in these sites, which can kill the spiders or reduce populations of other arthropods, and this compromise foraging in the entire food chain, affecting spiders, which are mostly secondary predators. Marliac *et al*. (2016) demonstrated that the continuous application of insecticides in apple orchards in southeast France negatively influenced the spider fauna, compared with the organic production of apple. Similar results were obtained by Mansour and Nentwig (1988) and Lefebvre *et al*. (2016), who also found a direct relationship between environments contaminated with chemical compounds and reduction in the biodiversity of spiders. The lack of information on the diversity of spiders in land use systems in the southern states of Brazil, especially Santa Catarina, compromises comparisons and more detailed discussions.

Besides the intrinsic conditions of the anthropized environment, i.e., entry of chemical compounds and reduction in the fluctuation of the population of other organisms, the composition of plants, their architecture and the microclimate formed in the different forms of land use system can affect the distribution of spiders (Peres *et al*. 2010, Bizuet-Flores *et al*. 2015, Rivera-Quiroz *et al*. 2016). These aspects tend to be more simplified in the cultivation areas, in relation to the native vegetation.

Spider families that are largely associated to the soil or vegetation, such as Theridiidae and Nemesiidae, are strongly affected by the movement of animals and/or machines, which can be an explanation for the reduction of these families in agricultural areas or areas used also by farm animals. Comparing the observed correlation of spider diversity with attributes such as Bd and WAD (Figure 2B) with the literature, we found that Downie *et al*. (2000) demonstrate that cattle grazing intensity drastically reduces the populations of spiders. The entry of sheep and cattle in pasture areas causes soil compaction and negatively interferes with the diversity and abundance of spiders (Dennis *et al*. 2015). Baretta *et al*. (2007) also report that the entry of production animals in pasture areas can be an aggravating factor for the reduction in spider fauna biodiversity as well as the effect of burning the vegetation.

The families Palpimanidae, Tetragnathidae, Araneidae, Hahniidae, Lycosidae, Gnaphosidae, and Salticidae showed direct correlation with the contents of Ca^{2+}, Mg^{2+}, and K^{+}. Those chemical attributes are commonly altered by management conditions, such as liming to correct acidity in agricultural areas, thus suggesting that these families easily adapt to land use and management conditions.

The correlation of Al^{3+} contents with Nesticidae, Amaurobiidae, Xenoctenidae, Dipluridae, Zodariidae, Ctenidae, Pholcidae, Corinnidae, Nemesiidae, Theridiidae, and Linyphiidae indicates adaptation to natural areas and acidic soils that have not been limed, differing from many soil organisms, which respond to the use of limestone (Baretta *et al*. 2011).

During the summer, besides physical and chemical attributes, a positive correlation was also found to at least one microbiological attribute, indicating interdependence between the carbon from the microbial biomass and the spider families. Thus, it should be pointed out that the higher the biological diversity of the soil, the greater the occurrence of secondary predators, with stabilization of the food chain and of the processes of maintenance of terrestrial ecosystems.

The content of organic matter is directly related to the litter decomposition rate, and these conditions are associated with some families of spiders, attracting primary decomposers and detrital fauna, and consequently favoring the appearance of predators, such as spiders. This mechanism was also described by Liu *et al*. (2015), who found positive relationships between litter decomposition rates and the abundance of spiders in native forest areas, reinforcing the results obtained in the present study.

Soil physical attributes such as Bd and WAD have a positive correlation with some families of spiders, which may be related to the effect of management on soil structure. The relationship between physical and chemical attributes and the diversity of spider families is proportional to the intensification of land use, so that the more managed and altered the environment, the greater the effects on the decrease in the diversity of spiders and, eventually, on possible losses in physical and chemical processes of the soil. Araújo *et al*. (2015), studying the relationship between soil degradation and key groups of soil organisms, observed that Araneae are strongly affected by the intensity of degradation of landscapes. Thus, a more adequate soil management in agricultural production areas can favor essential processes such as the biological control of pests and maintenance of energy flows in the soil (Haase & Balkenhol 2015, Brunbjerg *et al*. 2015).

Conclusions

Spider families are affected by the type of land use and management and by the seasonality in systems in the region of the Southern Plateau of Santa Catarina. The families Theridiidae and Nemesiidae are dependent on sites with low human intervention, being more abundant in the NF. The type of vegetation and management are the factors that most affect the spider families. Most spider families have a direct dependence on soil physical and chemical attributes, especially pH, soil density, weighted average diameter, organic matter, nitrogen, exchangeable aluminum, calcium, magnesium, potassium, and microporosity.

Acknowledgements

The authors thank the Research and Innovation Support Foundation of Santa Catarina (FAPESC) (Process number 6.309/2011-6/FAPESC) and the National Council for Scientific and Technological Development (CNPq) (Process number 563251/2010-7/CNPq). Dilmar Baretta thanks the CNPq for his research productivity grant (Process number 307162/2015-0/CNPq) and Antonio Domingos Brescovit (Process number 303028/2014-9/CNPq).

Author Contributions

Marcio Gonçalves da Rosa: Responsible for sampling of soil spiders and for data collection, contributed to manuscript preparation, critical revision, and adding intellectual content.

Antonio Domingos Brescovit: Contribution to taxonomic identification of soil spiders, critical revision, adding intellectual content.

Carolina Riviera Duarte Maluche Baretta: Contribution for data collection, contribution to critical revision, adding intellectual content.

Júlio Cesar Pires Santos: Contribution to critical revision, adding intellectual content.

Luís Carlos Iuñes de Oliveira Filho: Contribution to critical revision, adding intellectual content.

Dilmar Baretta: Substantial contribution in the concept and design of the study, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ANDERSON, J.M. & INGRAM, J.S.I. 1993. Tropical Soil Biology and Fertility: A Handbook on Methods. 2 ed. CAB International, Wallingford.

ARAÚJO, A.S.F., EISENHAUER, N., NUNES, L.A.P.L., LEITE, L.F.C. & CESARZ, S. 2015. Soil Surface-Active Fauna in Degraded and Restored Lands of Northeast Brazil. L. Degrad. Dev. 26:1-8.

BALKENHOL, B., HOHBERG, K. & PFANZ, H. 2016. Spiders in mofette fields—Survival of the toughest in natural carbon dioxide springs? Ecol. Indic. 69:749-757.

BARETTA, D., BRESCOVIT, A.D., KNYSAK, I. & CARDOSO, E.J.B.N. 2007. Trap and soil monolith sampled edaphic spiders (Arachnida: Araneae) in *Araucaria angustifolia* forest. Sci. Agric. 64:375-383.

BARETTA, D., SANTOS, J.C.P., SEGAT, J.C., GEREMIA, E.V., OLIVEIRA FILHO, L.C.I. & ALVES, M.V. 2011. Fauna edáfica e qualidade do solo. In Tópicos em Ciência do Solo (O. Klauberg-Filho, Á.L. Mafra & L.C. Gatiboni, Eds.). Sociedade Brasileira de Ciência do Solo, Viçosa, p.119-170.

BARSOUM, N., FULLER, L., ASHWOOD, F., REED, K., BONNET-LEBRUN, A.-S. & LEUNG, F. 2014. Ground-dwelling spider (Araneae) and carabid beetle (Coleoptera: Carabidae) community assemblages in mixed and monoculture stands of oak (*Quercus robur* L./*Quercus petraea* (Matt.) Liebl.) and Scots pine (*Pinus sylvestris* L.). For. Ecol. Manage. 321:29-41.

BARTZ, M.L.C., BROWN, G.G., ROSA, M.G., KLAUBERG FILHO, O., JAMES, S.W., DECAËNS, T. & BARETTA, D. 2014. Earthworm richness in land-use systems in Santa Catarina, Brazil. Appl. Soil Ecol. 83:59-70.

BIZUET-FLORES, M.Y., JIMÉNEZ-JIMÉNEZ, M.L., ZAVALA-HURTADO, A. & CORCUERA P. 2015. Diversity patterns of ground dwelling spiders (Arachnida: Araneae) in five prevailing plant communities of the Cuatro Ciénegas Basin, Coahuila, Mexico. Rev. Mex. Biodivers. 86:153-163.

BORCHARD, F., BUCHHOLZ, S., HELBING, F. & FARTMANN, T. 2014. Carabid beetles and spiders as bioindicators for the evaluation of montane heathland restoration on former spruce forests. Biol. Conserv. 178:185-192.

BRUNBJERG, A.K., JØRGENSEN, G.P., NIELSEN, K.M., PEDERSEN, M.L., SVENNING, J.-C. & EJRNÆS, R. 2015. Disturbance in dry coastal dunes in Denmark promotes diversity of plants and arthropods. Biol. Conserv. 182:243-253.

BUCHHOLZ, S. 2016. Natural peat bog remnants promote distinct spider assemblages and habitat specific traits. Ecol. Indic. 60:774-780.

CAMARA, R., CORREIA, M.E.F. & VILLELA, D.M. 2012. Effects of eucalyptus plantations on soil arthropod communities in a Brazilian Atlantic forest conservation unit. Biosci. J. 28:445-455.

CHEN, S., FATH, B.D. & CHEN, B. 2011. Information-based Network Environ Analysis: A system perspective for ecological risk assessment. Ecol. Indic. 11:1664-1672.

DENNIS, P., SKARTVEIT, J., KUNAVER, A. & MCCRACKEN, D.I. 2015. The response of spider (Araneae) assemblages to structural heterogeneity and prey abundance in sub-montane vegetation modified by conservation grazing. Glob. Ecol. Conserv. 3:715-728.

DOWNIE, I.S., RIBERA, I., MCCRACKEN, D.I., WILSON, W.L., FOSTER, G.N., WATERHOUSE, A., ABERNETHY, V.J. & MURPHY K.J. 2000. Modelling populations of *Erigone atra* and *E. dentipalpis* (Araneae: Linyphiidae) across an agricultural gradient in Scotland. Agric. Ecosyst. Environ. 80:15-28.

EMBRAPA - Empresa Brasileira de Pesquisa Agropecuária. 2011. Manual de métodos de análise de solo. 2 ed. Embrapa Solos, Rio de Janeiro.

GEE, G.W. & BAUDER, J.W. 1986. Particle size analysis. In Methods of soil analysis. Part 1. Physical and mineralogical methods. (A. KluteE, ed.). 2 ed: American Society of Agronomy and Soil Science Society of America, Madison, p.383-411.

HAASE, H. & BALKENHOL, B. 2015. Spiders (Araneae) as subtle indicators for successional stages in peat bogs. Wetl. Ecol. Manag. 23:453-466.

JESUS, E.N., FERREIRA, R.A., ARAGÃO, A.G., SANTOS, T.I.S. & ROCHA, S.L. 2015. Estrutura dos fragmentos florestais da Bacia Hidrogáfica do Rio Poxim-SE, como subsídio à restauração ecológica. Rev. Árvore. 39:467-474.

KEMPER, W.D. & CHEPIL, W.S. 1965. Size distribution of aggregates. In Methods of soil analysis. Part 1. Physical and mineralogical methods. (C.A. Black, D.D. Evans, J.L. White, L.E. Ensminger, F.E. Clark, eds.). American Society of Agronomy and Soil Science Society of America, Madison, p.499-510.

KOVÁCS-HOSTYÁNSZKI, A., ELEK, Z., BALÁZS, K., CENTERI, C., FALUSI, E., JEANNERET, P., PENKSZA, K., PODMANICZKY, L., SZALKOVSZKI, O. & BÁLDI, A. 2013. Earthworms, spiders and bees as indicators of habitat quality and management in a low-input farming region—A whole farm approach. Ecol. Indic. 33:111-120.

LEFEBVRE, M., FRANCK, P., TOUBON, J.-F., BOUVIER, J.-C. & LAVIGNE, C. 2016. The impact of landscape composition on the occurrence of a canopy dwelling spider depends on orchard management. Agric. Ecosyst. Environ. 215:20-29.

LIU, S., CHEN, J., GAN, W., SCHAEFER, D., GAN, J. & YANG, X. 2015. Spider foraging strategy affects trophic cascades under natural and drought conditions. Sci. Rep. 5:12396.

LOPES, J., PEREIRA, F., VIEIRA, V., MARÇAL, M., PAULA, M., PINTO, B. & CATELLI, L.L. 2009. Araneofauna capturada na mata e área aberta adjacente, no norte do Paraná, Brasil. Semina: Ciênc. Biol. Saúde. 41-46.

MADER, V., BIRKHOFER, K., FIEDLER, D., THORN, S., WOLTERS, V. & DIEHL, E. 2016. Land use at different spatial scales alters the functional role of web-building spiders in arthropod food webs. Agric. Ecosyst. Environ. 219:152-162.

MANSOUR, F. & NENTWIG, W. 1988. Effects of agrochemical residues on four spider taxa: Laboratory methods for pesticide tests with web-building spiders. Phytoparasitica. 16:317-325.

MARÍN, L., JACKSON, D. & PERFECTO, I. 2015. A positive association between ants and spiders and potential mechanisms driving the pattern. Oikos. 124:1078-1088.

MARLIAC, G., MAZZIA, C., PASQUET, A., CORNIC, J.-F., HEDDE, M. & CAPOWIEZ, Y. 2016. Management diversity within organic production influences epigeal spider communities in apple orchards. Agric. Ecosyst. Environ. 216:73-81.

MINEO, M.F., DEL-CLARO, K. & BRESCOVIT, A.D. 2010. Seasonal variation of ground spiders in a Brazilian Savanna. Zoologia. 27:353-362.

MORAES, M.C.P., MELLO, K., TOPPA, R.H. 2015. Análise da paisagem de uma zona de amortecimento como subsídio para o planejamento e gestão de unidades de conservação. Rev. Árvore. 39:1-8.

MUMME, S., JOCHUM, M., BROSE, U., HANEDA, N.F. & BARNES, A.D. 2015. Functional diversity and stability of litter-invertebrate communities following land-use change in Sumatra, Indonesia. Biol. Conserv. 191:750-758.

ODUM, H.T. 1988. Self-Organization, Transformity, and Information. Science. 242:1132-1139.

PERES, M.C.L., SOUZA-ALVES, J.P., BENATTI, K., DIAS, M.A., ALVES, A.O., MÁXIMO, C.O. & BRESCOVIT, A.D. 2010. Distribution of leaf litter spider (Araneae) in treefall gaps and on adjacent forest in an atlantic rainforest remnant in Bahia State, Brazil. Biociências. 16:109-115.

RICHMAN, D.B., BRANTLEY, S.L., HU, D.H.-C. & WHITEHOUSE, M.E.A. 2011. Spiders of the Chihuahuan Desert of Southern New Mexico and Western Texas. Southwest Nat. 56:44-53.

RIVERA-QUIROZ, F.A., GARCILAZO-CRUZ, U. & ÁLVAREZ-PADILLA, F. 2016. Spider cyberdiversity (Araneae: Araneomorphae) in an ecotouristic tropical forest fragment in Xilitla, Mexico. Rev. Mex. Biodivers. 87:1023-1032.

ROSA, M.G., KLAUBERG FILHO, O., BARTZ, M.L.C., MAFRA, Á.L., SOUSA, J.P.F.A. & BARETTA, D. 2015. Macrofauna edáfica e atributos físicos e químicos em sistemas de uso do solo no planalto catarinense. Rev. Bras. Cienc. Solo. 39:1544-1553.

SAS-KOVÁCS, É.-H., SAS-KOVÁCS, I. & URÁK, I. 2015. *Alopecosa psammophila* Buchar, 2001 (Araneae: Lycosidae): morphometric data and first record for Romania. Turkish J. Zool. 39:353-358.

SOIL SURVEY STAFF. 2014. Soil Taxonomy: A Basic System of Soil Classification for Making and Interpreting Soil Surveys. 12th ed. Washington: U.S. Department of Agriculture, Natural Resources Conservation Service. (Agriculture Handbook, 372)

TEDESCO, M.J., GIANELLO, C., BISSANI, C.A. & VOLKWEISS, S.J. 1995. Análises de Solo, Plantas e Outros Materiais. Universidade Federal do Rio Grande do Sul, Porto Alegre.

Received: 24/07/2018
Revised: 03/01/2019
Accepted: 14/01/2019

http://dx.doi.org/10.1590/1676-0611-BN-2018-0619

Biota Neotropica 19(2): e e20180541, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

A predictive multimetric index based on macroinvetebrates for Atlantic Forest wadeable streams assessment

Renata Bley da Silveira de Oliveira [1,2,3], *Riccardo Mugnai* [4], *Priscilla da Silva Pereira* [1], *Natália Freitas de Souza* [1,2]
& Darcílio Fernandes Baptista [1*]

[1] *Fundação Oswaldo Cruz, Instituto Oswaldo Cruz, Rio de Janeiro, RJ, Brasil*
[2] *Universidade Federal do Rio de Janeiro, Departamento de Ecologia, Rio de Janeiro, RJ, Brasil*
[3] *Agência Nacional de Águas, Brasília, Brasil*
[4] *Universidade Federal do Maranhão, São Luis, MA, Brasil*

[*]*Corresponding author: Darcílio F. Baptista, e-mail: darcilio@ioc.fiocruz.br*

OLIVEIRA, R. B. S., MUGNAI, R., PEREIRA, P. S., SOUZA, N. F., BAPTISTA, D. F. A predictive multimetric index based on macroinvetebrates for Atlantic Forest wadeable streams assessment. Biota Neotropica 19(2): e20180541. http://dx.doi.org/10.1590/1676-0611-BN-2018-0541

Abstract: Multimetric Indices (MMIs) have been widely applied for ecological assessment in freshwater ecosystems. Most MMIs face difficulties when scaling up from small spatial scales because larger scales usually encompass great environmental variability. Covariance of anthropogenic pressures with natural environmental gradients can be a confounding factor in assessing biologic responses to anthropogenic pressures. This study presents the development and validation of a predictive multimetric index to assess the ecological condition of Atlantic Forest wadeable streams using benthic macroinvertebrates. To do so, we sampled 158 sites for the index development. We adjusted each biological metric to natural variation through multiple regression analyses (stepwise-forward) and considered that the residual distribution describes the metric variation in the absence of natural environmental influence. For metric selection we considered normal distribution, variation explained by the models, redundancy between metrics and sensitivity to differentiate reference from impaired sites. We selected five metrics to the final index: total richness, %MOLD, %Coleoptera, EPT richness and Chironomidae abundance. The residuals were transformed into probabilities and the final index was obtained through the mean of these probabilities. This index performed well in discriminating the impairment gradient and it showed a high correlation (r = 0.85, p <0.001) with a specific index developed for a particular basin indicating a similar sensitivity. This index can be used to assess wadeable streams ecological condition in Atlantic Forest biome, so we believe that this type of approach represents an important step towards the application of biomonitoring tools in Brazil.
Keywords: *Biomonitoring, Bioassessment protocol, aquatic ecosystem, multimetric index, macroinvertebrate.*

Índice Multimétrico Preditivo baseado em macroinvertebrados para avaliação de riachos vadeáveis da Mata Atlântica

Resumo: Índices Multimétricos (MMIs) são ferramentas amplamente aplicadas ao monitoramento ecológico de ecossistemas aquáticos continentais. A maior dificuldade na utilização dos MMIs em amplas escalas espaciais consiste no aumento da variabilidade ambiental associado ao aumento da escala. A covariância entre os impactos antropogênicos e o gradiente ambiental natural pode atuar como fator de confusão, dificultando a avaliação das respostas biológicas às pressões antropogênicas. Este estudo consiste no desenvolvimento e validação de um índice multimétrico preditivo para avaliar a condição ecológica de riachos vadeáveis da Mata Atlântica, utilizando macroinvertebrados bentônicos. Todas as métricas biológicas foram ajustadas a variação natural através da análise de regressão múltipla ("stepwise-forward") e a distribuição dos resíduos foi utilizada para descrever variação de cada métrica na ausência de gradiente ambiental natural. Para a seleção das métricas foram considerados critérios de normalidade da distribuição, variação explicada pelos modelos, redundância entre métricas e sensibilidade para diferenciar gradiente de impacto. Foram selecionadas cinco métricas para compor o índice final: riqueza total, %MOLD (Mollusca+Diptera), %Coleoptera, riqueza de EPT (Ephemeroptera+Plecoptera+Trichoptera) e abundância de Chironomidae. Os resíduos das métricas selecionadas foram transformados em probabilidades e o valor final do índice foi obtido através da média dessas probabilidades. O índice teve ótimo desempenho em discriminar o gradiente de impacto e mostrou alta correlação (r = 0.85, p <0.001) com um índice desenvolvido especificamente para uma das bacias, indicando sensibilidade similar. O índice desenvolvido nesse trabalho pode ser utilizado em todo o estado do Rio de Janeiro e acreditamos que este tipo de abordagem constitui uma contribuição importante para o desenvolvimento e implementação de um programa de monitoramento biológico no Brasil.
Palavras-chave: *Biomonitoramento, Protocolo de Bioavaliação, ecossistemas aquáticos, macroinvertebrados.*

Introduction

The conservation and management of aquatic ecosystems is a great challenge worldwide as these ecosystems are highly threatened by several human activities. The knowledge and the accurate measurement of these impacts is a fundamental part for the decision process in conservation programs. Besides being based in ecological concepts, the protocols and tools for carrying out biological monitoring must be efficient, fast and consistently applied in different regions (Pont *et al* 2006). Also, the use of aquatic communities as biological indicators requires a detailed knowledge of the composition patterns and distribution of communities in water bodies in natural conditions and of the natural gradients that explain these patterns (Oberdorff et al. 2002). This is essential to evaluate biologic responses to anthropogenic pressures without the influence of natural environmental gradients.

Some studies in Brazil have shown the existence of a relationship between macroinvertebrate communities and natural gradients such as altitude (Henriques-Oliveira & Nessimian 2010) and river order (Baptista et al. 2001, Melo 2009). For the development of predictive indices, it is not enough to know the influence of environmental variables in the community as a whole, but it is important that direct relationships between these variables and metrics are studied.

At the moment the most used tool for assessment of biological conditions in aquatic ecosystems are the multimetric indices (Buss et al. 2015) and predictive models (Feio et al. 2007, Feio & Poquet 2011). A multimetric index (MMI) considers the effects of multiple impacts and aggregates individual biological, ecological and functional measures in a unique value that can be used to measure the general condition of the ecosystem (Karr 1981, Karr et al. 1986, Hering et al. 2006, Jun et al. 2012). The metrics based on multiple biological measures (diversity, composition, tolerance and trophic) are a characteristic or a measurable process of a biological system that alters in value along a gradient of human influence (Karr & Chu 2000). The strength of the MMI relies on its ability to integrate information of several aspects of a community, in order to provide a general classification of the ecosystem, without losing information from the individual metrics (Karr 1981, Karr et al. 1986, Hering et al. 2006). However, a great difficulty in the identification of the metrics sensitivity to anthropogenic impact is that they also can be affected by natural gradients such as altitude, precipitation and temperature, among others. Therefore, MMIs are generally developed for ecoregions or river typologies with the same natural conditions (Kosnicki et al. 2016, Munne & Prat 2009). Factors such as slope, altitude and type of substrate, for example, which may vary internally in previously defined ecoregions, may also influence the macroinvertebrate community and may even be more critical than the larger scale factors (Hawkins & Vinson 2000, Munn et al. 2009).

Covariance of anthropogenic pressures with natural environmental gradients can be a confounding factor in the evaluation of biologic responses to anthropogenic pressures (Stoddard et al. 2008, Hawkins et al. 2010, Moya et al. 2011). Aiming to overcome this methodological bottleneck, predictive multimetric indices are being developed in a way the metrics are adjusted to the variables that describe the natural gradients (Oberdorff et al. 2001, 2002, Pont et al. 2006, Tejerina-Garro et al. 2006, Moya et al. 2007, 2011, Chen et al. 2014, Macedo et al. 2016, Pereira et al. 2016, Chen et al. 2017). These indices take into account the influence of different natural gradients and allow that the metric value, in the absence of human interference, be predicted by the environmental characteristics of a specific locality. Besides the environmental variables, spatial variables should be included to avoid autocorrelation, which represents a problem for statistical inference and ecological patterns (Suriano et al. 2011).

Developing biomonitoring tools for large spatial scales is far more challenging than defining local tools for small regions, but this approach has been developed and studied in the USA (Barbour et al. 1996, Klemm et al. 2003, Blocksom & Johnson 2009, Flotemersch et al. 2014, Kosnicki et al. 2016) and in the EU (Böhmer et al. 2004, Gabriels et al. 2010, Mondy et al. 2012). The development of a national biomonitoring program as systematic management is under study in Brazil (Buss et al. 2015). The aim is to assess what would be the most appropriate tool to be deployed at the national level and applied to all natural biomes of Brazil. These studies have focused on developing mainly multimetric indices using macroinvertebrates. We highlight representative works for some Brazilian natural biomes: Amazon (Couceiro et al. 2012, Chen et al. 2017), Savanna (Ferreira et al. 2011, Macedo et al. 2016, Silva et al. 2017), Atlantic Forest (Baptista et al. 2007, 2011, 2013, Oliveira et al. 2011a, Jiménez-Valencia et al. 2014, Pereira et al. 2016).

In this study, we developed and tested a MMI based in the macroinvertebrate community that can discriminate natural from anthropogenic variability in wadeable streams in the Atlantic Forest in southeastern Brazil. This was the first predictive multimetric index developed in Brazil, and it is an important step towards the implementation of biomonitoring programs in the country.

Material and Methods

1. *Study area*

We sampled 158 sites in streams from 1st to 4th order (according to Strahler classification using 1:50,000 scale maps) that were divided into three categories: 64 minimally impaired areas, considered as reference sites, 50 severally altered by human activities sites; and 44 sites moderately affected by human influence in the Rio de Janeiro State. The sampling campaigns were carried out between 2006 and 2011 (during the dry season) using the same protocol and under the supervision of the same team. All these sites were located in watersheds between 20 m and 1900 m above sea level (Figure 1).

The area corresponding to all Rio de Janeiro state is 43,696 km². According to Alvares et al. (2013), 44% of Rio de Janeiro state's mid-lower portions are classified as tropical with a summer rainy season, with the most mountainous regions and plateaus classified as humid subtropical with hot summer, without dry season or with a dry winter. Temperatures oscillate between 15ºC and 28ºC, and annual rainfall is around 1000 – 1500 mm. The Atlantic Forest biome, which originally covered virtually the entire region, now represents less than 12% of its original extent and is mostly spread in the higher parts of the mountains and remnants interspersed with agriculture and pasture (Ribeiro et al. 2011).

2. *Sampled sites*

We classified the impairment classes of sampling sites using physicochemical and environmental parameters. We classified sampling sites in the field using the visual-based habitat assessment protocol (HAP; Barbour et al. 1999). The HAP evaluates ten environmental

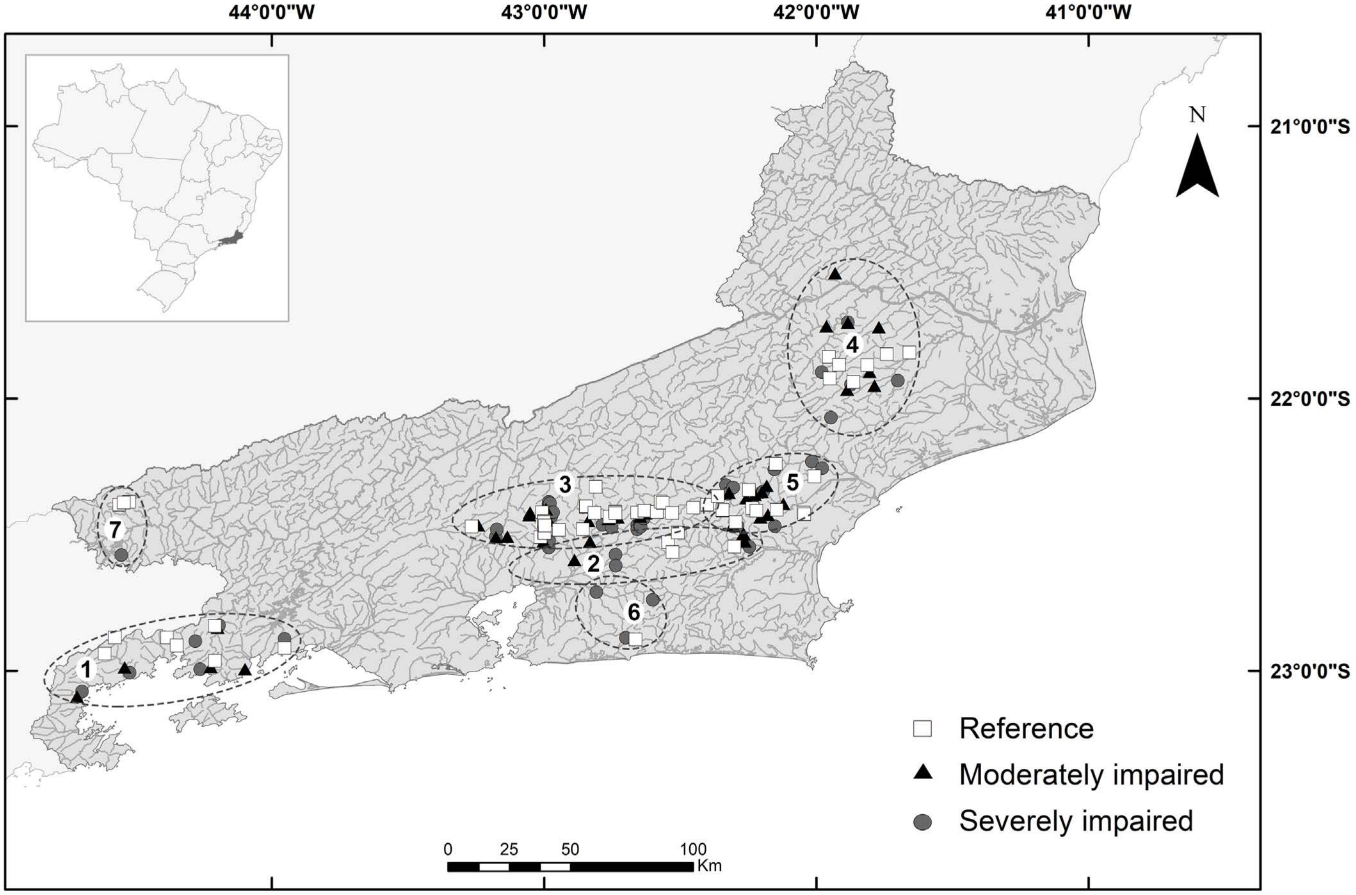


Figure 1. Map of the study area, showing the sampling sites in the Rio de Janeiro State. Squares indicate reference sites, and triangles and circles indicate moderately and severely impaired sites, respectively. Numbers represent river groups: 1- Ilha Grande Bay; 2- Central part Atlantic; 3- Central part Continental; 4- Northern region; 5- Northern Fluminense; 6- Coastal lowland; 7- Itatiaia.

parameters (e.g., sediment deposition, margin stability, and riparian vegetation) which the score ranges from 0 to 20. We used de mean score to obtain the site classification, as follows: 0-5 "Poor"; 5.1-9.9 "Marginal", 10–14.9 "Suboptimal" and 15–20 an "Optimal" environmental condition (Barbour et al., 1999). We classified as references sites if dissolved oxygen> 6.0 mg/L, an "Optimal" or "Suboptimal" environmental condition according to the HAP, no sign of channelization locally or upstream and if <25% area upstream land-use were urban (based on recent satellite images). We classified as impaired sites if they had "Poor" condition according to HAP and if recent satellite images showed >40% of the upstream area was affected by urban areas and agriculture. Intermediate sites had characteristics between these two classes.

The sampling sites were divided into groups for the development and test of the index according to Table 1. The sites of the group REF-CAL (reference calibration sites) were chosen in order to encompass all the watersheds sampled and represent the different natural gradients presented in the study area.

All these datasets were used in different steps of the process for developing the index as explained further in Figure 2.

3. *Sampling, screening, and identification of organisms*

For sampling macroinvertebrates we followed multi-habitat sampling procedure according to the availability of substrate in the reach using a Kick net with mesh size of 500 µm, using the protocol of the RBP III (Barbour et al. 1999). Twenty samples (around 20 m^2) were taken proportional to the substrates availability in each site. The percentage of available habitats was estimated by visual inspection. Substrates with less than 5% of the site area were not sampled. Samples were composited and conserved in the field in

Table 1. Sampling sites used to develop, validate and test the index.

Name of the group	Number of sampling sites	Objective
REF-CAL	50	Reference sites used to develop and calibrate the models that generated the index
SI-MET	42	Severely impaired sites used to test the sensitivity of metrics
REF-VAL	14	Reference sites used to validate the index
SI-VAL	8	Severely impaired sites used to test the index
MI-VAL	44	Sites with moderate impairment used to test the index

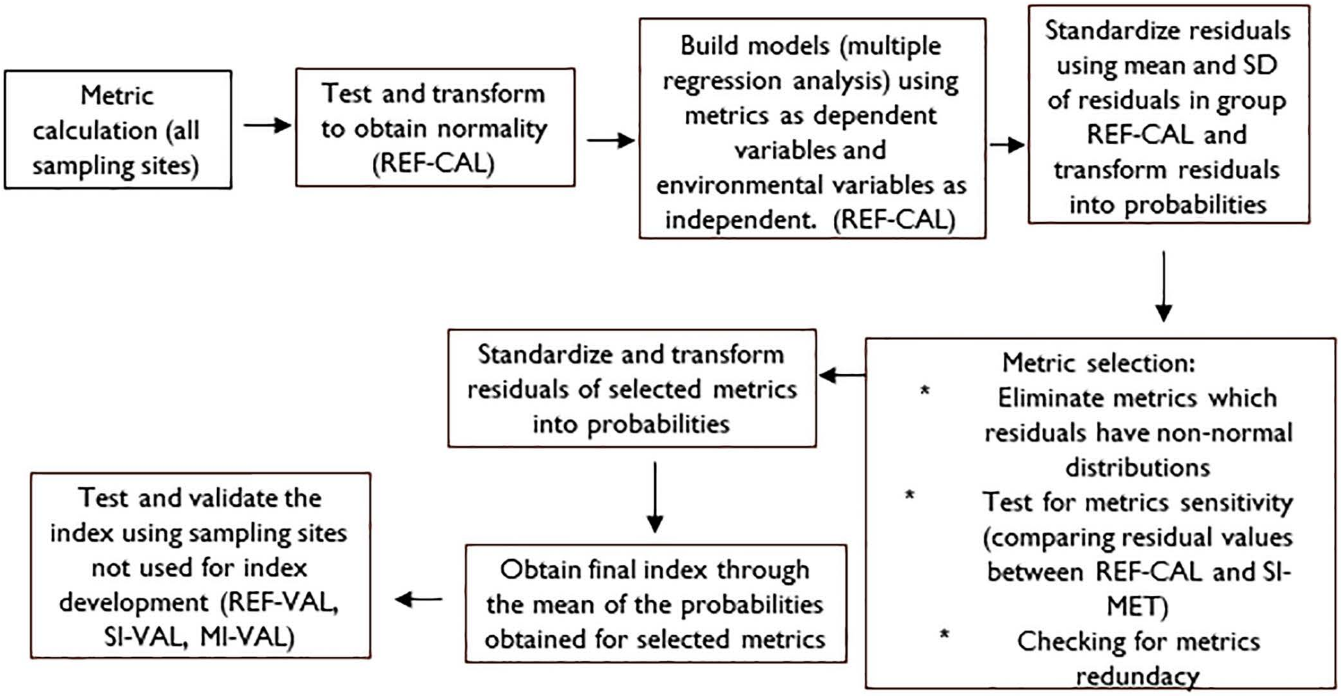


Figure 2. Overview of the process for developing the index and the groups of sampling sites used in each step.

80% ethanol and taken to the laboratory for further inspection. In the laboratory, samples were washed to remove organic matter, such as leaves and twigs in sieving of 500 µm. The remaining material was placed in a sub-sampler (64 × 36 cm), divided into 24 quadrats, each measuring 10.5 × 8.5 cm placed in the sub-sampling equipment – ESAM - Aquatic Macroinvertebrate Subsampling Equipment (patent number: EU 2572576; USA 9,513,94 B2).

The equipment was tested for its efficiency, and the number of sub-samples was defined for the metrics stability. After homogenization, six quadrats were randomly selected for sub-sampling. More two sub-samples were taken in case of the minimum number of 200 specimens were not reached (Oliveira et al. 2011b). The organisms were identified to the genus level, except Diptera, Hemiptera and Lepidoptera to the family level and Annelida to class level using the available taxonomic keys (Froehlich 1984, De Marmels 1990, Dominguez et al. 1992, Angrisano 1995, Merrit & Cummins 1996, Wiggins 1996, Nieser & de Melo 1997, Dorville & Froehlich 1999). For standardization, the level of taxonomic identification is treated here as operational taxonomic units (OTU).

4. *Environmental variables*

In each sampling site were taken measurements of environmental variables such as altitude (m), water temperature (ºC), precipitation (mm/year) and physical and chemical variables such as dissolved oxygen (mg/l), pH, conductivity (µS/cm), total dissolved solids (TDS). Dissolved oxygen (DO) was determined by using a YSI 550A analyzer, pH with a MPA 210p (LabConte) and conductivity and TDS by using MCA 150p (LabConte). Water samples were taken in sterile plastic bags (whirl-pak) according to APHA (2000). However, as recommended by Pont et al. (2009), only the variables that are not easily influenced by the anthropogenic impact such as channel slope and mean annual air temperature were used in the models, as we cannot guarantee that the reference sites do not suffer any kind of impact from human activities. Moreover, two variables were also measured: average width of the river (trough the measuring of 6 transversal sections in the reach sampled, considering only the wet width) and average depth.

The altitude was obtained using a digital model of ground elevation based in SRTM, using the software ESRI ArcGIS 9.0®. The area of the watershed was measured through the delimitation of polygons generated by this digital model. The average precipitation in each site was obtained from the map of total annual isoiets (period of 1968-1995) developed by CPRM (Centro de Pesquisa dos Recursos Minerais - Brasil). The temperature was obtained from the study of Araúјo et al. (2010) of monthly spatial distribution of the temperature in the State of Rio de Janeiro, based in linear models and SRTM.

A Shapiro-Wilk test was performed to test the normality of the data. The variables that had non-normal distributions were transformed to reach a normal distribution. For verification of redundancy between the environmental variables, a Pearson correlation test was performed. Variables with a correlation coefficient higher than 0.8 were considered redundant, and the most difficult to measure was eliminated.

5. *Identification of river groups determined by the fauna*

Some predictive multimetric indices use a variable that represents the river groups determined by their own fauna Pont et al. (2006). Analyses used in the initial stages of the predictive model development are performed to identify biologically relevant units. Other studies have grouped rivers as ecoregions or according to river typologies, using geographic, geologic and climatic characteristics (Moya et al. 2011, Oberdorff et al. 2001). In the present study, we used a methodology similar to Pont et al. (2006) for defining a variable called "river group". A Grouping Analysis (GA) was performed with macroinvertebrates abundances (transformed in logx+1) for 55 reference sites using the similarity of Bray-Curtis. After that, a Similarity Analyses (ANOSIM) was performed to verify the significance of the groups found in the grouping analyses. The ANOSIM calculates R-value that reflects the similarity between groups according to some factor, in this case, the fauna. An R-value close to zero indicates low difference and close to one a highly different fauna composition between river groups using the Bray-Curtis index.

6. *Metrics calculation, selection, and modeling*

We show a flow diagram with the general overview of this process and the datasets used in each step (Figure 2). Details regarding the description of analysis used are given further in the text.

Initially, 35 metrics chosen were calculated following previous studies of multimetric indices development (Barbour et al. 1996, Baptista et al. 2007, Oliveira et al. 2011b). All metrics were tested to verify the normality in the group REF-CAL. The metrics that even after transformations did not present normal distribution were eliminated. The metrics that had a small range were also eliminated (Klemm et al. 2003). Using the REF-CAL data, we generated models for each one of the metrics (dependent variables), using the environmental variables that represent the local factors (e.g., width and depth) and also the variable "river group" representing the spatial variation as predictive factors through multiple regression analyses (forward stepwise). The squares of the environmental variables were also considered for the identification of possible non-linear relationships. Each one of the river groups identified was used as variables and codified between zero and one. Only the river groups with determinant coefficients equal or higher than 0.3 were considered as adequate models. Regression analyses were performed in the software Statistica 7.0.

We also performed a regression analysis using the Akaike Information Criteria (AIC) for the selection of the best model. This analysis was carried out for confirming the selection of the model using the minimum square method, verifying if the selected model presented the lower values of AIC.

The residuals of each one of the metrics represent the metrics variation in the absence of natural gradients interference. The residuals were standardized subtracting the average of the residuals from the REF-CAL group and dividing by the standard deviation of that same data set, even when computed for the other groups of data (SI-MET, REF-VAL; SI-VAL; MI-VAL). The residual expected value in a reference site is equal to zero. For the metrics that decrease with the increase of anthropogenic impact (especially in severely impaired sites) negative residuals are expected and for metrics that increase with impact positive residuals are expected.

Assuming that residuals in the reference sites present normal distribution, with zero mean and standard deviation equal to one, it is possible to compute the probability of finding a lower residual value than the observed (for decreasing metrics) and higher than observed (for increasing metrics). The probability represents the chance that a specific site belongs to the distribution of the reference sites, that is, the lower the value the higher is the impairment in that site. The transformation of the residuals in probabilities makes the metrics comparable. All probabilities will vary between 0 and 1 and decrease with the impact. The expected probability distributions for a group of reference sites is a uniform distribution with average 0.5.

Residuals normality and variance homogeneity were tested in the reference sites. Metrics with non-normal residuals were eliminated. The models obtained were applied to the SI-MET data set and the residuals standardized. The residuals of the groups REF-CAL and SI-MET were compared by a paired t-test for verifying if the metrics are capable of differentiating minimally impaired from severely impaired sites.

A Pearson Correlation Analysis was performed to verify the redundancy of the metrics. If two metrics were highly correlated ($r > 0.8$ or < -0.8), the metrics with the lower p in the sensitivity test or the metrics which facilitates the application of the index (i.e., metrics which require identification only at the level of family or order) was retained.

7. *Index development and test*

The final index was obtained by the average of probabilities found for the selected metrics. Thus the index varied between 0 and 1 and was divided into five classes of anthropogenic impact. The index was denominated Predictive Multimetric Index for Rio de Janeiro State (PREMIER).

The index was validated in 3 independent data sets: REF-VAL, SI-VAL, and MI-VAL. If the index is working properly two conditions must be satisfied: (a) the average of the probabilities obtained for the group REF-VAL cannot be different from 0.5, and (b) the mean value of the group REF-VAL is higher than the mean value of the group MI-VAL which is, in turn, higher than the mean value of the group SI-VAL. Those conditions were tested by a unilateral t-test.

8. *Comparison between PREMIER and GMMI*

The Guapiaçu-Macacu Multimetric Index (GMMI) was developed by Oliveira et al. (2011a) for the drainage basins of the rivers Guapimirim, Guapiaçu e Macacu in Rio de Janeiro. The metrics used in this index were: richness family, Trichoptera richness family, Shannon index family, %Plecoptera, %EPT (Ephemeroptera+Plecoptera+Trichoptera), %MOLD (Mollusca+Diptera), %shredders, proportion Chironomidae/Diptera and proportion Hydropsychidae/Trichoptera.

The MMIs developed for a specific drainage basin are very sensitive to small disturbances in rivers of the same basin. However, they may not work well when applied in other basins with different characteristics. A predictive index based on a model developed for areas with distinct characteristics could present an inferior performance compared to the ones developed for specific areas when applied for the drainage basin for which it was developed.

For verifying if the PREMIER presented a lower performance than GMMI, both indices were tested in 10 sites in the Guapiaçu-Macacu drainage basin with different levels of anthropogenic impact, which were not used for the development of either index. The results were compared by a correlation test between the final values of the two indices.

Results

In this study, more than 110,000 benthic macroinvertebrate specimens were identified. In the reference sites that were used for the definition of the "river groups" by the fauna, more than 40,000 specimens were identified. The grouping analysis showed that the fauna is very similar in the following groups: Guapiaçu-Macacu, Itatiaia, Paquequer e Bocaina. Some sites located in lower areas (as São João river drainage basin) also demonstrated higher similarity among them. As the streams located in the northern Rio de Janeiro State are poorly studied and have different climatic conditions from other places it was decided to keep this group of rivers as a separate group. Criteria adopted here were both based on the analysis performed and in the knowledge of several prior works done in the region.

The results of ANOSIM considering the "river groups" show that, in most cases, the rivers did not differ significantly. The region that covers the area of Itatiaia National Park, which has specific features of a high degree of conservation of the aquatic ecosystems, was the most differentiated from other rivers groups. According to the ANOSIM, the following river groups were considered: coastal lowland (Maricá and São João rivers), northern Fluminense region (Macaé river), northern region (Desengano State Park), central part of Serra do Mar mountain region – continental side (drainage basin of the rivers Paquequer, Preto and Piabanha), central part of Serra do Mar mountain region –Atlantic side (drainage basin of the rivers Guapimirim, Guapiaçu and Macacu); "Ilha Grande" bay region (Bocaina National Park and Mambucaba river), Itatiaia (Itatiaia National Park, Serra da Mantiqueira). The R value obtained in the analysis was 0.48 ($p<0.001$), and all river groups differed significantly among them, except for the northern region of the State about the lowland coastal region (Table 2).

Characterizations of the sampling sites in the different determined river groups are presented. The higher sites are located on the continental side of the Serra do Mar, mainly in the Paquequer river basin. In this same region, the lower average temperature was found. The largest drainage basins are in the Macaé river basin, as well as the larger average width. The highest average depth was found in the rivers of the Itatiaia region. The region with the highest average precipitation was the Atlantic side of the central part of Serra do Mar (Table 3).

1. *Metric selection and index development*

From the 35 metrics calculated, nine were eliminated for not reaching normal distribution after transformations procedures. From the remaining 26 metrics, 16 presented more than 30% of its variation explained by the model. All 16 metrics with significant models presented residuals with normal distribution after the Shapiro-Wilk test ($p>0.05$). It was also performed a graphical analysis of residuals for verification of the homogeneity of variances. The results indicate that all metrics presented residuals randomly distributed around the mean. The sensitivity test, which compared the values of residuals of the 16 metrics in the group of reference sites with the ones of the severely impaired sites, showed that all were capable of significantly differentiate the groups (t-test; $p<0.01$).

The metric %Trichoptera had a slight increase in intermediate disturbance conditions and after that a decrease in severely impaired sites. To avoid assessment errors in the final index the metric %Trichoptera was eliminated (Table 4).

Several metrics presented a high correlation between each other. Therefore, in the face of a redundant metric, the one with the best result in the sensitivity test was chosen (t-test). In this step, five metrics were selected: richness (Family), %MOLD, %Coleoptera, EPT richness (OTU), Chironomidae abundance.

Using the dataset MI-VAL, a graphical evaluation was performed for verifying the relation of the metrics with the increase of anthropogenic impact, identifying if they were positively or negatively related with the disturbance gradient and also how it behaves in intermediate disturbance scenarios. All metrics presented a linear response to impairment with %MOLD and Chironomidae abundance presenting a positive relationship, and richness (Family), EPT richness (OTU) and %Coleoptera showed a negative relationship with the impact gradient. Finally, five metrics were kept for the index development (Figure 3). The models of the five selected metrics were confirmed by regression using the Akaike Information Criteria (AIC). The generated models presented the lower values of AIC. Table 5 presents the models of the five metrics retained to the final index.

The final value of the index was obtained by the average of the probability values of the five metrics. As expected, the average values obtained for the metrics of the group REF-CAL were not significantly different from 0.5 (Figure 4; Figure 5) and were not also different from the average obtained for the group REF-VAL ($p=0.33$). That means that the index is adequately calibrated with reference areas values mostly scoring over 0.5 and impaired sites scoring lower values (Figure 4). So, it can adequately asses the level of impairment of wadeable streams in Atlantic Forest.

2. *Index test*

The index was able to differentiate the classes of an impact since the values in the REF-VAL group were significantly higher than the MI-VAL group ($P = 0.02$) and the values MI-VAL were significantly higher than in SI-VAL ($p = 0.03$) (Figure 5).

3. *Comparison of the effectiveness of the predictive index with a specific index*

The GMMI and PREMIER indices were applied to 10 sampling sites with distinct natural characteristics and degrees of anthropogenic impact. It is noteworthy that the GMMI ranges from 0 to 100, divided into five classes: Very good (80-100), Good (60-80), Regular (40-60), Impaired (20-40) and Severely Impaired (0- 20). The PREMIER ranges from 0 to 1, divided into five classes: Very Good (≥ 0.5); Good (0.4-0.5); Regular (0.2-0.4); Impaired (0.1-0.2); Severely impaired (≤ 0.1). The correlation between the results of two indices was high (Pearson $r = 0.85$, $p < 0.001$), indicating that the PREMIER showed a

Table 2. Results of ANOSIM similarity of Bray-Curtis for the groups determined by fauna in the 55 reference sites.

Overall R: 0.48 p<0.001	Lowland	Northern-Fluminense	Northern	Central part- Atlantic	Ilha Grande Bay	Central part- Continental
Lowland						
Northern-Fluminense	0.1957*					
Northern	0.12697	0.2121*				
Central part - Atlantic	0.6496*	0.4277*	0.7344*			
Ilha Grande Bay	0.4434*	0.3975*	0.2627*	0.925*		
Central part – Continental	0.4329*	0.2618*	0.3022*	0.6948*	0.4839*	
Itatiaia	0.4435*	0.2635*	0.631*	0.7049*	0.9487*	0.6706*

*p<0.05

Table 3. Characterization of the sampling sites in the six river groups determined by the fauna in the reference sites (REF), moderately impaired (MI) and severely impaired sites (SI). In parenthesis Mean and Standard Deviation.

		Altitude (m)	Temperature (ºC)	Upstream area (Km2)	Average depth (cm)	Average width (m)	Precipitation (mm/year)
Ilha Grande Bay	REF	32-547 (156.6±196)	21-24 (22.6±1.14)	4.7-15.8 (9.77±4.8)	14-35 (20.1±8.8)	8.7-13 (10.6±1.8)	1400-1850 (1666.7±213.7)
	MI	8-546 (120.4±230.1)		5.7-50.5 (23.7±18.2)	14.4-35.5 (23.5±9)	6.5-17.5 (12.1±4.6)	1650-2150 (1890±227.5)
	SI	9-765 (226.3±337)		0.52-33.8 (12.5±11.7)	9.7-28.7 (15.4±7)	2.3-12 (7.5±3.1)	1400-2150 (1850±286.3)
Central part –Atlantic	REF	41-855 (462.9±296)	20-25 (22.5±1.8)	0.02-28.8 (5.7±7.6)	10-105 (33.5±21.5)	3-16 (7±3.4)	2100-2950 (2622±297)
	MI	8-470 (162.2±139.9)		1-487.4 (87.8±163.7)	16.3-75 (38.3±20.1)	4-16.5 (10±4.13)	1700-2650 (2255±304.1)
	SI	24-254 (66.5±44.6)		0.2-140.4 (34.4±42.3)	98.7-68.7 (26.16±15.4)	1.7±16 (7.7±5)	1500-2600 (2073.1±292.7)
Central part –Continental	REF	830-1194 (1016±158.2)	15-19 (17.3±1.7)	0.17-65.2 (12.1±23.5)	20.2-68 (32.1±16.9)	5-15 (9.1±3.6)	1550-2750 (2257.1±472)
	MI	834-1258 (986.9±141.3)		0.9-18.4 (8.9±6.8)	15.6-76 (43±20.7)	4-15 (7.5±3.6)	1550-2700 (2164.3±371.6)
	SI	818-897 (867.1±28.1)		5.8-72.7 (26.3±21.6)	8-75 (34.6±18.8)	2-14.5 (8.16±3.5)	1750-2600 (2294.4±297.3)
Northern State	REF	14-1054 (322.4±364.2)	18-25 (22.6±2.4)	1.1-56.3 (14.8±20.1)	20-98 (44.4±26.7)	3-20 (8.4±5.6)	1000-1250 (1121±95.1)
	MI	36-281 (113.9±89.5)		0.78-163.7 (61±63.4)	25.5-50 (36.4±9.4)	4.5-18.5 9.3±4.7	1000-1250 (1085.7±90)
	SI	35-468 (169±161.6)		0.3-155.1 (48.9±56.7)	20-55 (32.7±12.3)	4-10.5 (6.9±2.5)	1000-1450 (1200±161.2)
Northern-Fluminense	REF	33-1060 (568±360.9)	18-24 (20.4±2.3)	0.2-123.7 (22.6±38.3)	15-60 (30.1±14.2)	4-26 (9.9±6.3)	1600-2400 (2059±252.8)
	MI	48-705 (307.8±239.6)		3.4-329.4 (132.7±135.5)	17.4-55 (33.1±9.8)	5.3-12.3 (9.2±2.8)	1850-2300 (2016.7±152.1)
	SI	33-784 (366.8±303.7)		1.4-178.8 (36±62.8)	15.8-34.5 (23.5±5.7)	3-13.5 (6.5±3)	1550-2000 (1794.4±157)
Coastal lowland	REF	27-146 (76.4±41.6)	24-26 (25±0.8)	0.2-10.9 (3.4±4.2)	9-110 (30.5±32.8)	1-11 (4.7±3.4)	1250-2350 (1981.3±356.5)
	MI	23-66 (41.5±18.9)		14.6-121.6 (54.5±51)	13.5-60 (38.8±23.3)	8.3-11 (9.4±1.3)	2200-2350 2287.5±75
	SI	15-69 (39.3±27.4)		9.7-132.2 (59.6±64.3)	21.5-46 (32.8±12.4)	5.5-13 (10.3±4.2)	1250-2300 (1900±567.9)
Itatiaia	REF	838-1042 (881.3±54.1)	17-18 (17.7±0.6)	0.6-7.7 (4.9±3.8)	30-75 (57±23.8)	8-9 (8.7±0.6)	2000-2100 (2050±50)

similar sensitivity to the index developed specifically for the basin. Regarding the differences in ratings, it is important to note that when the classification was different, the PREMIER provided an inferior class than the GMMI. However, there has been no reverse case (Table 6).

Discussion

The natural condition of a river is a combined product of factors operating on different scales. On large scales, geological and climatic factors are the major determinants of the characteristics of streams, and the biota often responds to these factors along spatially distinct regions (Mykrä et al. 2004). In this work, environmental variables that represent local factors were used (width and depth, for example) and also the variable "river group" representing the spatial variation in wider geographical range. As in the study of Pont et al. (2006) the variable "river group" additively participates in the model, that is, they are only regional adjustments not changing the relationship between the metrics with the other variables in the model.

A metric positively related to altitude in a region will be positively related to altitude in the other region as well, requiring only the adjustment coefficient generated by "river group" variable. This variation among different river groups (the six regions defined in this case) may be related to biogeographical and historical factors and the natural gradients not measured by the variables used in the models (Pont et al. 2006). Thus, it will take more studies to identify the factors that are determining these regional differences and, preferably, turn them into quantitative variables that can be included in the models.

The PREMIER was able to assess most of the anthropogenic impacts on the macroinvertebrate community, regardless of various natural gradients in streams of biome of Atlantic Forest in southeastern

Table 4. Candidate metrics to integrate the PREMIER, response to impairment, if transformation to reach normal distribution, multiple regression analyses, if linear response to impairment and final evaluation.. OTU- operational taxonomic units; EPT- Ephemeroptera, Plecoptera and Trichoptera; %MOLD- Mollusca + Diptera; IBE-IOC- Índice Biótico Estendido Instituto Oswaldo Cruz.

Category	Metric	Response	Transformation	R^2	p	Linear response to impaiment	Final evaluation
Diversity	Richness (OTU)	Decrease	log10	0.54	<0.001	Yes	Valid
	Richness (Family)	Decrease		0.48	<0.001	Yes	Valid
	Trichoptera richness (OTU)	Decrease		0.35	0.003	Yes	Valid
	Trichoptera richness (Family)	Decrease		0.37	0.003	Yes	Valid
	EPT richness (OTU)	Decrease		0.46	<0.001	Yes	Valid
	EPT richness (Family)	Decrease		0.32	0.003	Yes	Valid
	Margalef (OTU)	Decrease		0.45	<0.001	Yes	Valid
	Margalef (Family)	Decrease		0.32	<0.001	Yes	Valid
	Shannon index (OTU)	Decrease		0.25	0.008	-	-
	Shannon index (Family)	Decrease		0.26	0.03	-	-
	Eveness (OTU)	Decrease		0.28	0.003	-	-
	Eveness (Family)	Decrease		0.13	0.027	-	-
	Dominance (OTU)	Decrease	-	-	-	-	-
	Dominance (Family)	Decrease	log10	0.14	0.055	-	-
	Equitability J (OTU)	Decrease	-	-	-	-	-
	Equitability J (Family)	Decrease		0.23	0.05	-	-
Composition	%Ephemeroptera	Decrease	-	-	-	-	-
	%Plecoptera	Decrease	Square root	0.24	0.02	-	-
	%Trichoptera	Decrease	Square root	0.35	<0.001	No	-
	%EPT	Decrease		0.25	0.04	-	-
	%Coleoptera	Decrease	Square root	0.33	0.002	Yes	Valid
	%Odonata	Variable	-	-	-	-	-
	%MOLD	Increase	Square root	0.46	<0.001	Yes	Valid
	%Diptera	Increase	Square root	0.46	<0.001	Yes	Valid
	%Chironomidae	Increase	Square root	0.37	<0.001	Yes	Valid
Tolerance	Baetidae/Ephemeroptera	Increase	-	-	-	-	-
	Hydropsychidae/Trichoptera	Decrease	-	0.15	0.08	-	-
	Diptera abundance	Increase	log10	0.44	<0.001	Yes	Valid
	Chironomidae abundance	Increase	log10	0.36	<0.001	Yes	Valid
	Chironomidae/Diptera	Increase	-	-	-	-	-
	IBE-IOC	Decrease	-	-	-	-	-
Trophic	% Scrapers	Decrease	Square root	0.37	<0.001	Yes	Valid
	% Shredders	Decrease	Square root	0.27	<0.001	-	-
	% Predators	Variable	-	-	-	-	-
	%Collector	Variable	-	-	-	-	-

Brazil. The metrics used in the index are known to be able to distinguish between different impairment levels and have been used in other indices (Baptista et al. 2007, Moya et al. 2007, Oliveira et al. 2011a, Lakew & Moog 2015, Melo et al. 2015). The richness metrics (richness and EPT richness) are considered useful due to their response to structural changes impaired gradients in macroinvertebrate assemblages (Suriano et al., 2013). The metrics that increase with impact (%MOLD, Chironomidae abundance) are associated organic pollution caused by untreated wastewater effluents in urban areas and to the increase of suspended organic particles in rural areas (Baptista et al. 2011). The %Coleoptera metric is often associated with the increase in primary production due to its function food web (Baptista et al. 2007).

Metrics based on trophic functional groups were not included in the index. The metrics %predators and %collectors were not included because it was not possible to normalize the data. The metric %shredders did not have the R-squared of its model larger than 0.3. The metric %scrapers had a high correlation with the metric %Coleoptera. Therefore it is indirectly represented in the index. The metric %Coleoptera was

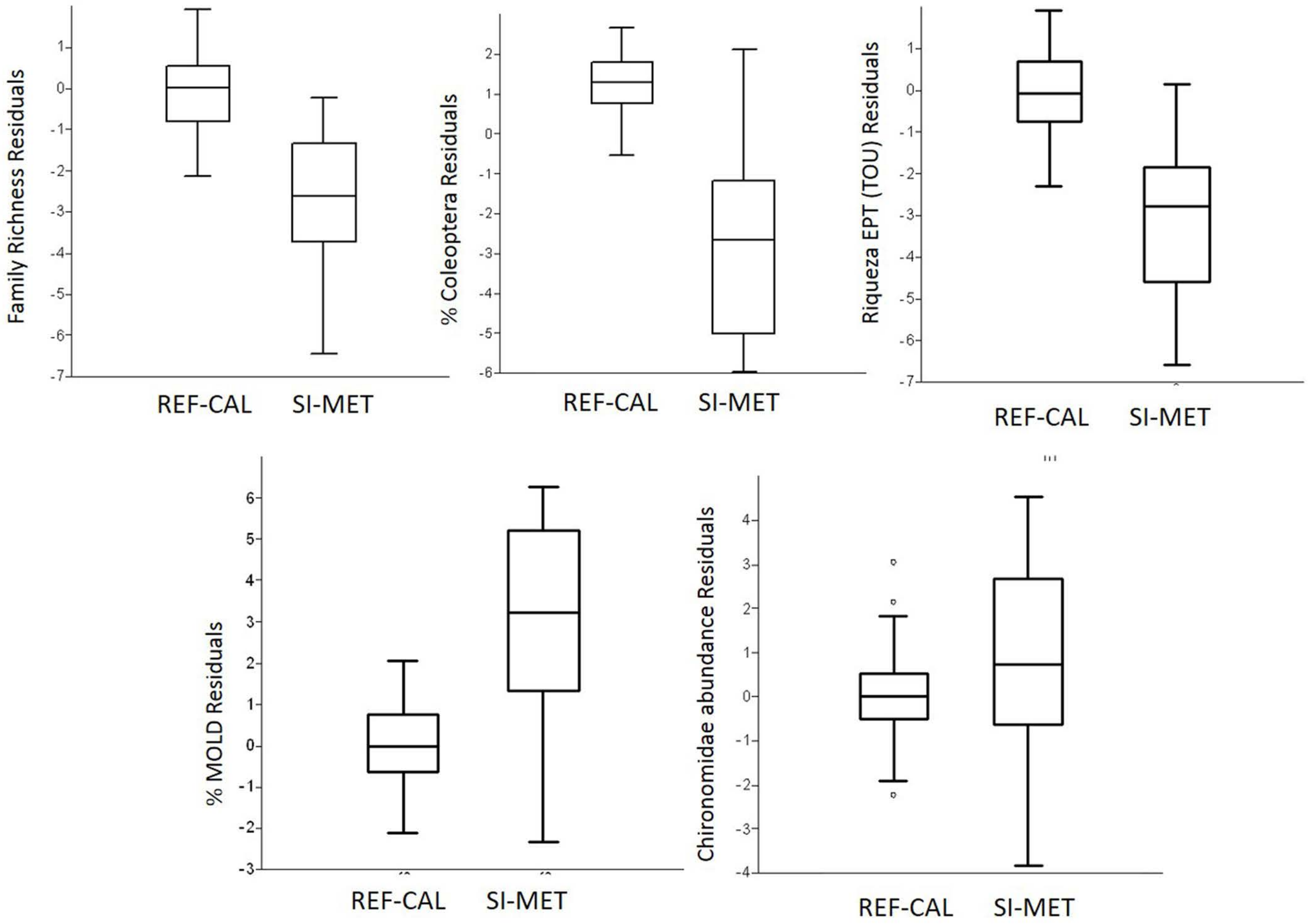


Figure 3. Box-plots of the selected metrics for the composition of PREMIER in the groups REF-CAL and SI-MET. The horizontal bars represent the median, the box represents the interquartile limits, and the vertical bars represent the minimum and maximum values.

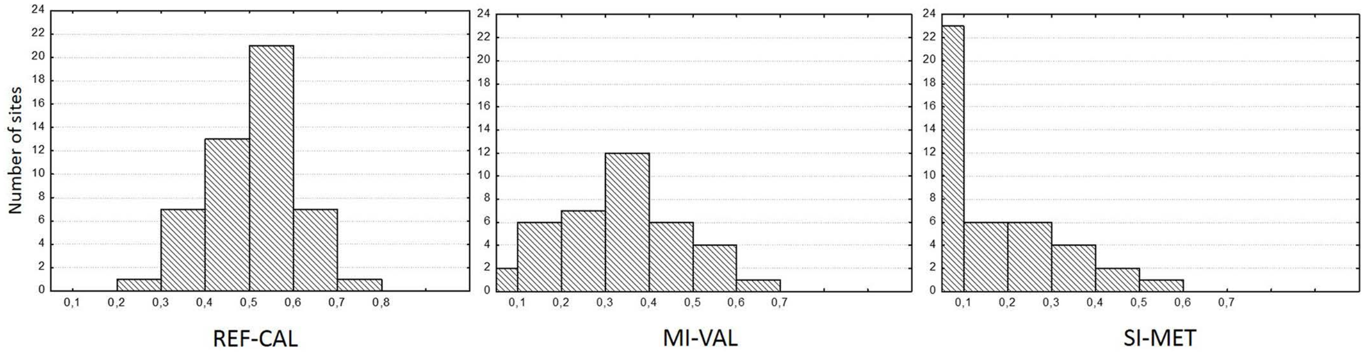


Figure 4. Histogram of PREMIER values for the calibration groups of the index, validation and selected metrics (REF-CAL, MI-VAL e SI-MET).

preferred because other studies found that functional metrics do not always respond to the impact in tropical environments (Moya et al. 2011, Melo et al. 2015, Pereira et al. 2016). Moreover, there is much debate about the feeding habits of some macroinvertebrate groups in the tropics and classifications have been discussed (Tomanova et al. 2006, Miserendino et al. 2007, Moya et al. 2007, Chará-Serna et al. 2012).

The metrics selected to compose the index require only that the groups Ephemeroptera, Plecoptera and Trichoptera are identified to the lowest taxonomic level. In the Diptera group, only Chironomidae should be identified to the family level. The other groups may be identified only to the order level or, in the case of Mollusca, to the phylum level. This is an important aspect for facilitating the use of the index for actual monitoring programs since it reduces the level of expertise and the time needed for applying it.

The results found in this study are very similar to other predictive multimetric indices already developed (Pont et al. 2006, 2009, Moya et al. 2011). It is noteworthy that, even using different groups of bioindicators, the final results of the indices are very similar. In the REF-CAL group the index ranged between 0.2 and 0.8, with an average of 0.502 (Figure 4). In the study of Moya et al. (2011) the index, also

Table 5. Description of the model for the five selected metrics in the index construction and standard deviation values for the group REF-CAL.

	Transformation	Richness (Family)	%MOLD	%Coleoptera	EPT richness (OTU)	Chironomidae abundance
Transformation			Square root	Square root		log10
Intercept		12.40242	4.29491	5.16658	12.43302	0.32836
Average depth	Log10		1.08272			
Average depth squared	Log10					0.16777
Altitude	Elevated 0.2	3.94061	0.52146	-0.27175	2.29832	
Altitude squared	Elevated 0.2					0.03019
Precipitation			0.00128			
Island Grande Bay		8.94388	1.86319	-0.58559	8.38662	1.57858
Northern		2.87168				1.07025
Northern-Fluminense		3.07517		0.57919		0.60490
Serra do Mar- Atlantic				1.39732		0.50598
Serra do Mar - Continental			-0.54982	1.43151	4.11806	0.83984
Coastal lowland		2.03961	1.98121		-2.66389	1.06934
Standard deviation REF-CAL		3.60272	1.02755	1.007	4.06217	0.34953

Table 6. Comparison of the classifications obtained with the GMMI and PREMIER indices.

	GMMI value	GMMI class	PREMIER value	PREMIER class
Site 1	90	Very Good	0.43	Good
Site 2	80	Very Good	0.41	Good
Site 3	99	Very Good	0.70	Very Good
Site 4	39	Impaired	0.11	Impaired
Site 5	88	Very Good	0.42	Good
Site 6	57	Regular	0.31	Regular
Site 7	97	Very Good	0.52	Very Good
Site 8	87	Very Good	0.68	Very Good
Site 9	52	Regular	0.29	Regular
Site 10	80	Very Good	0.73	Very Good
Site 11	34	Impaired	0.05	Severely Impaired

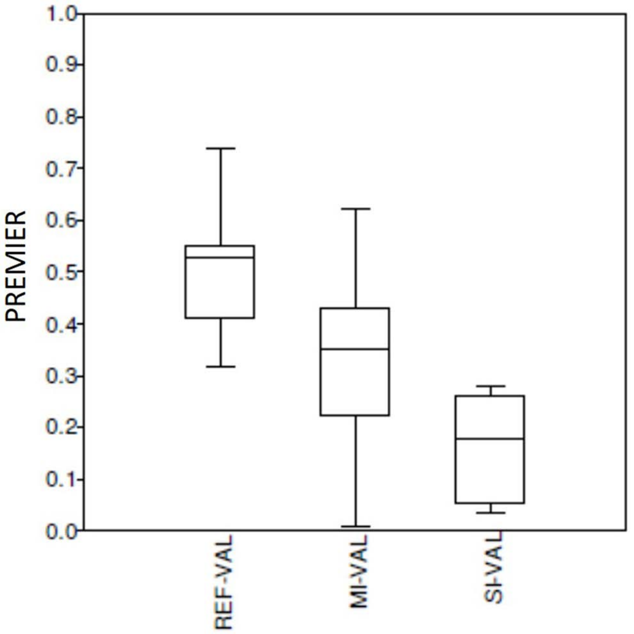


Figure 5. Box-plots of the PREMIER values in the groups REF-VAL, MI-VAL e SI-VAL. The horizontal bars represent the median, the box represents the interquartile limits, and the vertical bars represent the minimum and maximum values.

based on macroinvertebrates, varied in the calibration reference group between 0.3 and 0.8, with mean 0.5. In the study of Pont et al. (2006) the index based on fish community ranged from 0.1 to 0.8, averaging 0.5 in the reference sites. It is worth mentioning that in this study the index was developed to the whole Europe and the calibration group had 1000 reference sites distributed in several different countries. In the study of Pont et al. (2009) the index for aquatic vertebrates varied between 0.1 and 0.8 in the reference sites, having an average of 0.5. It is noticeable that the results are quite similar, especially that of Moya et al. (2011) that also developed using benthic macroinvertebrates.

For the development of PREMIER only variables that are not affected by disturbances were used. The altitude was kept in almost all models. The altitude gradient in small streams of mountainous regions tend to reflect other gradients such as water temperature (Pearson's correlation tested for the reference sites of 0.95) and slope. Both the temperature and slope gradients are known factors that affect the benthic community (Vinson & Hawkins 1998, Mykrä et al. 2008). Rivers groups were also retained in most of the models since the fauna actually appear to be different at these locations. Moya et al. (2011) also found that regions are important to determine the fauna. The test showed that the PREMIER is able to differentiate between different levels of impact and is sensitive to different types of disturbances. Ideally, it would be important to have a group of independent reference sites in the group used to calibrate the model, which presented different environmental characteristics, to verify if the index remains unchanged along the changes of natural gradients. However, priority was given to assemble a group of robust calibration and to ensure the spatial representation of the index. The test shows that even if there are still unmeasured natural gradients affecting the macroinvertebrate community, the index can assess the impact.

The PREMIER had its performance compared to a specific index developed for a particular basin (GMMI). The two indices agreed in most of the ratings and showed a high correlation, showing that the PREMIER has good sensitivity to different sources of disturbance. This shows that the PREMIER is a robust index and had the same sensitivity to disturbance as an index developed specially for the region.

One issue to be considered in the predictive multimetric approach is that the variance of the metrics not explained by the model must be the result of other unmeasured variables and also from human degradation gradients (Pont et al. 2006). That is, there must be natural gradients that covary with gradients of impact, or there are impact gradients, even in reference sites, influencing the variation of metrics. This brings us to another crucial aspect in the development of multimetric indices which is the definition of the reference condition. Even though the criteria are strict and equal for all sites, the ecological integrity of reference sites in certain regions may be higher than others. In the case of the State of Rio de Janeiro, it is much more difficult to find reference sites in the lower regions that meet the minimum established criteria. It is possible that in higher areas, due to the greater difficulty of occupation, the ecological integrity of reference sites is higher than the lower sites. A similar conclusion was also pointed out by Pinto et al. (2009).

It is noteworthy that in this study the temporal variation of the benthic community was not analyzed. As in Pont et al. (2006), samples collected at different times were used to increase the spatial representativeness of the index. However, Baptista et al. (2007) tested the stability of the selected metrics in Atlantic Forest streams to form a Multimetric index in three seasons and found that all had relative temporal stability.

Protecting the biotic integrity of our ecosystems depends on our ability to identify, measure and predict the effects that human activities have on them. This depends primarily on our ability to distinguish between natural variations and variations induced by anthropogenic disturbances (Oberdorff et al. 2002). The indices adjusted for natural variation has been shown to be more responsive and sensitive than standard indices (Chen et al. 2014, Macedo et al. 2016, Pereira et al. 2016). Using the methodology presented here is possible to assess the ecological status of Atlantic Forest wadeable streams. This allows that the monitoring can be performed on a large scale generating comparable data to facilitate management.

Brazil´s National Water Agency launched in 2013 the National Water Quality Monitoring Network which will be operated in a decentralized manner with partnerships established with states agencies (ANA 2014). At the moment, there is no goal within this network to include biological indicators. However, it may represent an important forum for discussion on the progress necessary for monitoring in the country, considering that some states already have biomonitoring in their programs. Furthermore, the use of cost-effective analysis (sampling, sorting, and identification) and the selection of a cost-benefit indicator group would be a great step forward for the development of practical biomonitoring programs. In this study, the protocols combined simple and inexpensive field equipment with optimized techniques for processing samples in the laboratory. The cost regarding time to process each sample generally is high, but the subsampling represented a saving of money, human resources and time (approximately 11 h in processing each sample) (see Oliveira et al. 2011b).

In Brazil, different multimetric indices were developed for the study area (Baptista et al. 2007, 2011, 2013, Oliveira et al. 2011a). However, these indices were developed specifically for each basin, considering specific characteristics. The PREMIER is an improvement over multimetric indices by considering the natural variability (e.g., topographic and climatic) and its application on wider geographical scale. The PREMIER index represents a robust methodology that can be easily reproduced in different areas and can easily be tested and applied for wadeable streams in Atlantic Forest. It can be an important tool for conservation and managing restoration areas in this important and severely damaged biome. Also, adequate water quality assessment is key to resolve, prevent and anticipate water conflicts in Brazil and can also be an important guide to financial investment in sanitation and conservation policies. We hope that the initiative of this study to produce a standardized protocol and an index that can be used on a broader scale contribute to improve ecological assessment in our country.

Supplementary material

The following online material is available for this article:
Appendix 1

Acknowledgments

We are grateful for financial support from CNPq/Universal (No 475359/2006-2); CNPq/PROEP/FIOCRUZ (No 400107/2011-2); PAEF/FIOCRUZ (IOC-023-FIO-18-2-4). Grant Productivity CNPq (No 301610/2009); Doctoral scholarship (CNPq-PPGE). We thank our colleagues of the LAPSA for in carrying out physicochemical and microbiological analysis of surface water (technical Valdinei Valin and Technologist Denise Assunção Borges).

Author Contributions

Renata Bley da Silveira de Oliveira: Substantial contribution in the concept and design of the study, contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Riccardo Mugnai: Contribution to manuscript preparation and to critical revision, adding intellectual content.

Priscilla da Silva Pereira: Contribution to manuscript preparation and to critical revision, adding intellectual content.

Natália Freitas de Souza: Contribution to manuscript preparation and to critical revision, adding intellectual content.

Darcílio Fernandes Baptista: Substantial contribution in the concept and design of the study, contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Conflicts of interest

The authors declare that there is no conflict of interest related to the publication of the data in this article.

References

ALVARES, C.A., STAPE, J.L., SENTELHAS, P.C., GONÇALVES, J.L.M., SPAROVEK, G. 2013. Köppen's climate classification map for Brazil. Meteorol. Z. 22(6):711–728.

ANGRISANO, E.B. 1995. Insecta Trichoptera. In Lopreto, E. C. & G. Tell (eds), Ecosistemas de Aguas Continentales. Metodologias para su estudio (vol III). Ediciones Sur, La Plata, p.1199–1237.

APHA, AWWA, WPCF. 2000. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association/American. Water Works Association/Water Environment Federation. Washington, DC.

ANA. 2014. Relatório Nacional de Conjuntura de Recursos Hídricos. Brasil.

ARAÚJO, F.F.S., SILVA, G.M., BAPTISTA, E.L.S., OLIVEIRA JUNIOR, J.F., LYRA, G.B. 2010. Espacialização da temperatura do ar mensal no estado do Rio de Janeiro baseada em modelos lineares e dados SRTM. In: IX Seminário de Atualização em Sensoriamento Remoto e Sistemas de Informações Geográficas Aplicados à Engenharia Florestal, Brasil, Curitiba.

BAPTISTA, D.F., BUSS, D.F., EGLER, M., GIOVANELLI, A., SILVEIRA, M.P., NESSIMIAN, J.L. 2007. A multimetric index based on benthic macroinvertebrates for evaluation of Atlantic Forest streams at Rio de Janeiro State, Brazil. Hydrobiologia 575:83-94.

BAPTISTA, D.F., DORVIIÉ, L.F.M., BUSS, D.F., NESSIMIAN, J.L. 2001. Spatial and temporal organization of aquatic insects assemblages in the longitudinal gradient of a tropical river. Braz. J. Biol. 61(2):295-304.

BAPTISTA, D.F., HENRIQUES-OLIVEIRA, A.L., OLIVEIRA, R.B.S., MUGNAI, R., NESSIMIAN, J.L., BUSS, D.F. 2013. Development of a benthic multimetric index for the Serra da Bocaina bioregion in Southeast Brazil. Braz. J. Biol. 73(3):573-583.

BAPTISTA, D.F., SOUZA, R.G., VIEIRA, C.A., MUGNAI, R., SOUZA, A.S., OLIVEIRA, R.B.S. 2011. Multimetric index for assessing ecological condition of running waters in the upper reaches of the Piabanha-Paquequer-Preto Basin (RJ, Brazil). Zoologia 28:619-628.

BARBOUR, M.T., GERRITSEN, J., GRIFFITH, G.E., FRYDENBORG, R., McCARRON, E., WHITE, J.S., BASTIAN, M.L. 1996. A framework for biological criteria for Florida streams using benthic macroinvertbrates. J. N. Am. Benthol. Soc. 15:185–211.

BARBOUR, M.T., GERRITSEN, J., GRIFFITH, G.E., FRYDENBORG, R., McCARRON, E., WHITE, J.S., BASTIAN, M.L. 1996. A framework for biological criteria for Florida streams using benthic macroinvertebrates. J. N. Am. Benthol. Soc. 15:185–211.

BARBOUR, M.T., GERRITSEN, J., SNYDER, B.D., STRIBLING, J.B. 1999. Rapid Bioassessment. Protocols for Use in Streams and Wadeable. Rivers: Periphyton, Benthic Macroinvertebrates, and Fish; EPA-841-B-99–002; U.S. Environmental Protection Agency, Office of Water: Washington, DC, USA.

BLOCKSOM, K.A. & JOHNSON, B.R. 2009. Development of a regional macroinvertebrate index for large river bioassessment. Ecol. Indic. 9(2):313-328.

BÖHMER, J., RAWER-JOST, C., ZENKER, A., MEIER, C., FELD, C.K., BISS, R., HERING, D. 2004. Assessing streams in Germany with benthic invertebrates: Development of a multimetric invertebrate based assessment system. Limnologica 34(4):416-432.

BUSS, D.F., CARLISLE, D.M., CHON, T.S., CULP, J., HARDING, J.S., KEIZER-VLEK, H.E., ROBINSON, W.A., STRACHAN, S., THIRION, C., HUGHES, R.M. 2015. Stream biomonitoring using macroinvertebrates around the globe: a comparison of large scale programs. Environ. Monit. Assess. 187:1-21.

CHARÁ- SERNA, A.M., CHARÁ, J.D., ZÚÑIGA, M.D.C., PEARSON, R.G., BOYERO, L. 2012. Diets of leaf litter-associated invertebrates in three tropical streams. J. Limnol. 48:139-144.

CHEN, K., HUGHES, R.M., XU, S., ZHANG, J., CAI, D., WANG, B. 2014. Evaluating performance of macroinvertebrate-based adjusted and unadjusted multi-metric indices (MMI) using multi-season and multi-year samples. Ecol. Indic. 36:142-151.

CHEN, K., HUGHES, R.M., BRITO, J.G., LEAL, C.G., LEITÃO, R.P., de OLIVEIRA-JÚNIOR, J.M., HAMADA, N. 2017. A multi-assemblage, multi-metric biological condition index for eastern Amazonia streams. Ecol. Indic. 78:48-61.

COUCEIRO, S.R.M., HAMADA, N., FORSBERG, B.R., PIMENTEL, T.P., LUZ, S.L.B. 2012. A macroinvertebrate multimetric index to evaluate the biological condition of streams in the Central Amazon region of Brazil. Ecol Indic 18: 118-125.

De MARMELS, J. 1990. Key to the ultimate instar larvae of the Venezuelan Odonate families. Opuscula Zoologica Fluminensis 50:1–6.

DOMINGUEZ, E., HUBBARD, M.D., PETERS, W.D. 1992. Clave para ninfas y adultos de las familias y generos de Ephemeroptera Sudamericanos. Biol. Acuat. 16:5–39.

DORVILLIÉ, L.F.M., FROEHLICH, C.G. 1999. Additional characters to distinguish the nymphs of the Perlidae genera from Southeastern Brazil (Insecta, Plecoptera). Aquat. Insects 21:281–284.

FEIO, M. J., REYNOLDSON, T. B., FERREIRA, V., GRAÇA, M. A. S. 2007. A predictive model for freshwater bioassessment (Mondego River, Portugal). Hydrobiologia, 589(1): 55-68.

FEIO, M. J., & POQUET, J. M. 2011. Predictive models for freshwater biological assessment: statistical approaches, biological elements and the Iberian Peninsula experience: a review. Intern. Rev. of Hydrobiology 96(4): 321-346.

FERREIRA, W.R., PAIVA, L.T., CALLISTO, M. 2011. Development of a benthic multimetric index for biomonitoring of a neotropical watershed. Braz. J. Biol. 71:15-25.

FLOTEMERSCH, J.E., NORTH, S., BLOCKSOM, K.A. 2014. Evaluation of an alternate method for sampling benthic macroinvertebrates in low-gradient streams sampled as part of the National Rivers and Streams Assessment. Environ. Monit. Assess. 186:949–959.

FROEHLICH, C.G. 1984. Brazilian Plecoptera 4. Nymphs of perlid genera from southeastern Brazil. Annales de Limnologie 20:43–48.

GABRIELS, W., LOCK, K., De PAUW, N., GOETHALS, P.L. 2010. Multimetric Macroinvertebrate Index Flanders (MMIF) for biological assessment of rivers and lakes in Flanders (Belgium). Limnologica 40(3):199-207.

HAWKINS, C.P., CAO, Y., ROPER, B. 2010. Method of predicting reference condition biota affects the performance and interpretation of ecological indices. Freshw. Biol. 55(5):1066–1085.

HAWKINS, C.P., VINSON, M.R. 2000. Weak correspondence between landscape classifications and stream invertebrate assemblages: implications for bioassessment. J. N. Am. Benthol. Soc. 19(3):501-517.

HENRIQUES-OLIVEIRA, A.L., NESSIMIAN, J.L. 2010. Aquatic macroinvertebrate diversity and composition in streams along an altitudinal gradient in Southeastern Brazil. Biota Neotrop. 10(3):115-128.

HERING, D., FELD, C. K., MOOG, O., OFENBÖCK, T. 2006. Cook book for the development of a multimetric index for biological condition of aquatic ecosystems: Experiences from the European AQEM and STAR projects and related initiatives. Hydrobiologia 566:311-342.

JIMÉNEZ-VALENCIA, J., KAUFMANN, P.R., SATTAMINI, A., MUGNAI, R., BAPTISTA, D.F. 2014. Assessing the ecological condition of streams in a southeastern Brazilian basin using a probabilistic monitoring design. Environ. Monit. Assess. 186(8):4685-4695.

JUN, Y.C., WON, D.H., LEE, S.H., KONG, D.S., HWANG, S-J. 2012. A multimetric benthic macroinvertebrate index for the assessment of stream biotic integrity in Korea. Int. J. Environ. Public Health 9:3599-3628.

KARR, J.R. & Chu, E.W. 2000. Sustaining living rivers. Hydrobiologia 422(423):1-14.

KLEMM, D.J., BLOCKSOM, K.A., FULK, F. A., HERLIHY, A.T, HUGHES, R.M., KAUFMANN, P.R., PECK, D.V., STODDARD, J.L., THOENY, W.T., GRIFFITH, M.B., DAVIS, W.S. 2003. Development and evaluation of a macroinvertebrate biotic integrity index (MBII) for regionally assessing Mid-Atlantic highlands streams. Environ. Manage. 31(5):656-669.

KOSNICKI, E., SEFICK, S.A., PALLER, M.H., JERRELL, M.S., PRUSHA, B.A., STERRETT, S.C., FEMINELLA, J.W. 2016. A Stream Multimetric Macroinvertebrate Index (MMI) for the Sand Hills Ecoregion of the Southeastern Plains, USA. Environ. Manage. 58(4):741-751.

LAKEW, A., MOOG, O. 2015. A multimetric index based on benthic macroinvertebrates for assessing the ecological status of streams and rivers in central and southeast highlands of Ethiopia. Hydrobiologia 751(1):229-242.

MACEDO, D.R., HUGHES, R.M., FERREIRA, W.R., FIRMIANO, K.R., SILVA, D.R., LIGEIRO, R., CALLISTO, M. 2016. Development of a benthic macroinvertebrate multimetric index (MMI) for Neotropical Savanna headwater streams. Ecol. Indic. 64:132-141.

MELO, A.S. 2009. Explaining dissimilarities in macroinvertebrate assemblages among stream sites using environmental variables. Zoologia 26(1):79-84.

MELO, S., STENERT, C., DALZOCHIO, M.S., MALTCHIK, L. 2015. Development of a multimetric index based on aquatic macroinvertebrate communities to assess water quality of rice fields in southern Brazil. Hydrobiologia 742(1):1-14.

MERRIT, R.W. & CUMMINS, K.W. 1996. An Introduction to the Aquatic Insects of North America, 3rd edn. Kendall/Hunt, Dubuque.

MISERENDINO, M.L. 2007. Macroinvertebrate functional organization and water quality in a large arid river from Patagonia (Argentina). J. Limnol. 43:133-145.

MONDY, C.P., VILLENEUVE, B., ARCHAIMAULT, V., USSEGLIO-POLATERA, P. 2012. A new macroinvertebrate-based multimetric index (I 2 M 2) to evaluate ecological quality of French wadeable streams fulfilling the WFD demands: a taxonomical and trait approach. Ecol. Indic. 18:452-467.

MOYA, N., HUGHES, R.M., DOMÍNGUEZ E., GIBON, F.-M., GOITTIA, E., OBERDORFF, T. 2011. Macroinvertebrate-based multimetric predictive models for evaluating the human impact on biotic condition of Bolivian streams. Ecol. Indic. 11:840-847.

MOYA, N., TOMANOVA, S., OBERDORFF, T. 2007. Initial development of a multi-metric index based on aquatic macroinvertebrates to assess streams condition in the Upper Isiboro-Sécure Basin, Bolivian Amazon. Hydrobiologia 589:107-116.

MUNNÉ A. & PRAT, N. 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628(1):203.

MUNN, M.D., WAITE, I.R., LARSEN, D.P., HERLIHY, A.T. 2007. The relative influence of geographic location and reach-scale habitat on benthic invertebrate assemblage in six ecoregions. Environ. Monit. Assess. 154:1-14.

MYKRÄ, H., AROVIITA, J., KOTANEN, J., HÄMÄLÄINEM, H., MUOTKA, T. 2008. Predicting the stream macroinvertebrate fauna across regional scales: influence of geographical extent on model performance. J. N. Am. Benthol. Soc. 25(3):705-716.

MYKRÄ, H., HEINO, J., MUOTKA, T. 2004. Variability of lotic macroinvertebrate assemblages and stream habitat characteristics across hierarchical landscape classifications. Environ. Manage. 34(3):341-352.

NIESER, N., de MELO, A.L. 1997. Os heterópteros aquáticos de Minas Gerais, guia introdutório com chave de identificação para as espécies de Nepomorpha e Gerromorpha. Editora UFMG, Belo Horizonte, 180 pp.

OBERDORFF, T., PONT, D., HUGUENY, B., CHESSEL, D. 2001. A probabilistic model characterizing fish assemblages of French rivers: a framework for environmental assessment. Freshw. Biol. 46:399-415.

OBERDORFF, T., PONT, D., HUGUENY, B., PORCHER, J.P. 2002. Development and validation of a fish-based index for the assessment of 'river health' in France. Freshw. Biol. 47:1720-1734.

OLIVEIRA, R.B.S., BAPTISTA, D.F., MUGNAI, R., CASTRO, C.M., HUGHES, R.M. 2011a. Towards rapid bioassessment of wadeable streams in Brazil: Development of the Guapiaçu-Macacu Multimetric Index (GMMI) based on benthic macroinvertebrates. Ecol. Indic. 11:1584-1593.

OLIVEIRA, R.B.S., MUGNAI, R., CASTRO, C.M., BAPTISTA, D.F. 2011b. Determining subsampling effort for the development of a rapid bioassessment protocol using benthic macroinvertebrates in streams of Southeastern Brazil. Environ. Monit. Assess. 175:75-85.

PEREIRA, P.S., SOUZA, N.F., BAPTISTA, D.F., OLIVEIRA, J.L., BUSS, D.F. 2016. Incorporating natural variability in the bioassessment of stream condition in the Atlantic Forest biome, Brazil. Ecol. Indic. 69:606-616.

PINTO, B.C.T., ARAUJO, F.G., RODRIGUES, V.D., HUGHES, R.M. 2009. Local and ecoregion effects on fish assemblage structure in tributaries of the Rio Paraíba do Sul, Brazil. Freshw. Biol. 54:2600-2615.

PONT, D., HUGHES, R.M., WHITTIER, T.R., SCHMUTZ, S.A. 2009. Predictive index of biotic integrity model for aquatic-vertebrate assemblages of western U.S. streams. Trans. Am. Fish Soc. 138:292-305.

PONT, D., HUGUENY, B., BEIER, U., GOFFAUX, D., MELCHER, A., NOBLE, R., ROGERS, C., ROSET, N., SCHMUTZ, S. 2006. Assessing river biotic condition at the continental scale: a European approach using functional metrics and fish assemblages. J. Appl. Ecol. 43:70-80.

RIBEIRO, M.C., MARTENSEN, A.C., METZGER, J.P., TABARELLI, M., SCARANO, F., FORTIN, M.J. 2011. The Brazilian Atlantic Forest: a shrinking biodiversity hotspot. In: Zachos, F.E., Habel, J.C. (Eds.), Biodiversity Hotspots: Distribution and Protection of Conservation Priority Areas. Springer, Heidelberg, Berlin, pp.405–434.

SILVA, D.R.O., HERLIHY, A.T., HUGHES, R.M., CALLISTO, M. 2017. An improved macroinvertebrate multimetric index for the assessment of wadeable streams in the neotropical savanna. Ecol. Indic. 81:514–525.

STODDARD, J.L., HERLIHY, A.T., PECK, D.V., HUGHES, R.M., WHITTIER, T.R., TARQUINIO, E. 2008. A process for creating multimetric indices for large-scale aquatic surveys. J. N. Am. Benthol. Soc. 27:878–891.

SURIANO, M. T., FONSECA-GESSNER, A. A., ROQUE, F. O., & FROEHLICH, C. G. 2011. Choice of macroinvertebrate metrics to evaluate stream conditions in Atlantic Forest, Brazil. Environ. Monit. Assess. 175(1-4): 87-101.

SURIANO, M. T. and FONSECA-GESSNER, A.A. 2013. Structure of benthic macroinvertebrate assemblages on a gradient of environmental integrity in Neotropical streams. Acta Limnologica Brasiliensia. 25 (4): 418-428.

TEJERINA-GARRO, F.L., MÉRONA, B., OBERDORFF, T., HUGUENY, B. 2006. A fish-based index of large river quality for French Guiana (South America): methods and preliminary results. Aquat. Living Resour. 19:31–46.

TOMANOVA, S., GOITTIA, E., HELEZIC, J. 2006. Trophic levels and functional feeding groups of macroinvertebrates in neotropical streams. Hydrobiologia 556:251-264.

VINSON, M.R., HAWKINS, C.P. 1998. Biodiversity of stream insects: variation at local, basin and regional scales. Annu. Rev. Entomol. 43:271-293.

WIGGINS, G.B. 1996. Larvae of the North American caddisfly genera (Trichoptera), 2nd edn. University of Toronto Press, Toronto: XIII 457 pp.

Received: 12/03/2018
Revised: 23/11/2018
Accepted: 12/12/2018
Published Online: 16/05/2019

Biota Neotropica 19(2): e20180561, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Use of a species-rich and highly eutrophic tropical estuary in the South Atlantic by Pleuronectiformes (Teleostei: Acanthopterygii)

***Luiz Constantino Silva-Junior*[1]*, Sérgio Ricardo Santos*[1]*, *Márcio Chagas Macedo*[1]*, *Gustavo Wilson Nunan*[2]**

***& Marcelo Vianna*[1]**

[1]*Universidade Federal do Rio de Janeiro, Departamento de Biologia Marinha, Laboratório de Biologia e Tecnologia Pesqueira, Rio de Janeiro, RJ, Brasil.*
[2]*Universidade Federal do Rio de Janeiro, Departamento de Vertebrados – Ictiologia, Museu Nacional do Rio de Janeiro, Rio de Janeiro, RJ, Brasil.*
**Corresponding author: Sérgio Ricardo Santos, e-mail: srbs.ufrj@gmail.com*

SILVA-JUNIOR, L. C., SANTOS, S. R., MACEDO, M. C., NUNAN, G. W., VIANNA, M. **Use of a species-rich and highly eutrophic tropical estuary in the South Atlantic by Pleuronectiformes (Teleostei: Acanthopterygii)**. Biota Neotropica. 19(2): e20180561. http://dx.doi.org/10.1590/1676-0611-BN-2018-0561

Abstract: A total of 1,471 specimens of 16 species of flatfishes (Pleuronectiformes) were caught during 48 sampling campaigns between July 2005 and June 2007 at ten stations in Guanabara Bay, Rio de Janeiro, Brazil. Paralichthyidae was the dominant family, with *Etropus crossotus* as the dominant species. The outer stations, especially those on the western side of the lower estuary, were distinguished as a result of their higher abundance of flatfishes and number of species. The spatial distribution of *E. crossotus* and its population structure indicate that this species is an estuarine resident despite the apparent reduction in its area of occupation within the estuarine complex. Among the other species, nine were classified as marine stragglers (*Achirus declivis, Bothus ocellatus, Cyclopsetta chittendeni, Etropus longimanus, Paralichthys orbignyanus, P. patagonicus, Syacium micrurum, Symphurus diomedeanus* and *Trinectes paulistanus*) and three as estuarine opportunists (*Bothus robinsi, Citharichthys macrops* and *Syacium papillosum*); another three could not be classified due to the small number of captures or lack of previous data (*S. tessellatus, A. lineatus* and *C. spilopterus*).

Keywords: *biodiversity, community, estuarine fishes, flatfishes, Paralichthyidae.*

Uso de um estuário tropical rico em espécies e altamente eutrofizado no Atlântico Sul por Pleuronectiformes (Teleostei: Acanthopterygii)

Resumo: Um total de 1.471 espécimes de 16 espécies de linguados (Pleuronectiformes) foram capturados durante 48 campanhas de amostragem entre julho de 2005 e junho de 2007 em dez estações na Baía de Guanabara, Rio de Janeiro, Brasil. Paralichthyidae foi a família dominante, com *Etropus crossotus* como a espécie dominante. As estações externas, especialmente aquelas no lado ocidental do baixo estuário, foram distinguidas como resultado de sua maior abundância de linguados e número de espécies. A distribuição espacial de *E. crossotus* e sua estrutura populacional indicam que esta espécie é uma residente estuarina, apesar da aparente redução em sua área de ocupação dentro do complexo estuarino. Dentre as outras espécies, nove foram classificadas como migrantes marinhos (*Achirus declivis, Bothus ocellatus, Cyclopsetta chittendeni, Etropus longimanus, Paralichthys orbignyanus, P. patagonicus, Syacium micrurum, Symphurus diomedeanus* e *Trinectes paulistanus*) e três como oportunistas estuarinos (*Bothus robinsi, Citharichthys macrops* e *Syacium papillosum*); outras três não puderam ser classificados devido ao pequeno número de capturas ou falta de dados prévios (*S. tessellatus, A. lineatus* e *C. spilopterus*).

Palavras-chave: *biodiversidade, comunidade, peixes estuarinos, linguados, Paralichthyidae.*

Introduction

The species of Pleuronectiformes, commonly known as flatfishes, can be easily identified by their asymmetrically compressed body, being usually pigmented on the side with eyes and depigmented on the blind side. They are demersal fishes with a carnivorous diet and have no swim bladder (Nelson et al. 2016). Pleuronectiformes is composed of 772 living species, primarily marine, distributed among 129 genera and 14 families (Nelson et al. 2016). In Brazilian waters, 56 marine species have been recorded and are distributed in 20 genera and five families (Menezes et al. 2003).

Despite the economic importance of Pleuronectiformes, their interactions with the varied ecosystems in which they are found are poorly understood. This is evident in environments such as estuaries, essential ecosystems for fish populations that are used as breeding and feeding grounds for several species, many of commercial value (Silva-Júnior et al. 2012, Blaber 2013). Pleuronectiformes contains species of high abundance in estuarine ecosystems (Vieira et al. 1998, Chaves et al. 2003), and these species undergo part or all of their reproductive cycle within these environments (Allen & Baltz 1997, Vieira et al. 1998, West et al. 2003, Chaves & Bouchereau 2004). Furthermore, flatfishes play an important role in the trophic chain of estuaries by acting as top or second-order predators (West et al. 2003, Bouchereau & Chaves 2003, Chaves & Bouchereau 2004).

Despite the ecological and socioeconomic importance of estuaries, they experience constant processes of degradation caused by human activities. However, studies on the biology of estuarine fishes have attracted the attention of researchers (e.g., Silva-Júnior et al. 2012, Bodin et al. 2014). Some studies have explored the interaction between flatfishes and the estuary environment, both tropical and temperate (Martinho et al. 2010, Oliveira & Favaro 2011), despite only a few being from estuaries located in the western South Atlantic coast.

In the tropical estuary of Guanabara Bay (Rio de Janeiro State, Brazil), the harvest of flatfishes is associated with intense fishing as bycatch of shrimp trawl fisheries (Jablonski et al. 2006). However, very little information is available on the fish fauna of this estuary (Rodrigues et al. 2007; Andrade-Tubino et al. 2009; Rosenfelder et al. 2012; Silva-Junior et al. 2012; Franco et al. 2014). The scarcity of information about the flatfish community of Guanabara Bay, a tropical estuary with a history of environmental degradation, demands a thorough assessment of the fish community. Continuous efforts have been undertaken to understand the ecological processes and patterns that drive the local ichthyofauna, leading to relevant contributions to the comprehension of composition and abundance of Tetraodontiformes (Andrade et al. 2015, Santos et al. 2015) and Gerreidae (Côrrea & Vianna 2016), as well as the population biology of *Genidens genidens* (Silva-Júnior et al. 2013) and the whitemouth croaker (*Micropogonias furnieri*) (Mulato et al. 2015).

The present study aims to determine the specific composition and abundance of flatfishes in Guanabara Bay. The data collected from all sampled species are compared to the data in the literature in order to classify the use of the estuarine complex and identify temporal and spatial changes in richness and evenness. Previous studies of other taxonomic fish groups occupying a large variety of niches in Guanabara Bay have shown a high diversity when compared to other Brazilian estuaries, but with a distinct predominance of one species over all others from each group (Andrade et al. 2015, Santos et al. 2015, Silva-Júnior et al. 2013, Silva-Júnior et al. 2016).

Materials and Methods

1. Study area

The tropical estuary of Guanabara Bay is located on the western South Atlantic coast (22º40'–23°00'S and 043º00'–043º20'W). The Guanabara Bay is considered to be a large estuarine complex, covering approximately 324 km^2 and with an estimated water volume of 1.87 × 10^9 m^3. It is supplied by a drainage basin that extends over 4,000 km^2 and includes 35 rivers. The opening of the bay is 1,500 m wide and is divided into two channels by Cotunduba Island. The estuary is located in the metropolitan region of the city of Rio de Janeiro, the fourth largest city in Latin America, with over 11 million inhabitants (Forstall et al. 2009). The tidal regime is semidiurnal, with a maximum height of 1.4 m. The depths range from less than 1 m to over 30 m in the central channel. The bay suffers from continuous and extensive land reclamation (recent and historical), silting, solid waste and sewage disposal (Carreira et al. 2004), intense industrial activity (Soares-Gomes et al. 2016), unplanned urban growth with continual deficiencies in sanitation (Costa et al. 2018), and pollution by heavy metals (Neto et al. 2006) and petroleum and its by-products (Soares-Gomes et al. 2010). The relevance of such impacts increases as a result of the importance of this ecosystem to the inhabitants of the metropolitan region of Rio de Janeiro because of its various uses, such as fishing, shipping, port activities, recreation, and tourism.

The sampling stations were selected on the basis of Mayr et al. (1989), who characterized the hydrobiology of Guanabara Bay and divided it into five areas according to the water quality. Sampling was conducted at ten stations, two in each of the five predefined areas (Figure 1):

Area 1 – The upper estuary, north of Governador Island. This region is severely impacted by pollution from domestic sewage, industrial waste and garbage dumps. This area also suffers from impaired circulation caused by historical land reclamation, which reduces the removal of pollutants by coastal waters (Carreira et al. 2004; Silva Jr. et al. 2016). It is distinguished by muddy sediment and low salinities (27.0–29.5) and temperatures between 24.0 and 25.9°C at the bottom (Mayr et al. 1989).

Area 2 – The upper estuary near Paquetá Island. This area is characterised by (i) the discharge of the least polluted rivers, (ii) the presence of a large extent of the remaining original mangrove coverage, which is protected under a Federal Conservation Unit (EPA of Guapimirim), and (iii) the influence of the central channel, a main source of less polluted waters and the origin of the salt wedge, which favours a less degraded environment than that found in Area 1 (Carreira et al. 2004). This portion of the estuary is characterised by muddy sediment and waters with salinities and temperatures at the bottom ranging from 27.0 to 29.5 and 22.5 to 23.9°C, respectively (Mayr et al. 1989).

Area 3 – The middle estuary, which is directly influenced by the central channel and is an area with a greater exchange of water and better environmental conditions than the upper estuary (Carreira et al. 2004). The substrate is composed of muddy sediments and fine sand, and the waters have high salinity, above 30.0, and bottom temperatures below 25°C (Mayr et al. 1989).

Area 4 – As the most exposed portion of the bay, the lower estuary is strongly influenced by the coastal waters and marine conditions.

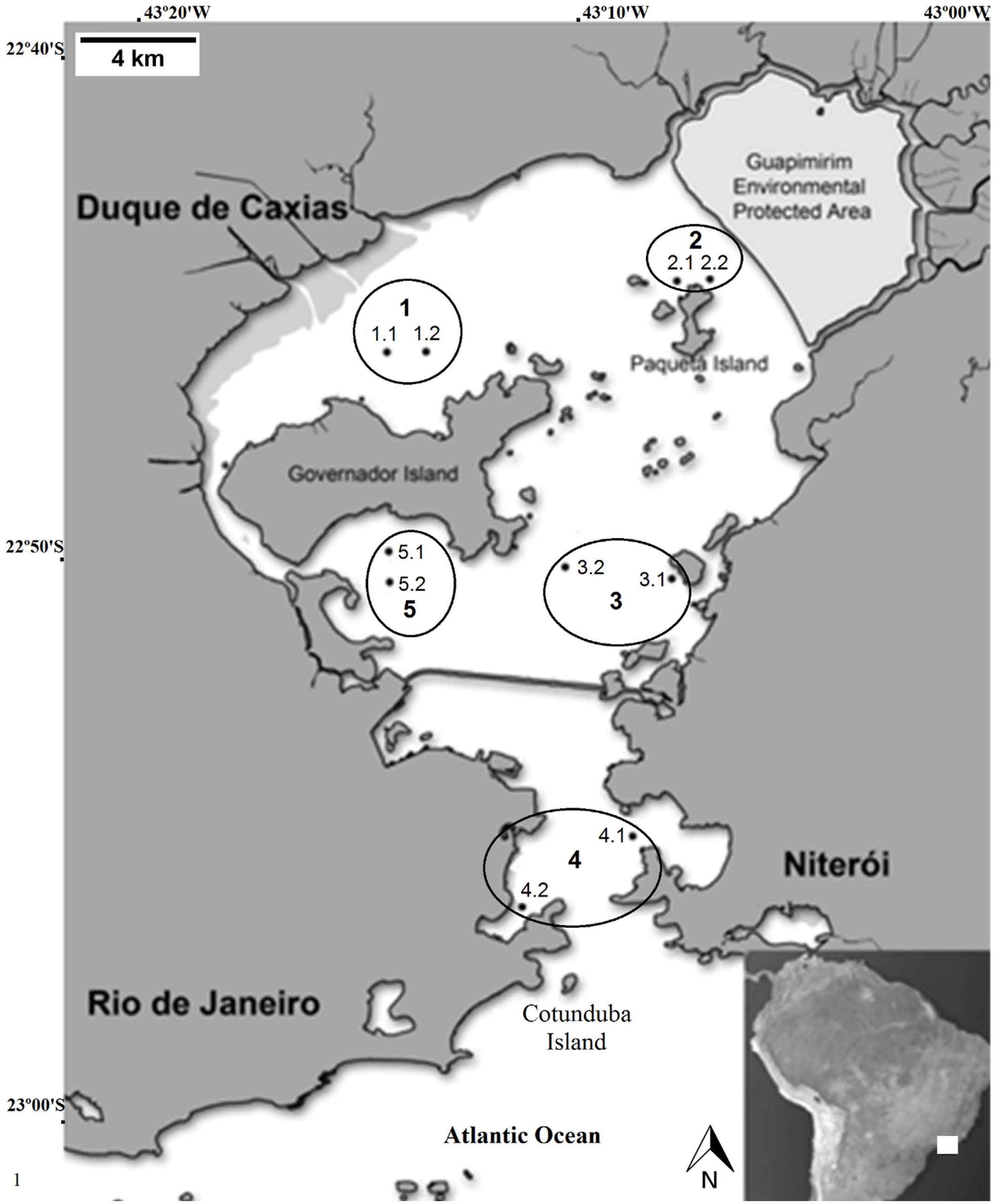


Figure 1. Map of Guanabara Bay, an estuary in the southeastern Brazil. The map shows all ten sampling stations divided into five areas (1 to 5) and distributed from the upper estuary to the lower estuary. The limits of the Guapimirim Environmental Protected Area are also indicated.

The stations are located near the estuary mouth, and this area is subjected to contributions of raw sewage from the cities of Rio de Janeiro (4.2) and Niterói (4.1) (Figure 1) (Silva Jr. et al. 2016). Fine or medium sand covers the bottom of this area, while water in the region has high salinity (always > 32.0) and low temperatures (always < 25°C), closer to the conditions found in the adjacent coastal waters (Mayr et al. 1989).

Area 5 - South of Governador Island. This area is subjected to high intake of the sewage produced on the island. Despite these conditions, the close proximity to the central channel allows relatively good water circulation and the dilution of pollutants (Carreira et al. 2004; Silva Jr. et al. 2016). The waters found in this area are characterised by salinities ranging from 29.5 to 32.0 and temperatures similar to those in Area 2. The substrate is composed of muddy sediment (Mayr et al. 1989).

2. *Biological sampling*

Specimens were collected every two weeks between July 2005 and June 2007 at ten stations (two per area), for a total of 48 campaigns and 480 hauls. The campaigns were organised in seasons: winter (July, August and September), spring (October, November and December), summer (January, February and March), and autumn (April, May and June). Samplings were conducted with a commercial fishing boat. The wooden trawler was 9.5 m long and had a 36 hp motor. The fishing gear included a 7.0 m-long net with a 14 m ground rope and mesh of 18 mm. Each trawl lasted 30 min at a constant speed of 1.5 knots. The depth, temperature and salinity were measured at the bottom using a YSI multiparameter.

All flatfishes caught were identified, and the total length (TL, cm) and total weight (TW, g) were recorded for each individual. Identification was based on the literature (Gutherz 1967, Figueiredo & Menezes 2000, Carpenter 2002). The original descriptions of *Citharichthys spilopterus* (Günther, 1862) and *Citharichthys arenaceus* (Evermann & Marsh, 1902) and the voucher specimens from the Ichthyological Collection of the Museu Nacional / Universidade Federal do Rio de Janeiro (MNRJ) were consulted in order to check for precise identification of specimens. The following lots were examined: *C. arenaceus* – MNRJ 18234, MNRJ 30616 and MNRJ 30768; *C. spilopterus* – MNRJ 9135, MNRJ 25695, MNRJ 29412, MNRJ 30628 and MNRJ 30672. A voucher specimen of *C. spilopterus* from this study was deposited in the Ichthyological Collection of the MNRJ.

The catch per unit effort (CPUE), based on the number of individuals (ind./h) and weight (g/h), and the seasonal and spatial distribution were calculated. The evaluation of the distribution of species per station was based on absolute frequencies. The effectiveness of the sampling design and the number of campaigns was determined based on the stabilisation of the sample-based rarefaction curve (Gotelli & Colwell 2010). Furthermore, for each station, Margalef's richness and Shannon's diversity and evenness were calculated using the natural logarithm (Magurran 2004).

A principal component analysis (PCA) was performed in XLSTAT statistical software for Excel using Pearson's r as an index of similarity. In addition, a hierarchical cluster analysis (HCA) was conducted to identify the associations among the different areas of the tropical estuary. UPGMA was employed to group species and areas and the Bray-Curtis dissimilarity index was calculated with the program PAST 3.08 (Hammer et al. 2001). We used the Bray-Curtis similarity index expressed as 1-B. A cophenetic correlation coefficient, a measure of the accuracy of the dendrogram using the matrix of distances, was also calculated for each index (Valentin 2012). Multivariate clustering analysis and ordination were applied using the total number of individuals per species as descriptors of the ten sampling points. The normality of the data was verified using Shapiro-Wilk's test. Given the non-normal distribution of the data, the Wilcoxon signed-rank test was chosen and conducted using PAST 3.08 and IBM SPSS Statistics 22. The results were considered significant at the 95% level.

When possible, each species was classified in a functional group and according to its use of the estuary, based on observations of this study and the available literature (Allen & Baltz 1997, Reichert 1998, Chaves & Serenato 1998, Walsh et al. 1999, Reichert 2003, Chaves & Bouchereau 2004, Dias et al. 2005). The functional group classification followed Elliott et al. (2007).

Results

1. *Species composition, abundance and spatial distribution*

The trawl survey resulted in the capture of 1,471 flatfishes belonging to 16 species, nine genera, and four families, with a total weight of 31.1 kg. There was a clear predominance of Paralichthyidae in terms of both number of individuals and richness, followed by Achiridae, Bothidae and Cynoglossidae (Table 1). The most abundant species was *Etropus crossotus*, with 49.7% of all specimens caught and occurring in all samples, followed by *Symphurus tessellatus*, with 23.7% of all specimens caught and occurring in 97.9% of the samples (Table 1). The collector's curve suggests that the number of samples was sufficient to provide a reasonably accurate inventory of the flatfish assemblage in Guanabara Bay, as it was stabilised on the 16th campaign, with just one additional species added in the following campaigns (Figure 2). Only three species were distributed across all five areas of the estuary: *E. crossotus*, *S. tessellatus* and *C. spilopterus*. Other species of Pleuronectiformes were rare (less than 10 specimens) or had restricted distributions (Table 2). Five species showed a frequency of occurrence that was greater than 50% in all studied months: *E. crossotus*, *S. tessellatus*, *Citharichthys macrops*, *Syacium papillosum* and *C. spilopterus*. While *Bothus robinsi* was captured in 42% of the samples, the other species were present in fewer than 20%.

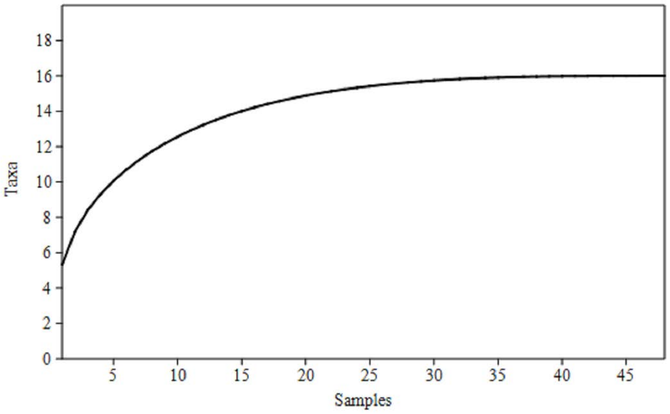


Figure 2. Sample-based rarefaction curve of species of Pleuronectiformes collected during each campaign conducted between July 2005 and June 2007 in Guanabara Bay, southeastern Brazil.

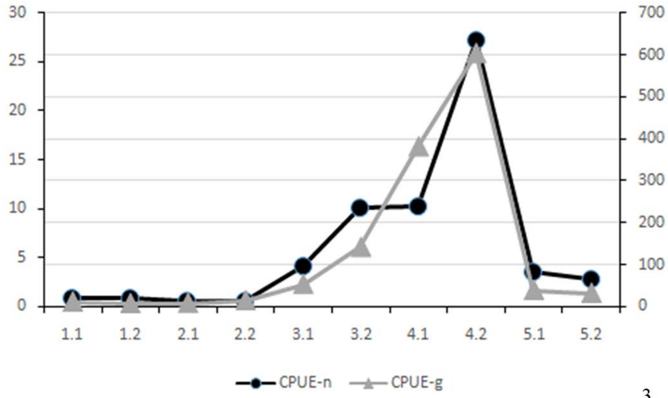
Table 1. Number of individuals (N), frequency of occurrence (%), and amplitude of the length (TL) and weight (TW) of the pleuronectiform species captured between July 2005 and June 2007 in Guanabara Bay, south-eastern Brazil.

Species	**N**	**FO%**	**TL (cm)**	**Mean TL**	**St.d. TL**	**TW (g)**	**Mean TW**	**St.d. TW**
PARALICHTHYIDAE								
Citharichthys macrops Dresel, 1885	112	72.9	11.2-18.5	13.07	3.13	14.3-66.6	27.82	18.96
Citharichthys spilopterus Günther, 1862	59	52.1	9.9-14.7	12.81	3.27	9.3-30.3	24.24	20.55
Cyclopsetta chittendeni Bean, 1895	3	6.3	6.4-14.4	9.70	4.18	2.2-27.4	11.73	13.68
Etropus crossotus Jordan & Gilbert, 1882	731	100.0	5.0-18.8	11.66	2.44	1.2-76.6	19.82	12.18
Etropus longimanus Norman, 1833	4	6.3	8.1-10.7	9.50	1.12	4.6-12.0	7.93	3.19
Paralichthys orbignyanus (Valenciennes, 1837)	3	6.3	5.8-25.3	18.37	10.90	1.8-190.0	119.60	102.66
Paralichthys patagonicus Jordan, 1889	5	6.3	7.9-10.6	9.60	1.42	3.6-9.2	7.80	4.13
Syacium micrurum Ranzani, 1842	19	16.7	5.6-12.4	8.63	2.59	3.1-22.6	8.63	8.45
Syacium papillosum (Linnaeus, 1758)	112	66.7	5.2-20.7	15.65	4.31	1.7-105.6	51.24	31.80
BOTHIDAE								
Bothus ocellatus (Agassiz, 1831)	10	16.7	6.8-14.4	10.56	2.59	7.7-47.9	19.90	13.83
Bothus robinsi Topp & Hoff, 1972	40	41.7	4.9-13.2	9.14	2.22	1.2-34.5	12.25	8.38
ACHIRIDAE								
Achirus declivis Chabanaud, 1940	3	6.3	9.7-12.7	10.80	1.65	5.5-41.2	17.90	20.19
Achirus lineatus (Linnaeus, 1758)	11	16.7	4.6-9.6	7.75	3.30	1.8-19.4	15.08	26.88
Trinectes paulistanus (Miranda-Ribeiro, 1915)	6	12.5	7.7-17.4	10.95	2.32	6.4-50.9	30.83	18.93
CYNOGLOSSIDAE								
Symphurus diomedeanus (Goode & Bean, 1885)	5	8.3	10.9-14.9	12.78	2.11	12.4-32.9	19.20	12.12
Symphurus tessellatus (Quoy & Gaimard, 1824)	348	97.9	7.3-17.4	12.24	1.48	2.0-37.0	12.69	5.34
TOTAL	**1,471**							

Table 2. Abundance per sampling station of all Pleuronectiform species collected between July 2005 and June 2007, in five areas of Guanabara Bay, Brazil (ER – Estuarine Resident, MO – Marine estuarine opportunist, MS – Marine straggler).

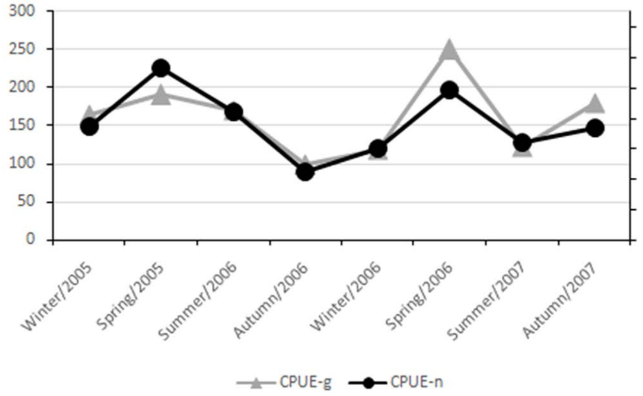
Species	**Guild**	**1.1**	**1.2**	**2.1**	**2.2**	**3.1**	**3.2**	**4.1**	**4.2**	**5.1**	**5.2**
A. declivis	MS	2							1		
A. lineatus	ER	3	4		2		1		1		
B. ocellatus	MS								10		
B. robinsi	MO							12	28		
C. macrops	MO							76	36		
C. spilopterus	ER	4	1	11	7	4	12	1	18		1
E. crossotus	ER	3	2	2	3	61	26	92	451	45	46
E. longimanus	MS							1	3		
P. orbignyanus	MS	1			1		1				
P. patagonicus	MS								5		
C. chittendeni	MS						2		1		
S. micrurum	MS							2	17		
S. papillosum	MO							59	53		
S. diomedeanus	MS						4		1		
S. tessellatus	ER	9	15	3	2	33	197	2	26	39	22
T. paulistanus	MS		1			1	1		2	1	
TOTAL		**22**	**23**	**16**	**15**	**99**	**244**	**245**	**653**	**85**	**69**

The spatial analysis of the CPUE indicated a clear distinction between the lowest values found in the upper estuary (1.1, 1.2, 2.1 and 2.2) and the highest values in the lower estuary (3.2, 4.1 and 4.2). The middle estuary (3.1, 5.1 and 5.2) indicated CPUE values slightly above those found in the upper estuary (Figure 3). The seasonal variation in the CPUE revealed that the highest values in terms of number of individuals and TW were found during spring. While the difference in CPUE-n values between the first and second years showed a slightly larger number of individuals captured in the first year, there was a clear difference in CPUE-g, with larger values found in the second year. This indicates that the specimens caught during the first year, especially during spring, were smaller than those captured in the second year (Figure 4).



3

Figure 3. Spatial variation in CPUE in terms of the number and total weight of specimens of Pleuronectiformes captured in Guanabara Bay, an estuary in the southeastern Brazil, between July 2005 and June 2007.



4

Figure 4. Seasonal variation in CPUE in terms of number and total weight of specimens of Pleuronectiformes captured in Guanabara Bay, an estuary in the southeastern Brazil, between July 2005 and June 2007.

The analysis of the CPUE values and percentage of individuals per sampling station showed that station 4.2, located on the western side of the lower estuary, was the locality where most of the pleuronectiforms (44.4%) were captured, with the abundance and biomass being far greater than those recorded in other stations (Figures 3 and 4). While a significant difference was found in terms of the total length for the total catch of Pleuronectiformes between the two years of the study (Wilcoxon, p<0.001), the data reflect a significant change in size (Wilcoxon, p<0.001) and mass (Wilcoxon, p=0.006) for *Syacium papillosum* in the second year, with means of 11.7 cm TL and 28.0 g TW for the first year, increasing to 17.2 cm TL and 60.6 g TW in the second year.

2. Richness, evenness and diversity

A total of 15 species was represented among specimens collected in the first year, while 16 were recorded in the second year (Table 3). Margalef's species richness was 2.06 when considering both sampled years; specifically, 2.09 in the first year and 2.31 in the second year. The highest richness was obtained during the summer of 2006, with a value of 2.26 (13 species), and the lowest was recorded in the winter of 2006, with a value of 1.21 (7 species). The sampling station with the highest richness was 4.2, with a value of 2.16 (15 species), and the lowest richness values were found at stations 5.2 and 5.1, with values of 0.47 and 0.45, respectively (3 species each).

The diversity value obtained for the two years was 1.55; 1.44 in the first year and 1.60 in the second. The period with the highest diversity value (1.83) was autumn of 2006, and the lowest value (1.14) occurred in the winter of the same year. The station with the highest diversity was 1.1 (1.58), and the lowest value was found at 5.2 (0.70) (Table 3). The evenness obtained for the first and second years was 0.53 and 0.58, respectively, whereas the value for both years was 0.56. The period with the highest value of evenness was autumn of 2006, with a value of 0.76, and the lowest occurred during the spring of 2005, with a value of 0.52. The stations with the highest values of evenness were 1.1 and 2.2, with values of 0.88 and 0.87, respectively. The lowest values were recorded at stations 3.2 (0.35) and 4.2 (0.47) (Table 3).

3. Principal component analysis (PCA) and hierarchical clustering (HCA)

The PCA (Figure 5) helped to characterise the sampled area using the correlations between different values of abundance among species. The factorial plan 1-2 explains 72% of the variability (50% and 22% for axes 1 and 2, respectively). Stations 3.2 and 4.2 have a characteristic distribution and are isolated in the graph. To a lesser extent, station 4.1 was also isolated from the other stations. Three sampling stations could be defined based on their distinct groups of species: 4.2 was distinguished because of the dominance of *E. crossotus* and the presence of *Paralichthys patagonicus*, *Bothus ocellatus*, *B. robinsi*, *Etropus longimanus* and *Syacium micrurum*, species that occurred only or primarily at this site. Station 3.2 was segregated by the dominance of *S. tessellatus* and the exclusive presence of *Symphurus diomedeanus* and *Cyclopsetta chittendeni*. Lastly, station 4.1 showed a dominance of *C. macrops* and *S. papillosum* and a low frequency of *S. tessellatus*.

The HCA (Figure 6) identified three large groups. The first was composed of areas 3 and 5, representing the middle estuary; the second was composed of area 4 in the lower estuary; and areas 1 and 2 were associated with the third group, representing the upper estuary. When considering the more closely related stations, six other associations were identified. Station 3.1 and area 5 (5.2 and 5.1) shared a similarity greater than 0.80. Area 1 (1.1 and 1.2) had a similarity of 0.65, and area 2 (2.1 and 2.2) scored 0.70. The remaining stations, 3.2, 4.1 and 4.2, were isolated from the others. The cophenetic correlation coefficient for this analysis was 0.91.

Table 3. Seasonal and spatial variation in the values of richness, diversity and evenness of Pleuronectiform species collected between July 2005 and June 2007, in five areas of Guanabara Bay, Brazil (standard deviation in parenthesis).

	Species	Richness	Diversity	Evenness
winter/05	8	1.35 (±0.25)	1.24 (±0.16)	0.60 (±0.04)
spring/05	10	1.61 (±0.20)	1.19 (±0.12)	0.52 (±0.02)
summer/06	13	2.26 (±0.27)	1.43 (±0.33)	0.56 (±0.14)
autumn/06	11	2.14 (±0.20)	1.83 (±0.13)	0.76 (±0.06)
winter/06	7	1.21 (±0.30)	1.14 (±0.26)	0.59 (±0.11)
spring /06	10	1.65 (±0.43)	1.55 (±0.04)	0.67 (±0.19)
summer /07	11	1.99 (±0.37)	1.47 (±0.22)	0.61 (±0.09)
autumn /07	12	2.13 (±0.14)	1.50 (±0.17)	0.60 (±0.08)
1.1	6	1.62 (±0.75)	1.58 (±0.50)	0.88 (±0.03)
1.2	5	1.28 (±0.60)	1.07 (±0.33)	0.66 (±0.10)
2.1	3	0.72 (±0.51)	0.83 (±0.25)	0.76 (<0.01)
2.2	5	1.48 (±0.63)	1.40 (±0.32)	0.87 (±0.06)
3.1	4	0.65 (±0.33)	0.84 (±0.30)	0.61 (±0.13)
3.2	8	1.27 (±0.55)	0.73 (±0.41)	0.35 (±0.14)
4.1	8	1.27 (±0.57)	1.33 (±0.40)	0.64 (±0.12)
4.2	15	2.16 (±0.48)	1.26 (±0.28)	0.47 (±0.19)
5.1	3	0.45 (±0.54)	0.75 (±0.31)	0.68 (±0.09)
5.2	3	0.47 (±0.40)	0.70 (±0.30)	0.63 (±0.08)

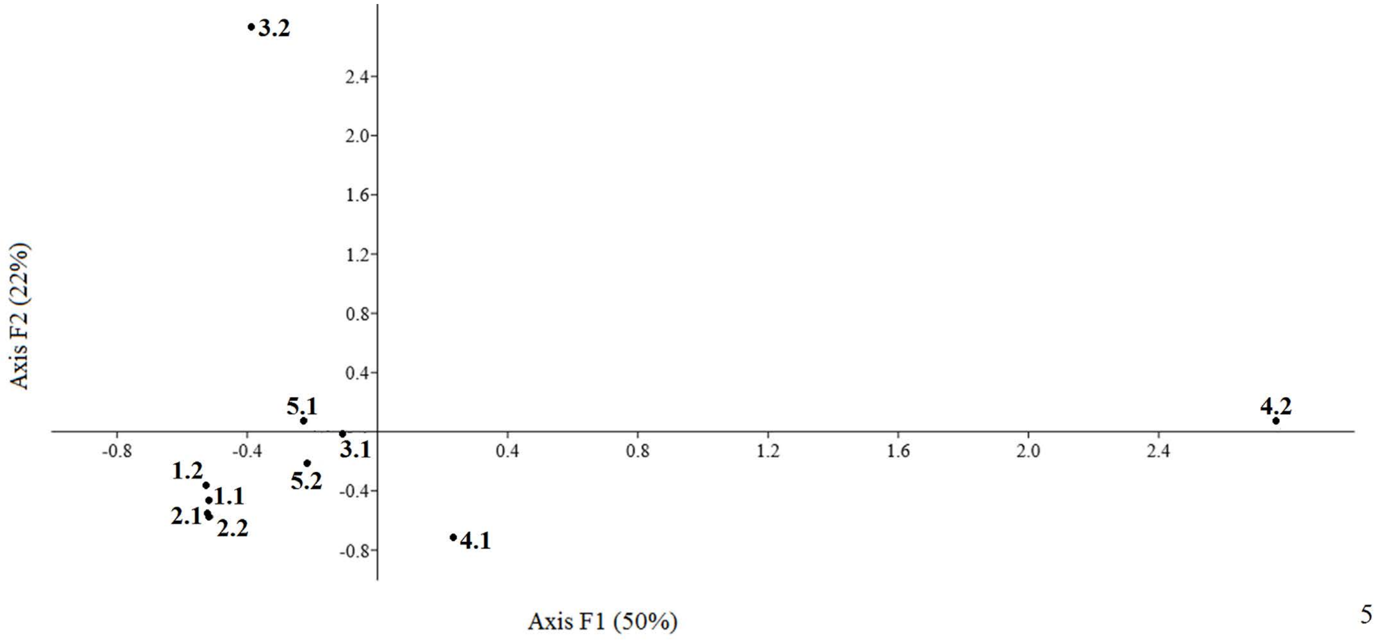


Figure 5. Principal component analysis (PCA) of the Pleuronectiformes species captured in Guanabara Bay, an estuary in the southeastern Brazil, between July 2005 and June 2007.

Discussion

1. Composition

The sampling strategy was based on a trawl net due to the low selectivity of this fishing gear, thus enabling a fairly accurate characterisation of the diversity of demersal fish associated with unconsolidated substrates (Vianna & Almeida 2005). The same sampling design was used in a previous study by Rodrigues et al. (2007), who identified only six species (all at low abundance) of pleuronectiforms in Guanabara Bay. The results presented here, a total of 16 flatfish species, indicate that the Richness of Pleuronectiformes in the Guanabara Bay was not properly assessed by earlier studies. The specific composition and predominance of Paralichthyidae in Guanabara Bay was similar to the diversity of flatfishes found in other tropical estuaries from South-eastern Brazil.

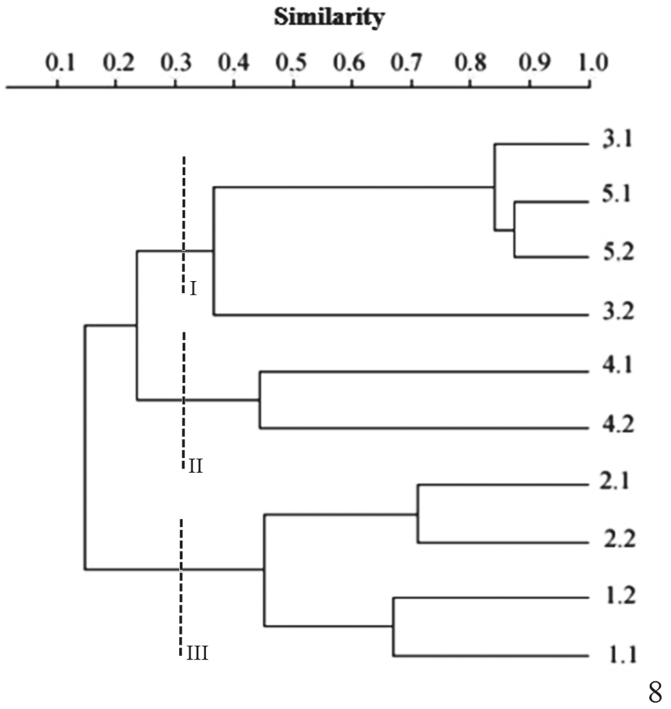


Figure 6. Hierarchical cluster analysis (HCA) of species of Pleuronectiformes recorded in Guanabara Bay, an estuary in the southeastern Brazil, between July 2005 and June 2007, showing the three main identified groups.

Andreatta et al. (2002) identified 11 pleuronectiform species in Ribeira Bay (22º59'S / 44º22'W), while Mendonça & Araújo (2002) found 14 species in Sepetiba Bay (22º59'S / 44º22'W); in both studies, Paralichthyidae was the most speciose family, with eight and nine species, respectively. Further north, in the bay of Vitória (20º17'S / 40º16'W), seven Paralichthyidae species were recorded among a total of 14 flatfishes (Chagas et al. 2006; Catelani et al. 2014).

Guanabara Bay is an open ecosystem that experiences the periodic influence of different water masses. Nevertheless, even with the stabilisation of the species rarefaction curve, the addition of another species, *Etropus longimanus*, in the 37th sampling campaign was not unexpected. Different water masses allows the presence of species associated with coastal waters and colder and deeper water masses. Consequently, it is likely that other species may be recorded in the future. A thorough analysis of the abiotic data for the same samples but extended to all fish community can be seen in Silva Jr. et al. (2016). The comparison of the species composition among estuaries showed an interesting difference. The flatfish *Citharichthys arenaceus* was the only species absent in this study but present in other tropical estuaries along the Rio de Janeiro coast. This inconsistency may be due to difficulties associated with the taxonomy of the species. There are problems in distinguishing *C. arenaceus* and *Citharichthys spilopterus*, which have overlapping morphometric and meristic characters (Carpenter 2002). There is a lack of comparative studies between both species (Figueiredo & Menezes 2000). The comparison of the specimens captured in Guanabara Bay in this study with many specimens of both species from the Ichthyological Collection of the MNRJ allowed us to conclude that the specimens previously identified as *C. arenaceus* in Guanabara Bay corresponded to *C. spilopterus*. No significant change in total length or total weight was identified between the first and second years of the study, except in *Syacium papillosum*.

2. Abundance

The decline in abundance of species of Pleuronectiformes collected between the two years was a direct reflection of the strong decrease in the capture of *E. crossotus*. The highest captures occurred during springtime due to various factors. The peak in the spring of 2005 was a consequence of the increased abundance of *E. crossotus*, which is in contrast to the peak during the spring of 2006, characterised by increased captures of other species of flatfishes despite the low abundance of *E. crossotus*. The lowest capture rate for all flatfishes occurred on the autumn of 2006. There was no marked seasonality among the pleuronectiform species in Guanabara Bay. However, Barletta et al. (2003) reported an increase in the abundance of species in the rainy season and identified it as a pattern that is common in tropical estuaries.

The high abundance of flatfishes in the lower estuary (4.2) was due to the elevated capture of specimens of *E. crossotus* along with the presence of species associated with coastal waters, such as *Bothus ocellatus*, *B. robinsi*, *Citharichthys macrops*, *Syacium micrurum* and *S. papillosum* (Figueiredo & Menezes 2000). This behaviour was not recorded on the other side of the bay mouth (4.1), presumably due to the different environmental conditions. Originally, it was expected that the two sampling stations would be similar in terms of their fish assemblages due to their location at the entrance of the bay and the predominant marine environmental conditions (high salinity and sandy bottom). The source for this unexpected difference may be the disposal of organic matter through an outfall located close to the sampling station. Banks covered in algae and bryozoans are indicative of the disposal of organic matter in wastewater and were recorded during sampling at station 4.1 but not at 4.2.

Even though the central channel (3.2) had the same abundance in terms of the number of specimens as the lower estuary station (4.1), the recorded biomass was lower. This result reflects the dominance of *Symphurus tessellatus*, a species that is smaller than the more abundant species at station 4.1 (*E. crossotus*, *S. papillosum* and *C. macrops*). The highest abundance of *S. tessellatus* found in the central channel may be a consequence of the characteristics of the area, especially the muddy bottom. This species naturally inhabits areas with such characteristics (Figueiredo & Menezes 2000), which contrasts with the sandy bottom found at the entrance of the bay.

3. Richness

Seasonally, lower values of richness were recorded in the winter and spring of both sampling years, with the lowest values found during the winter. Conversely, the highest values of richness occurred in the rainy season, during the summer and autumn. This seasonal variation in richness was consistent with the pattern observed by Barletta et al. (2003), with more species found in the rainy season. Results, however, differ from those presented by Vendel et al. (2002), which reported larger values of fish richness in the autumn and winter in a Brazilian subtropical estuary. In another subtropical estuary from South-eastern Brazil, Pinheiros Bay (Paraná), Schwarz-Junior et al. (2006) found no seasonal pattern in the richness of the fish fauna.

Spatially, the number of species followed the same pattern observed for the abundance, with considerably higher values detected in the western part of the estuary mouth (4.2) than in any of the other sampling stations, including the central channel (3.2) and the eastern part of the lower estuary (4.1). However, Margalef's richness, which considers the number of individuals in the sample, showed a different picture;

the western lower estuary was the richest, followed by stations 1.1 and 2.2. This result is a consequence of the low abundance and relatively high number of species in the upper estuary. An increase in the number of species in the outermost regions of the estuary, in addition to an increased presence of marine species, has been observed in Guanabara Bay (Rodrigues et al. 2007), Sepetiba Bay (Araújo et al. 1998), both in the State of Rio de Janeiro, and Guaratuba Bay, in the State of Paraná (Bouchereau & Chaves 2003) and appears to be a trend in tropical and subtropical estuaries (Blaber 2000; Blaber 2013; Catelani et al. 2014). A two-way ANOVA based on fish metrics (CPUE, richness, diversity and equitability) was conducted by Silva Jr. et al. (2016) based on the same two years of sampling but including all fishes caught. While changes in abundance, diversity and equitability were independently associated with differences in area and season, shifts in richness were exclusively related to spatial variability. The greatest diversity and abundance values for Pleuronectiformes were found in the lower estuary and the central channel, which is in accordance with what was reported for other trophic fish groups (Silva Jr. et al. 2016). No seasonal pattern was observed in the variation of the diversity and evenness indices. The lack of patterns in these indices was also recorded by Vendel et al. (2002) and Spach et al. (2003) in two estuaries in Paranaguá Bay, by Schwarz-Junior et al. (2006) in Pinheiros Bay and by Chagas et al. (2006) in Vitória Bay.

4. Principal component analysis and hierarchical cluster analysis

Eutrophic environments commonly present conditions of hypoxia or anoxia in their deeper layers, which do not mix with the upper layers due to the formation of a cline (Breitburg 2002). Intense eutrophication and consequent hypoxia are reported to occur in large portions of Guanabara Bay (Ribeiro & Kjerfve 2002; Aguiar et al. 2011). While the lower estuary is characterized by greater exposure to less polluted coastal waters, the upper portions of the bay, especially the western and northwestern sectors, receive the majority of the drainage from metropolitan Rio de Janeiro (Ribeiro & Kjerfve 2002). The existing facilities for sewage treatment and waste disposal are insufficient to halt the environmental degradation. Ribeiro & Kjerfve (2002) estimated that in order to achieve pre-1950 conditions, it would be necessary to properly treat 80-90% of all domestic and industrial sewage, far from the 15% that is presently treated. The data presented in this study therefore address flatfish populations that have been exposed to a long history of human impacts in an already naturally stressed environment.

The PCA analysis separated the three stations (3.2, 4.1 and 4.2) that had the most diverse environmental features, while the remaining seven stations were grouped in a single cluster. At these three stations, the number of species was higher than at the others. Coincidentally, these are the stations with the best estuarine environmental conditions, where the dissolution of the organic load is higher due to the action of the tidal regime. The isolation of the western (4.2) and eastern (4.1) sides of the lower estuary and central channel (3.2) was expected based on the field observations. The hierarchical cluster analysis showed some similarity, albeit small, between 4.1 and 4.2 and grouped areas 1 and 2 and 3.1 and 5. This result indicated that the composition of Pleuronectiformes varied between four zones: the upper estuary, above Governador and Paquetá islands, represented by areas 1 and 2; the middle of the estuary, formed by stations 5 and 3.1; the lower estuary, indicating the difference between the eastern (4.1) and western (4.2) sides of the estuary mouth; and the central channel (3.2), which is variable in size according to rainfall. The spatial distribution observed for the pleuronectiform species follows the pattern recorded by Rodrigues et al. (2007) for the entire demersal fish assemblage of Guanabara Bay. Patterns of distribution influenced by local rainfall are supported by data from other species in the same estuary. This is the case with the abundance and distribution of the pink shrimp *Farfantepenaeus brasiliensis* (Gomes et al. 2013) and the blue crab *Callinectes ornatus* (Keunecke et al. 2012) with differences according to environmental conditions, among them the seasonal spawning peaks in the first and distinctive patterns of sexual distribution in the latter.

5. Use of the estuary

Despite the recent review of the classification of estuarine fish guilds (Potter et al. 2013), due to the scarcity of data on the fish community of the Guanabara Bay, this study follows Elliot et al. (2007). The most abundant species, *Etropus crossotus*, was classified as an estuarine resident in this study and in Guaratuba Bay, which is a subtropical estuary in southern Brazil (Chaves & Bouchereau 2004). This close relationship between *E. crossotus* and the estuarine environment is supported by the present study due to its regular presence throughout the sampling campaigns, high relative abundance, various size classes and wide distribution in the estuary. Sánchez-Gil et al. (2008) considered *E. crossotus* to be an estuarine species but found recruitment in the estuarine plume on the inner continental shelf off an estuary of the southern Gulf of Mexico. The reduction in the abundance of *E. crossotus* between the first and second years was responsible for the observed decrease in the whole flatfish assemblage. This decrease in catch should be investigated through continuous monitoring of the area; it may indicate an early depletion of the population or only a natural fluctuation.

The reduction in the abundance of *E. crossotus*, despite the lack of a seasonal pattern, may be associated with its life cycle. This reduction coincides with the presence of larger individuals prior to the recruitment observed in the second year. It should be noted, however, that no population data are available for the species in coastal areas besides studies on bycatch of the shrimp fishery, where *E. crossotus* was the most abundant species among the pleuronectiforms (Vianna & Almeida 2005), indicating that the estuarine resident *Etropus crossotus* may also explore areas outside of the estuary. Differences in rainfall patterns may affect the living conditions and the annual cycle of flatfish populations. Increased freshwater inflow in the rainy season, usually with a high content of organic matter, deteriorates the environmental conditions in the bay (Valentin et al. 1999) and increases the size of the area under hypoxia, forcing individuals of *E. crossotus* to move to areas with better conditions in the estuary and adjacent coastal areas (the species was found up to 40 m deep) (Vianna & Almeida 2005).

The wide size range and presence of larvae (Castro et al. 2005) suggest that *E. crossotus* completes its life cycle in the estuary. A similar pattern was described for the south-eastern United States, where it was observed that the species development had a strong dependency on a nursery area with plenty of resources (Reichert 1998). Other groups of species were also observed: Marine stragglers, characterised by rare species, were represented only in low abundance and did not show dependence on estuaries in the literature. This group includes the majority of the collected species: *A. declivis*, *B. ocellatus*, *C. chittendeni*, *E. longimanus*, *P. orbignyanus*, *P. patagonicus*, *S. micrurum*, *S. diomedeanus* and *T. paulistanus*.

Marine estuarine opportunist species are present in varying abundance, are restricted to the lower estuary and include smaller, most likely young, individuals. This group is composed of *B. robinsi*, *C. macrops* and *S. papillosum*. Three other species, two rare and one abundant (*S. tessellatus*, *A. lineatus* and *C. spilopterus*), are indicated as estuarine residents by Chaves & Bouchereau (2004); however, in Guanabara Bay, the data led to a different classification or precluded any determination.

The tonguefish *S. tessellatus* was represented mostly by adults and a few small, most likely young, specimens. However, the population of *S. tessellatus* may have been subsampled since young individuals of this species can escape through the mesh of the net. *Achirus lineatus* and *C. spilopterus* had low capture rates in Guanabara Bay; hence, the ability to classify these two species was hindered. However, both are commonly found in Brazilian estuaries and were considered as estuarine residents in previous studies (Allen & Baltz 1997, Chaves & Serenato 1998, Mendonça & Araújo 2002, Chaves & Bouchereau 2004, Chaves & Bouchereau 2004, Chagas et al. 2006, Schwarz-Junior et al. 2006). The conclusion that *A. lineatus* as a resident estuarine species is also supported by the presence of larvae in the Guanabara Bay (Castro 1998). The results presented here revealed a relatively high diversity of Pleuronectiformes in the Guanabara Bay estuary. This diversity included species of flatfishes at different niches as indicated by different trophic levels and their distinct morphologies, which has already been indicated in other estuaries, including in Brazil (Guedes & Araújo 2008; Russo et al. 2008; Lima et al. 2018). The pattern observed in this study is similar to the reports for other fish groups, in which a high diversity of species was accompanied by the biomass being predominantly represented by a single species: *Etropus crossotus* for Pleuronectiformes, as revealed by this study, *Chilomycterus spinosus* for Tetraodontiformes (Andrade et al. 2016), *Genidens genidens* for Siluriformes (Silva Jr. et al. 2013), *Eucinostomus argenteus* for Gerreidae (Corrêa & Vianna 2016) and *Micropogonias furnieri* for Sciaenidae (Silva Jr. et al. 2016).

In conclusion, pleuronectiform species have a number of unique characteristics, such as limited swimming ability due to the absence of a swim bladder and demersal habits, which make them especially vulnerable to low-oxygen environments (Tallqvist et al. 1999, Breitburg 2002, Bell & Eggleston 2005). In the present study, the sensitivity to these adverse conditions appears to affect the distribution of flatfishes. The specific composition shows that, in Guanabara Bay, the main species caught are small in size and that while the species with the highest market value are present, *Paralichthys* spp. and *Syacium* spp., they are not commercially attractive because of their small size and low abundance. The greatest diversity was found in areas of the lower estuary, while both the middle and upper estuary showed low richness and abundance. New studies are necessary to establish the extent to which flatfish populations are affected by the ongoing environmental degradation, such as urban pollution and dredging, and the effects on the observed distribution patterns.

Acknowledgements

The authors would like to thank all who were involved in this study, especially the BioTecPesca colleagues for their assistance in collecting samples and in the biometric analyses. We thank Dr. Fabio Di Dario and one anonymous reviewer whose comments helped improve and clarify this manuscript. This study was part of the "Environmental Assessment of Guanabara Bay" program, coordinated and funded by CENPES-PETROBRAS, supported by the Long Term Ecological Program – CNPq (403809/2012-6) and FAPERJ (E-26/110.114/2013).

Author Contributions

Luiz Constantino Silva-Júnior: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Sérgio Ricardo Santos: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Márcio Chagas Macedo: Contribution to data collection.

Gustavo Wilson Nunan: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Marcelo Vianna: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AGUIAR, V.M.C.; NETO, JAB & RANGEL, C.M. 2011. Eutrophication and hypoxia in four streams discharging in Guanabara Bay, RJ, Brazil, a case study. Mar Poll Bull 62 (8): 1915-1919.

ALLEN, R.L. & BALTZ, D.M. 1997. Distribution and microhabitat use by flatfishes in a Louisiana estuary. Env Biol Fish 50: 85-103.

ANDRADE, A.C.; SANTOS, S.R.; VERANI, J.R. & VIANNA, M. 2016. Guild composition and habitat use by Tetraodontiformes (Teleostei, Acanthopterygii) in a south-western Atlantic tropical estuary. J Mar Biol Assoc UK 96(6): 1251-1264.

ANDRADE-TUBINO, M.F.; FIORE-CORREIA, L.B. & VIANNA, M. 2009. Morphometrics and length structure of Micropogonias furnieri (Desmarest, 1823) (Perciformes, Sciaenidae) in Guanabara bay, State of Rio de Janeiro, Brazil. Bol Inst Pesca 35: 239 - 246.

ANDREATA, J.V.; MEURER, B.C.; BAPTISTA, M.G.S.; MANZANO, F.V.; TEIXEIRA, D.E.; LONGO, M.M. & FRERET, N.V. 2002. Composição da assembléia de peixes da Baía da Ribeira, Angra dos Reis, Rio de Janeiro, Brasil. Rev Bras Zool 19 (4): 1139-1146.

ARAÚJO, F.G.; CRUZ-FILHO, A.G.; AZEVEDO, M.C. & SANTOS, A.C.A. 1998. Estrutura da comunidade de peixes demersais da Baía de Sepetiba, RJ. Rev Bras Biol 58 (3): 417-430.

BARLETTA, M.; BARLETTA-BERGAN, A.; SAINT-PAUL, U. & HUBOLD, G. 2003. Seasonal changes in density, biomass and diversity of estuarine fishes in tidal mangrove creeks of the lower Caeté Estuary (northern Brazilian coast, east Amazon). Mar Ecol Prog Ser 256: 217-228.

BELL, G.W. & EGGLESTON, D.B. 2005. Species-specific responses by blue crabs and fish to chronic and episodic hypoxia. Mar Biol 146: 761-770.

BLABER, S.J.M. 2000. Fish and Aquatic Resources Series, Vol 7: Tropical Estuarine Fishes: Ecology, Exploitation and Conservation. Oxford, UK. Blackwell Science Ltd.

BLABER, S.J.M. 2013. Fish and fisheries in tropical estuaries: The last 10 years. Estuar Coast Shelf Sci 135: 57–65.

BODIN, N.; TAPIE, N.; LE MÉNACH, K.; CHASSOT, E.; ELIE, P.; ROCHARD, E. & BUDZINSKI, H. 2014. PCB contamination in fish community from the Gironde Estuary (France): Blast from the past. Chemosphere 98: 66-72.

BOUCHEREAU, J.L. & CHAVES, P.T.C. 2003. Ichthyofauna in the ecological organisation of a South-West Atlantic mangrove ecosystem: The bay of Guaratuba, South East Brazil. Vie et Millieu 53 (2-3): 103-110.

BREITBURG, D. 2002. Effects of Hypoxia, and the Balance between Hypoxia and Enrichment, on Coastal Fishes and Fisheries. Estuaries 25 (4b): 767-781.

CARPENTER, K.E. 2002. The living marine resources of the Western Central Atlantic. Volume 3: Bony fishes part 2 (Opistognathidae to Molidae), sea turtles and marine mammals. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists Special Publication No. 5. 1375-2127. Roma.

CARREIRA R.S.; WAGENER A.L.R. & READMAN, J.W. 2004. Sterols as markers of sewage contamination in a tropical urban estuary (Guanabara Bay, Brazil): space-time variations. Estuar Coast Shelf Sci 60: 587-598.

CASTRO, M.S. 1998. Variação temporal das larvas de peixes em um ponto fixo da Entrada da Baía de Guanabara, RJ (Brasil). Dissertação apresentada na Universidade Federal Fluminense, Niterói.

CASTRO, M.S.; BONECKER, A.C.T. & VALENTIN, J.L. 2005. Seasonal variation fish larvae at the entrance of Guanabara bay, Brazil. Braz Arch Biol Technol 48 (1): 121–128.

CATELANI, PA; PETRY, A.C.; DARIO, F.D.; SANTOS, V.L.M. & MINCARONE, M.M. 2014. Fish composition (Teleostei) of the estuarine region of the Macaé River, southeastern Brazil. Check List 10(4): 927-935.

CHAGAS, L.P.; JOYEUX, J.-C. & FONSECA, F.R. 2006. Small-scale spatial changes in the estuarine fish: subtidal assemblages in tropical Brazil. J Mar Biol Assoc UK 86(4): 861-875.

CHAVES, P.T.C. & BOUCHEREAU, J.-L. 2004. Trophic organization and functioning of fish populations in the bay of Guaratuba, Brazil, on the basis of a trophic contribution factor. Acta Adriat 45 (1): 83-94.

CHAVES, P.T.C. & SERENATO, A. 1998. Diversidade de dietas na assembléia de linguados (Teleostei, Pleuronectiformes) do manguezal da Baía de Guaratuba, Paraná, Brasil. Rev Bras Oceanogr 46 (1): 61-68.

CHAVES, P.T.C.; COVA-GRANDO, G. & CALLUF, C. 2003. Ictiofauna demersal numa região de plataforma continental do sul do Brasil submetida à pesca camaroneira. Acta Biol Parana 32 (1,2,3,4): 69-82.

CORRÊA, B. & VIANNA, M. 2016. Spatial and temporal distribution patterns of the silver mojarra Eucinostomus argenteus (Perciformes: Gerreidae) in a tropical semi-enclosed bay. J Fish Biol 89: 641-660.

COSTA, L.A.A.; PESSOA, D.M.M. & CARREIRA, R.S. 2018. Chemical and biological indicators of sewage river input to an urban tropical estuary (Guanabara Bay, Brazil). Ecol Indic 90: 513-518.

DIAS, J.F.; FIADI, C.B.; SILBIGER, H.L.N. & SOARES, L.S.H. 2005. Reproductive and population dynamics of the bay whiff Citharichthys spilopterus Günther, 1862 (Pleuronectiformes: Paralichthyidae) in the Mamanguá inlet, Rio de Janeiro, Brazil. Neotrop Ichthyol 3 (3): 411-419.

ELLIOTT, M.; WHITFIELD, A.K.; POTTER, I.C.; BLABER, S.J.M.; CYRUS, D.P.; NORDLIE, F.G. & HARRISON, T.D. 2007. The guild approach to categorizing estuarine fish assemblages: a global review. Fish Fish 8: 241-268.

EVERMANN, B.W. & MARSH, M.C. 1902. The Fishes of Porto Rico. In: Investigations of the Aquatic Resources and Fisheries of Porto Rico by the United States Fish Comission Steamer Fish Hawk: 49-350.

FIGUEIREDO, J.L. & MENEZES, N.A. 2000. Manual de peixes marinhos do sudeste do Brasil: VI. Teleostei (5). Museu de Zoologia USP. São Paulo.

FORSTALL, R.L.; GREENE, R.P. & PICK, J.B. 2009. Which are the largest? Why lists of major urban areas vary so greatly. Tijdschr Econ Soc Geogr 100 (3): 277-297.

FRANCO, A.C.S.; BROTTO, D.S.; ZEE, D.M.W. & SANTOS, L.N. 2014. Reproductive biology of Cetengraulis edentulus (Cuvier, 1829), the major fishery resource in Guanabara Bay, Brazil. Neotrop Ichthyol 12 (4): 819-826.

GOMES, A.P.P.; KEUNECKE, K.A.; SILVA-JÚNIOR, D.M. & VIANNA, M. 2013. Modulating reproduction of Penaeidae shrimps: ecological responses of two sympatric species (Decapoda: Dendrobranchiata) on south-eastern Brazilian coast. J Mar Biol Assoc UK 93 (3): 733–740.

GOTELLI, N.J. & COLWELL, R.K. 2010. Estimating species richness. In Biological diversity: frontiers in measurement and assessment (Magurran, A.E., McGill, B.J., ed), pp. 39–54 Oxford, UK: Oxford University Press

GUEDES, A.P.P.; ARAÚJO, F.G. 2018. Trophic resource partitioning among five flatfish species (Actinopterygii: Pleuronectiformes) in a tropical bay in south-eastern Brail. J Fish Biol 72(4): 1035-1054.

GUTHERZ, E.J. 1967. Field Guide to the flatfishes of the family Bothidae in the western North Atlantic. United States Fish and Wildlife Service Circular 263: 1-47.

HAMMER, Ø.; HARPER, D.A.T. & RYAN, P.D. 2001. PAST: Paleontological statistics software package for education and data analysis. Paleontol Electronica 4 (1): 9pp.

JABLONSKI, S.; AZEVEDO, A.F. & MOREIRA, L.H.A. 2006. Fisheries and Conflicts in Guanabara bay, Rio de Janeiro, Brazil. Braz Arch Biol Technol 49 (1): 79-91.

LIMA, C.S.S.; CLARK, F.J.K.; SALES, N.S. & PESSANHA, A. 2018. Strategies of resource partitioning between two sympatric puffer fishes in a tropical hypersaline estuary, Brazil. Environ Biol Fish: 1-15. https://doi.org/10.1007/s10641-018-0729-z.

KEUNECKE, K.A.; D'INCAO, F.; VERANI, J.R. & VIANNA, M. 2012. Reproductive strategies of two sympatric swimming crabs Callinectes danae and Callinectes ornatus (Crustacea: Portunidae) in a estuarine system, south-eastern Brazil. J Mar Biol Assoc UK 92 (2): 343–347.

MAGURRAN, A.E. 2004. Measuring biological diversity. Oxford, UK. Blackwell Science Ltda.

MARTINHO, F.; DOLBETH, M.; VEGAS, I.; BAPTISTA, J.; CABRAL, H.N. & PARDAL, M.A. 2010. Does the flatfish community of the Mondego estuary (Portugal) reflect environmental changes? J Appl Ichthyol 26: 843-852.

MAYR, L.M.; PARANHOS, R.; TENENBAUM, D.R.; NOGUEIRA, C.R.; BONECKER, A.C.T.; VILLAC, M.C. & BONECKER, S.L.C. 1989. Hydrobiological Characterization of Guanabara bay. In: Coastlines of Brazil (Ed. NEVES, C.). American Society of Civil Engineers, NY.

MENDONÇA, P. & ARAÚJO, F.G. 2002. Composição das populações de linguados (Osteichthyes, Pleuronectiformes) da Baía de Sepetiba, Rio de Janeiro, Brasil. Rev Bras Zool 19 (2): 339-347.

MENEZES, N.A.; BUCKUP, P.A.; FIGUEIREDO, J.L. & MOURA, R.L. 2003. Catálogo das espécies de peixes marinhos do Brasil. MZUSP, São Paulo.

MULATO, I.P.; CORRÊA, B. & VIANNA, M. 2015. Distribuição espaço-temporal de Micropogonias furnieri (Perciformes, Sciaenidae) em um estuário tropical no sudeste do Brasil. Bol Inst Pesca 41(1): 1-18.

NELSON, J.S. 2006. Fishes of the World. 4a. Ed. 624 pp. John Wiley & Sons, Inc., NY.

NETO, J.A.B.; GINGELE, F.X.; LEIPE, T. & BREHME, I. 2006. Spatial distribution of heavy metals in surficial sediments from Guanabara Bay: Rio de Janeiro, Brazil. Environ Geol 49: 1051-1063.

OLIVEIRA, E.C. & FAVARO, L.F. 2011. Reproductive biology of the flatfish Etropus crossotus (Pleuronectiformes: Paralichthyidae) in the Paranaguá Estuarine Complex, Paraná State, subtropical region of Brazil. Neotrop Ichthyol 9 (4): 795-805.

POTTER, I.C.; TWEEDLEY, J.R.; ELLIOT, M. & WHITFIELD, A.K. 2013. The ways in which fish use estuaries: a refinement and expansion of the guild approach. Fish Fish 16 (2): 230-239.

REICHERT, M.J.M. 1998. Etropus crossotus, an annual flatfish species; age and growth of the fringed flounder in South Carolina. J Sea Res 40: 323-332.

REICHERT, M.J.M. 2003. Diet, consumption, and growth of juvenile fringed flounder (Etropus crossotus); a test of the 'maximum growth/optimum food hypothesis' in a subtropical nursery area. J Sea Res 50: 97-116.

RIBEIRO, C.H.A. & KJERFVE, B. 2002. Anthropogenic influence on the water quality in Guanabara Bay, Rio de Janeiro, Brazil. Reg Environ Change 3:13–19.

RODRIGUES, C.; LAVRADO, H.P.; FALCÃO, A.P.C. & SILVA, S.H.G. 2007. Distribuição da ictiofauna capturada em arrastos de fundo na Baía de Guanabara – Rio de Janeiro, Brasil. Arq Mus Nac 65 (2): 199-210.

ROSENFELDER, N.; LEHNERT, K.; KAFFARNIK, S.; TORRES, J.P.M.; VIANNA, M.; VETTER, W. 2012. Thorough analysis of polyhalogenated compounds in ray liver samples off the coast of Rio de Janeiro, Brazil. Environ Sci Pollut R 19: 379-389.

RUSSO, T.; PULCINI, D.; O'LEARY, Á; CATAUDELLA, S. & MARIANI, S. 2008. Relationship between body shape and trophic niche segregation in two closely related sympatric fishes. J Fish Biol 73(4): 809-828.

SÁNCHEZ-GIL, P.; YÁÑEZ-ARANCIBIA, A.; TAPIA, M.; DAY, J.W.; WILSON, C.A. & COWAN JR., J.H. 2008. Ecological and biological strategies of Etropus crossotus and Citharichthys spilopterus (Pleuronectiformes:Paralichthyidae) related to the estuarine plume, Southern Gulf of Mexico. J Sea Res 59: 173-185.

SANTOS, S.R.; ANDRADE, A.C.; VERANI, J.R. & VIANNA, M. 2015. Population explosion of the burrfish Chilomycterus spinosus spinosus (Diodontidae, Tetraodontiformes) in a eutrophic tropical estuary. Mar Biol Res 11(9): 955-964.

SCHWARZ-JUNIOR, R.; FRANCO, A.C.N.P.; SARPEDONTI, V.; PICHLER, H.A. & QUEIROZ, G.M.L.N. 2006. Composição e estrutura da ictiofauna demersal na Baía dos Pinheiros, Paraná. Braz J Aquat Sci Technol 10 (1): 27-39.

SILVA-JÚNIOR, D.R.; GOMES, V.S.; LINDE-ARIAS, A.R. & VIANNA, M. 2012. Metallothionein in the pond perch Diplectrum radiale (Teleostei) as a biomarker of pollution in Guanabara bay estuary, Brazil. J Braz Soc Ecotoxicol 7 (1): 83 - 88.

SILVA-JUNIOR, D.R.; CARVALHO, D.M.T. & VIANNA, M. 2013. The catfish Genidens genidens (Cuvier, 1829) as a potential sentinel species in Brazilian estuarine waters. J Appl Ichthyol 29:1297–303.

SILVA-JUNIOR. D.R.; PARANHOS, R. & VIANNA, M. 2016. Spatial patterns of distribution and the influence of seasonal and abiotic factors on demersal ichthyofauna in an estuarine tropical bay. J Fish Biol 89: 821-846.

SOARES-GOMES, A.; GAMA, B.A.P.; NETO, J.A.B.; FREIRE, D.G.; CORDEIRO, R.C.; MACHADO, W.; BERNARDES, M.C.; COUTINHO, R.; THOMPSON, F.L. & PEREIRA, R.C. 2016. An environmental overview of Guanabara Bay, Rio de Janeiro. Regional Studies in Marine Science 8(2): 319-330.

SOARES-GOMES, A.; NEVES, R.L.; AUCÉLIO, R.; VAN DER VEN, P.H.; PITOMBO, F.B.; MENDES, C.L.T. & ZIOLLI, R.L. 2010. Changes and variations of polycyclic aromatic hydrocarbon concentrations in fish, barnacles and crabs following an oil spill in a mangrove of Guanabara Bay, Southeast Brazil. Mar Poll Bull 60: 1359-1363.

SPACH, H.L.; SANTOS, C. & GODEFROID, R.S. 2003. Padrões temporais na assembléia de peixes na gamboa do Sucuriú, Baía de Paranaguá, Brasil. Rev Bras Zool 20 (4): 591-600.

TALLQVIST, M.; SANDBERG-KILPI, E. & BONSDORFF, E. 1999. Juvenile flounder, Platichthys flesus (L.), under hypoxia: effects on tolerance, ventilation rate and predation efficiency. J Exp Mar Biol Ecol 242: 75-93.

VALENTIN, J.L.; TENENBAUM, D.R.; BONECKER, A.C.T.; BONECKER, S.L.C.; NOGUEIRA, C.R.; PARANHOS, R. & VILLAC, M.C. 1999. Caractéristiques hydrobiologiques de la Baie de Guanabara (Rio de Janeiro, Brésil). J Oceanogr Rech 24 (1): 33-41.

VALENTIN, J.L. 2012. Ecologia numérica. Uma introdução à análise multivariada de dados ecológicos. 2nd edition. Interciência, Rio de Janeiro.

VENDEL, A.L.; SPACH, H.L.; LOPES, S.G. & SANTOS, C. 2002. Structure and Dynamics of Fish Assemblages in a Tidal Creek Environment. Braz Arch Biol Technol 45 (3): 365-373.

VIANNA, M. & ALMEIDA, T. 2005. Bony fish bycatch in the southern Brazil pink shrimp (Farfantepenaeus brasiliensis and F. paulensis) fishery. Braz Arch Biol Technol 48 (4): 611-623.

VIEIRA, J.P.; CASTELLO, J.P. & PEREIRA, L.E. 1998. Ictiofauna In. SEELIGER, U.; ODEBRECHT, C. & CASTELLO, J.P. (eds.). Os ecossistemas costeiro e marinho do extremo sul do Brasil. Rio Grande, Ecoscientia.

WALSH, H.J.; PETERS, D.S. & CYRUS, D.P. 1999. Habitat utilization by small flatfishes in a North Carolina estuary. Estuaries 22: 803–813.

WEST, J.M.; WILLIAMS, G.D.; MADON, S.P. & ZEDLER, J.B. 2003. Integrating spatial and temporal variability into the analysis of fish food web linkages in Tijuana Estuary. Environ Biol Fish 67: 297-309.

Received: 14/04/2018
Revised: 19/11/2018
Accepted: 14/01/2019
Published online: 11/02/2019

Biota Neotropica 19(2): e20180670, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Cetacean movements in coastal waters of the southwestern Atlantic ocean

Marcos C. de O. Santos[1]*, José Laílson-Brito[2], Leonardo Flach[3], Júlia E. F. Oshima[4], Giovanna C. Figueiredo[1], Rafael R. Carvalho[2], Elisa S. Ventura[1], Julia M. B. Molina[1] & Alexandre F. Azevedo[2]

[1]Universidade de São Paulo, Departamento de Oceanografia Biológica, Instituto Oceanográfico, Laboratório de Biologia da Conservação de Mamíferos Aquáticos, Praça do Oceanográfico, 191, Butantã, 05508-120, São Paulo, SP, Brasil
[2]Universidade do Estado do Rio de Janeiro, Faculdade de Oceanografia, Laboratório de Mamíferos Aquáticos de Bioindicadores "Professora Izabel Gurgel", Rua São Francisco Xavier, 524, Maracanã, 20550-013, Rio de Janeiro, RJ, Brasil
[3]Instituto Boto-Cinza, Rua Gastão de Carvalho, Itacuruçá, 23860-000, Rio de Janeiro, RJ, Brasil
[4]Universidade Estadual Paulista, Programa de Pós-graduação em Zoologia, Instituto de Biociências, Laboratório de Ecologia Espacial e Conservação, Campus Rio Claro, Avenida 24-A, 1515, Bela Vista, 13506-900, Rio Claro, SP, Brasil
**Corresponding author: Marcos C. de O. Santos, e-mail: sotalia@gmail.com*

SANTOS, M.C.O., LAÍLSON-BRITO, J., FLACH, L., OSHIMA, J.E.F., FIGUEIREDO, G.C., CARVALHO, R.R., VENTURA, E.S., MOLINA, J.M.B., AZEVEDO, A.F. **Cetacean Movements in Coastal Waters of the Southwestern Atlantic Ocean**. Biota Neotropica. 19(2): e20180670. http://dx.doi.org/10.1590/1676-0611-BN-2018-0670

Abstract: Cetaceans were monitored along *ca.* 700 km of the southeast coast of Brazil (22°S to 25°S) from 1995 to 2014 using photo-identification. The objective of this study was to identify any presence of long-distance movements for monitored cetacean species and discuss implications. Data on long-range movements of four of the monitored species are presented after the analysis of 321,765 photographs taken for individual identification. Seven individuals from four populations of Guiana dolphins (*Sotalia guianensis*) considered resident to particular estuaries or bays were reported in dispersal involving movement between pairs of protected areas over long-range distances varying between 86 and 135 km. Three cataloged rough-toothed dolphins (*Steno bredanensis*), first seen in Guanabara Bay, Rio de Janeiro state (22°46'S) in November 2011, were sighted 240 km southwards as members of the same group in coastal waters of São Paulo state (23°46'S) in July 2014. Water depth for those sightings ranged from 16 to 52.7 m; local sightings of rough-toothed dolphins in Brazil have frequently been in shallow waters, but the species global distribution is usually associated with deeper waters. In a 27-day interval in the spring of 2012, a group of 16 orcas (*Orcinus orca*) travelled *ca.* 277 km in shallow coastal waters ranging from 20 to 30 m deep. Orcas are commonly observed between November and February in southeast Brazil, probably in search for prey. In summer months between 2012 and 2014, three Bryde's whales (*Balaenoptera edeni*) sighted in waters ranging from 14 to 49 m deep, moved between 218 and 327 km. Bryde's whales are usually found in local coastal waters where they spend summer months feeding on sardines. To date, these are the longest estimated movements reported to *S. guianensis*, *S. bredanensis*, *O. orca* and *B. edeni* in the Southwestern Atlantic Ocean.
Keywords: *Movements; Sotalia guianensis; Steno bredanensis; Orcinus orca; Balaenoptera edeni.*

Movimentos de cetáceos pelas águas costeiras do sudoeste do oceano Atlântico

Resumo: Cetáceos foram monitorados em cerca de 700 km da costa sudeste do Brasil (22°S a 25°S) entre 1995 e 2014 com o uso da fotoidentificação. O objetivo deste estudo foi de identificar quaisquer presenças de movimentos de longa distância de indivíduos das espécies monitoradas e discutir suas implicações. Dados de movimentos de longa distância de quatro das espécies monitoradas são apresentados após a análise de 321.765 fotos obtidas para identificações individuais. Sete indivíduos de quatro populações de boto-cinza (*Sotalia guianensis*) consideradas residentes a estuários e baías particulares foram reportados em movimentos de dispersão envolvendo pares de áreas protegidas em distâncias que variaram entre 86 e 135 km. Três golfinhos-de-dentes-rugosos (*Steno bredanensis*) catalogados e avistados primeiramente na Baía de Guanabara, Rio de Janeiro (22°46'S), em novembro de 2011, foram avistados 240 km mais ao sul como membros de um mesmo grupo nas águas costeiras do Estado de São Paulo (23°46'S) em julho de 2014. A profundidade da água onde essas avistagens aconteceram variou entre 16 e 52,7 m; avistagens de golfinhos-de-dentes-rugosos têm sido efetuadas em águas rasas na costa sudeste do Brasil, embora a distribuição global da espécie esteja associada a águas profundas. Em um intervalo de 27 dias na primavera de 2012, um grupo de 16 orcas (*Orcinus orca*) se desclocou cerca de 277 km em águas rasas variando entre 20 e 30 m. Orcas são comumente avistadas entre novembro e fevereiro no sudeste do Brasil, possivelmente em busca de presas. Nos meses de verão entre 2012 e 2014, três baleias-de-Bryde (*Balaenoptera edeni*) avistadas em águas de 14 a 49 m de profundidade, moveram-se entre 218 e 327 km. baleias-de-Bryde são encontradas em águas costeiras locais onde passam os meses de verão se alimentando de sardinhas. Até o presente momento, esses são os deslocamentos estimados como os de maiores distâncias observados para *S. guianensis*, *S. bredanensis*, *O. orca* and *B. edeni* no Atlântico Sudoeste.
Palavras-chave: *Movimentos; Sotalia guianensis; Steno bredanensis; Orcinus orca; Balaenoptera edeni.*

Introduction

Movement is a hallmark of marine mammal behavior and an important component to understanding how they use their world (Stern 2009). Movements play pivotal roles in shaping biodiversity across spatiotemporal scales, determining patterns in species distribution and interactions, changing genetic traits and diversity, and modifying habitat structures and resource levels (Jeltsch et al. 2013). It is thus not a coincidence that "movement ecology" turned into a new ecological discipline, rapidly expanding worldwide (see Nathan et al. 2008).

As of October 2018, 47 cetacean species were reported in Brazilian aquatic environments (Santos et al. 2010a, Cypriano-Souza et al. 2017), which represents *ca*. 50% of all described species worldwide (see Committee on Taxonomy 2017). Among cetacean species found in the Southwestern Atlantic Ocean (SAO), four are of interest in this study due to the lack of prior focus on their long-range movements in this region: the Guiana dolphin - *Sotalia guianensis* (Van Bénéden 1864), Bryde's whale - *Balaenoptera edeni* Anderson 1879, orca - *Orcinus orca* (Linnaeus 1758), and rough-toothed dolphin - *Steno bredanensis* (G. Cuvier in Lesson 1828). The most common cetacean species found within Brazilian waters is the Guiana dolphin, dwelling in shallow coastal and estuarine waters; Brazilian occurrences extends from the country's northern border shared with French Guiana down the coast to the southern limit of the species distribution, in the state of Santa Catarina in southern Brazil (Da Silva et al. 2010).

Bryde's whales are usually found in coastal waters of southeast Brazil, where they spend mainly spring and summer months feeding on sardines (Siciliano et al. 2004). Stranding events of adults and juveniles are reported year-round in this region without a marked seasonality (Moura & Siciliano 2012). Bryde's whales are amongst the least known of the larger baleen whales and are commonly found in tropical and subtropical waters worldwide (Kato & Perrin 2009). Along the northeast coast of Brazil, a few sightings and catches in whaling operations with possible misidentifications with sei (*B. borealis*) and Omura's whales (*B. omurai*) were reported in the past decades (Andriolo et al. 2010). Little is known about movements and migration patterns of all stocks.

Orcas are cosmopolitan cetaceans found in all oceans, most commonly observed in coastal and temperate waters, especially in areas of high productivity (Heyning & Dahlheim 1988, Ford 2009). They are known to display both short and long-scale movements, the latter reaching *ca*. 5,400 km to cope with biological and ecological requirements such as finding food patches, adapting to a seasonally changing environment, and investing in interactions with other social units (Dahlheim et al. 2008, Matthews et al. 2011, Fearnbach et al. 2014). Migration was recently attributed to orcas in the southern hemisphere, possibly evidence of coping with physiological requirements (Durban & Pitman 2012). Sightings have been occasionally observed along the Brazilian coast, with a higher number of records in southeastern waters between November and February (Siciliano et al. 1999). Unpublished aerial images have been showing orcas preying on rays in local shallow waters. However, no information is available on their local abundance, movements and distribution.

While rough-toothed dolphins are widely distributed throughout tropical and warm-temperate waters worldwide, little is known about this species anywhere in their range (Baird et al., 2008). Their global distribution is usually associated to deeper waters, but they have been frequently sighted in shallow waters in southeast Brazil (Lodi 1992, Lodi & Hetzel 1999, West et al. 2011). The only known information on rough-toothed dolphin movements in the SAO was reported by Lodi et al. (2012). Those authors witnessed the movement of four cataloged individuals along the coast of Rio de Janeiro, reaching up to 119.7 km in a short window of time between August and September 2011.

A large proportion of what is known about cetaceans in Brazilian waters comes from studies using dead individuals collected from stranding events (*e.g*. Zerbini et al. 1997, Greig et al. 2001; Ramos et al. 2001, Costa et al. 2017) and/or incidental catches in fishing operations conducted since the end of the 1970s (*e.g*. Siciliano 1994). Starting only at the end of the 1980s, live cetaceans are now also being studied in their element along the *ca*. 8,500 km shoreline. Based on their distribution and close proximity to scientific institutions, long-term studies have been conducted on resident bottlenose dolphin populations in southern Brazil (*e.g*. Simões-Lopes & Fabián 1999, Fruet et al. 2011; 2015) and resident Guiana dolphin populations in southern and southeastern Brazil (*e.g*. Flores 1999, Santos et al. 2000, Azevedo et al. 2004).

For the most part, these studies focused on several aspects of behavioral observations, then most used the technique of photo-identification to follow individuals on a relatively restricted spatial and temporal scale. Throughout its range, investigations on area utilization by *S. guianensis* was based on photo-identification and showed that surveyed populations are resident to the investigated sites (see Flores 1999, Santos et al. 2001, Azevedo et al. 2007), with travel distances reaching up to 30 km, and home ranges averaging between 13.5 to 72.4 km^2 (Flores & Bazzalo, 2004, Oshima & Santos 2016). Most studies on *S. guianensis* have been conducted in protected bays and estuaries, a different scenario when compared to cetacean species found in open waters. Open water investigations have recently been providing the first insights on the occurrence, movements and relation to physical ocean conditions for several other cetaceans (see Di Tullio et al. 2016), but insignificant in scope when compared to the larger body of work devoted to *S. guianensis* and *T. truncatus* in bays and estuaries. As a consequence, information on individual movements out of the home ranges of considered resident populations is still scarce over an extensive area of the (SAO), where more than half of the global cetacean species had already been reported.

Photo-identification is the most economically accessible technique in developing regions across the globe to gather important information regarding cetacean ecology, but until now has not been used comprehensively to understand long-range movements. In recent years, satellite tags have been used as one of the main tools to analyze movements of long-ranging aquatic species such as cetaceans (see Hussey et al. 2015), but such techniques would be limited to a small pool of individuals due to economic and import restrictions. Sampling a considerable number of individuals of different gender, age and personality is essential to avoid bias when describing population traits. Improvements in photo quality in recent years have made image analysis more robust due to the higher density of pixels available to identify features (Urian et al. 2015). This study thus provides new information on long-range movements of four cetacean species (*S. guianensis*, *B. edeni*, *O. orca* and *S. bredanensis*) along the southeast coast of Brazil using photo-identification to follow cataloged individuals.

Materials and Methods

Data were collected in different studies conducted from 1995 to 2014 dedicated to investigating cetacean ecology using photo-identification in estuaries, bays and coastal waters of southeast Brazil (Fig. 1), all based on onboard observations.

Four studies were undertaken in four protected bays and estuaries to investigate population parameters and aspects of ecology of Guiana dolphins (*Sotalia guianensis*). The Lagamar Estuary is composed of the Paranaguá Estuarine Complex (PEC), 3,870 km^2 on the northern coast of Paraná state (Noernberg et al. 2006), and the Cananéia Estuary (CE), a 234 km^2 area on the southern tip of São Paulo state (SMA, 1991). Both areas are connected through a 5 km long man-made channel dug in the 1950s. Investigations in the CE took place from 2000 to 2010 in 132 km^2 of inner estuarine waters used by Guiana dolphins (see details in Oshima et al. 2010, Oshima & Santos 2016). In the PEC, investigations on Guiana dolphins were conducted between 2006 and 2009 in 124 km^2 of protected estuarine waters (see Santos et al. 2010b). The Sepetiba Bay (SPB), on the coast of Rio de Janeiro state, is 526 km^2, with *ca*. 425 km^2 of that area surveyed for Guiana dolphins since 2005 (see Flach et al. 2008, Dias et al. 2009). Also along the coast of Rio de Janeiro, the 328 km^2 Guanabara Bay (GB) (Kjerfve et al. 1997) and adjacent coastal waters have been surveyed for Guiana dolphins since 1995 (details in Azevedo et al. 2007).

Three investigations have been directed towards mapping the occurrence, movements and sound emissions of cetaceans along the shoreline of Paraná, São Paulo and Rio de Janeiro states. Since 2008, GB and adjacent coastal waters up to the 50 m isobath have been surveyed for a cetacean multi-species study to describe their distribution, movements and sound emissions. Moving southwards, the marine protected area known as Parque Estadual Marinho da Laje de Santos (PEMLS), on the São Paulo state coast, was the target of a two-year cetacean multi-species monitoring program conducted between May 2013 and June 2015 to map their occurrence (see Santos et al. 2017). From December 2012 to December 2014, *ca*. 650 km of coastal waters in a straight line along the states of São Paulo and Paraná was covered by another multi-species study of cetaceans. The area was surveyed using 6-day cruises screening a corridor of water depths ranging between 15 m and 40 m. The aims were to map cetacean occurrence, movements and sound emissions. In Table 1, efforts and results gathered to multi-species surveys along the coast of São Paulo were merged, as they were conducted in the same area. These listed efforts were not evenly distributed in time and space.

Outboard-powered vessels with sizes ranging from 5 to 16 m were the mobile platforms used in all studies. All surveyed subsets of the monitored estuaries, bays and coastline had specific tracks to be followed to optimize the chances of encountering cetaceans. Cruises were undertaken in sea states up to 3 Beaufort on the wind scale. When cetaceans were found, the specific sighting location (GPS position), water depth, salinity and superficial temperature at most occasions were recorded. Afterwards, vessels approached cetaceans to estimate group size and invest in gathering photographs to identify individuals. Photographs of dorsal fins of all sighted cetaceans and flukes for humpback whales were taken using film (1995 to 2005) or digital SLR cameras with 300 to 400 mm zoom lenses. Printed photos of past catalogs were scanned. Catalogs were organized by each species to check for the addition of new identified individuals of the investigated populations/stocks and to confirm re-sightings of known and previously sighted individuals following procedures described by

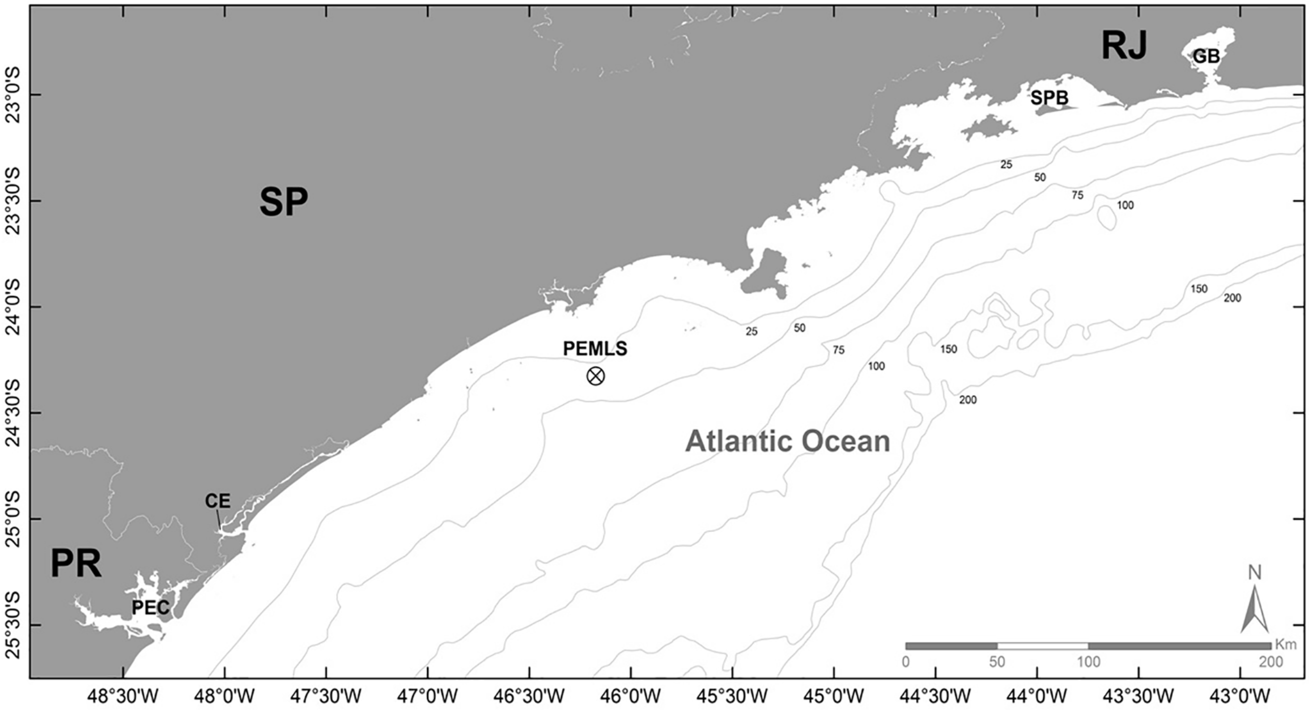


Figure 1. Map of the surveyed area used to investigate cetacean movements along the southeast coast of Brazil, covering part of Paraná (PR) state coast, São Paulo (SP) state coast, and Rio de Janeiro (RJ) state coast. The following subsets were the main targets of Guiana dolphin investigations: The Paranaguá Estuarine Complex (PEC) in PR state, the Cananéia Estuary (CE) in SP state, and the Sepetiba (SPB) and Guanabara (GB) Bays in RJ state. PEMLS means "Parque Estadual Marinho da Laje de Santos", a coastal Marine Protected Area.

Table 1. Summarized efforts invested in the investigation of cetacean movements along the southeast coast of Brazil from 1995 to 2014 using photo-identification. Target species (SG means *Sotalia guianensis*; Multi means multi-species surveys), length of time of surveys in years, main surveyed regions, their estimated area, the number of photographs taken and analyzed, and the number of identified individuals are presented. Acronyms: CE = Cananeia Estuary; PEC = Paranaguá Estuarine Complex; SPB = Sepetiba Bay; GB = Guanabara Bay; SP = São Paulo; RJ = Rio de Janeiro.

Region	Species	Years	Area (km^2)	# Photos	# Ids
CE	SG	2000-2010	132	85,925	199
PEC	SG	2006-2009	124	49,921	188
SPB	SG	2005-2014	425	130,000	820
GB	SG	1995-2014	328	12,154	108
Coast RJ	Multi	2008-2014	1,200	13,224	181
Coast SP	Multi	2012-2014	11,000	30,541	397
TOTAL	Multi	1995-2014	13,209	333,919	1,893

Würsig & Jefferson (1990). Photographs were analyzed and compared by at least two investigators using the Nikon View software and, more recently, Darwin (Stewman et al. 2006). Catalogs were compared among areas to find matches of identified individuals, the main evidence of movement between two different locations. Distances travelled by cetaceans were estimated using ArcGIS 9.1 (ESRI), linking distant locations involving a sighting and a re-sighting of the same individual. When bays and estuaries were included in the analysis, the estimate evaluated the shortest distance to connect the inner location to the further distant location in another protected area, leaving the protected site and travelling along the coast. For sightings reported exclusively in open shoreline waters, only the shortest connection between the two most distant locations was used to estimate the travelled distance.

Results

1. Guiana dolphins, Sotalia guianensis

Considering the surveyed bays and estuaries where Guiana dolphins were found, four catalogs were organized for PEC, CE, SPB, and GB. The number of identified individuals, showing the number of analyzed photographs in parenthesis, were: PEC = 188 (49,921), CE = 199 (85,925), SPB = 820 (130,000), and GB = 108 (12,154) (see Table 1). Overall, 1,315 Guiana dolphins were cataloged after the analysis of 278,000 dorsal fin photographs. Two individuals (KN 159 and 194) from the considered resident population of Guiana dolphins, sighted between 2000 and 2008 in CE inner waters, were reported for the first time in coastal waters in May 2005, *ca*. 10 km distant from the main estuarine entrance. This short-range movement is the first evidence of the plasticity in the use of coastal waters by Guiana dolphins monitored in local protected inner estuaries. Both individuals were female adults and had at least two calves in eight years, with several sightings in the same group, close to the main entrance of the CE. In an interval ranging from one to seven years, another four individuals switched between the PEC and CE estuaries (Table 2, Figure 2). Their movements, considering the most distant locations in inner estuarine basins through coastal waters, ranged between 86 and 124 km, the longest distances reported so far for the species in local waters. The first cataloged dolphin sighted once in the PEC (PR 001) in May 2002 was re-sighted in August 2009 in the CE, where it was cataloged as KN 363. Two adult dolphins, sighted on one (KN 251) and four (KN 300) occasions at the main entrance of the CE in 2001, between 2005 and 2007, respectively, were seen in the same group in the PEC (PR 116 and 115) in October 2008. An adult female (KN 209), sighted on 10 different occasions all over the CE between 2000 and 2006, was seen in November 2008 in the PEC (PR 121). This dolphin had a calf in 2003, which was previously seen with its mother up to 2006 in the CE. The mother was again observed with another calf in 2008 in the PEC.

Three adult individuals (SEP 411, 481 and 783), sighted from one (SEP 783) to seven (SEP 481) distinct occasions in the SPB between March 2006 and January 2012, were photographed in the same group 135 km northwards in December 2012 at the main entrance of the GB, where they were assigned as BG 097, 096 and 098, respectively (Table 2, Figure 3). In previous sightings, SEP 411 and 481 shared the same group on at least three occasions, while SEP 783 was sighted only once in the SPB. These three Guiana dolphins have been sighted in the same group inside the GB. For *S. guianensis*, the estimated range of 135 km is so far the longest distance reported for an individual dolphin.

2. Multi-species

Along the coast of São Paulo state, eleven cetacean species (23% of the total reported in Brazilian waters) were reported between December 2012 and December 2014 after 13 cruises. The analysis of 30,541 photographs resulted in 11 catalogs, with the number of identified individuals represented in parenthesis: Guiana dolphin, *S. guianensis* (2), common bottlenose dolphin, *Tursiops truncatus* (121), Atlantic spotted dolphin, *Stenella frontalis* (162), long-beaked common dolphin, *Delphinus delphis* (14), rough-toothed dolphin, *Steno bredanensis* (71), orca, *Orcinus orca* (6), La Plata dolphin, *Pontoporia blainvillei* (1), humpback whale, *Megaptera novaeangliae* (1), Bryde's whale, *Balaenoptera edeni* (6), dwarf minke whale, *Balaenoptera acutorostrata* (3), and Antarctic minke whale, *B. bonaerensis* (1). On the Rio de Janeiro state coast, the following species (n=4; 8% of the total reported in Brazilian waters) were reported to have individually marked individuals: *T. truncatus* (21), *S. bredanensis* (139), *O. orca* (9), and *B. edeni* (12). These individuals were identified from the analysis of 13,224 photographs.

3. Rough-toothed dolphins, Steno bredanensis

Three cataloged rough-toothed dolphins (SBRJ 005, 025 and 030), first seen in coastal waters of Rio de Janeiro state in November 2009, were reported in the same group in December 2013 in areas closer to shore and 240 km southwards near the shoreline of São Paulo state (SB 027, 029 and 030) in July 2014 (Table 2, Figure 4). Water depth where these dolphins were observed ranged from 16 to 52.7 m.

Table 2. Matching of 15 cataloged individuals of 4 cetacean species monitored along the southeast coast of Brazil from 1995 to 2014. Catalog numbers are presented (ID), as well as date and locations of first and last sightings related to the longest travelled distances, which is shown in the last column.

Species	ID	Date 1st Sighting	Lat/Long 1st Sighting	ID	Date Last Sighting	Lat/Long Last Sighting	Distance (km)
Sotalia guianensis	PR 001	10 MAY 02	25°17'S/48°19'W	KN 363	21 AUG 09	24°58'S/47°56'W	124
	KN 300	23 JAN 05	25°03'S/47°54'W	PR 115	10 FEB 08	25°28'S/48°19'W	86
	KN 251	21 OCT 01	25°03'S/47°54'W	PR 116	10 FEB 08	25°20'S/48°14'W	89
	KN 209	09 NOV 00	25°04'S/47°56'W	PR 121	11 FEB 08	25°19'S/48°12'W	121
	SEP 481	18 MAR 06	23°00'S/43°57S'W	BG 096	12 NOV 14	22°50'S/43°09'W	135
	SEP 411	19 FEB 08	22°59'S/43°57S'W	BG 097	12 NOV 14	22°50'S/43°09'W	135
	SEP 783	19 JAN 12	22°59'S/43°58S'W	BG 098	12 NOV 14	22°50'S/43°09'W	135
Steno bredanensis	SBRJ 005	12 NOV 09	22°46'S/43°08'W	SB 027	31 JUL 14	23°46'S/45°00'W	240
	SBRJ 025			SB 029			
	SBRJ 030			SB 030			
Orcinus orca	ORJ 003	15 NOV 12	22°59'S/43°05'W	OO 006	07 DEC 12	23°58'S/45°33'W	277
	ORJ 004			OO 002			
Balaenoptera edeni	BE 001	06 DEC 12	23°43'S/45°07'W	RJ 002	22 FEB 13	22°58'S/42°52'W	250
	BE 004	27 NOV 13	24°23'S/46°04'W	RJ 005	04 APR 14	23°04'S/43°12'W	327
	BE 005	29 JAN 14	23°31'S/45°05'W	RJ 008	16 MAR 14	23°00'S/43°02'W	218

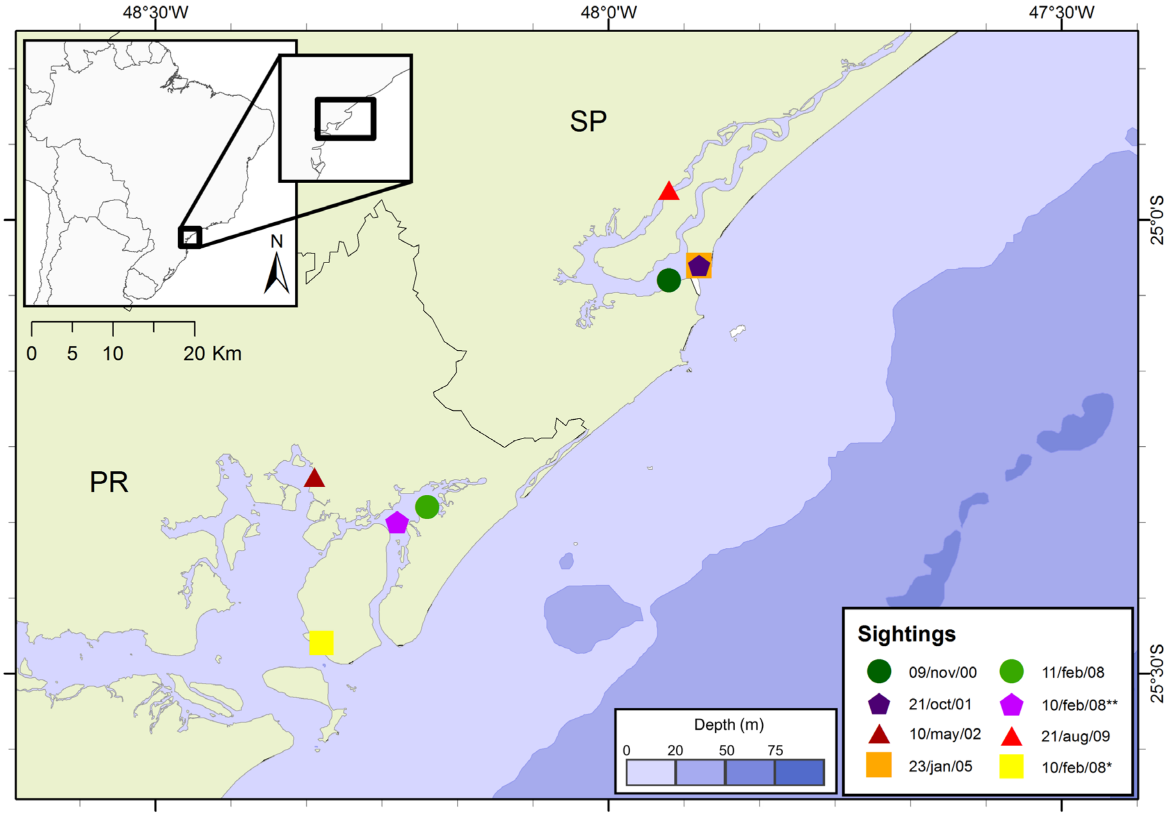


Figure 2. Map showing the estimated distances precluded by four Guiana dolphins switching between the Paranaguá Estuarine Complex in the state of Paraná (PR) and the Cananeia Estuary in the state of São Paulo (SP), southeastern Brazil, from November 2000 to August 2009. Each symbol represents one individual dolphin. The first sighting date is shown at the left column and the latest at the right one. Icons (*) and (**) represent individuals sighted in the same date but in different groups.

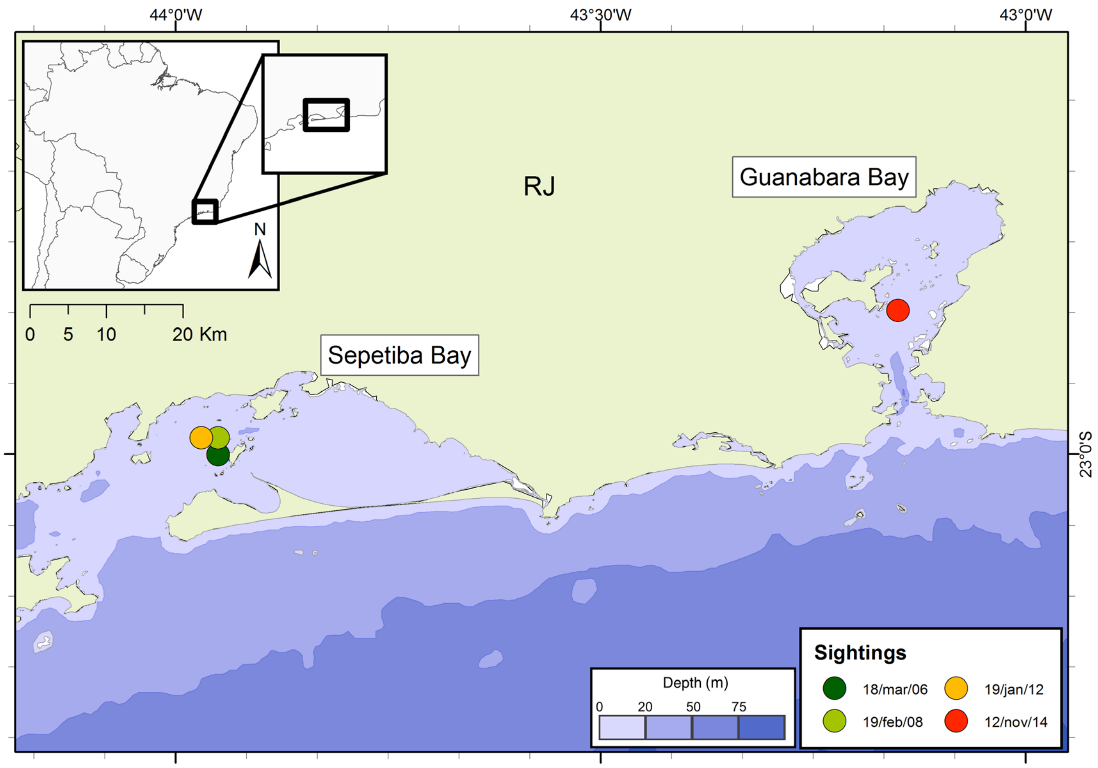


Figure 3. Map showing the estimated distances precluded by three Guiana dolphins switching between the Sepetiba bay and the Guanabara bay, both in the state of Rio de Janeiro (RJ), southeastern Brazil. The first sightings of each cataloged individual occurred in Sepetiba bay between March 2006 and January 2012, and the latest sighting of the three cataloged individuals in the same group occurred in November 2014.

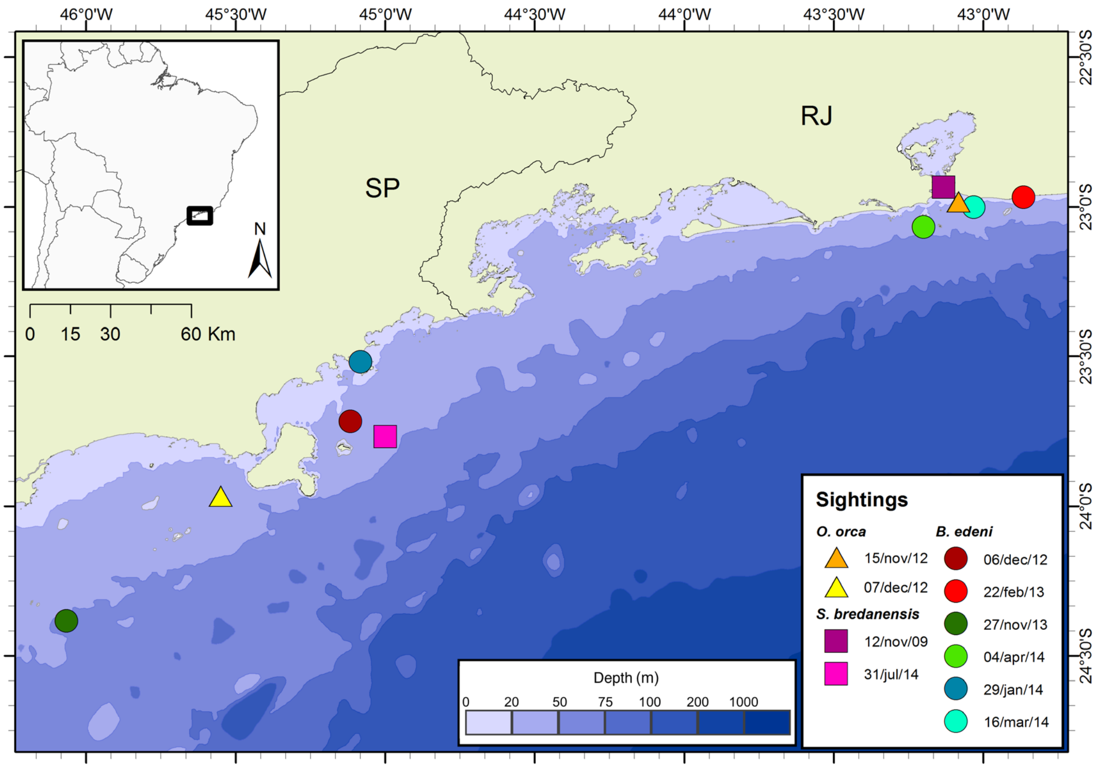


Figure 4. Map showing the estimated distances precluded by two identified rough-toothed dolphins in the same group in both sightings (△), two killer whales (□) also in the same group in both sightings, and three Bryde's whales never reported together (○) between 2009 and 2014 along the coast of the states of São Paulo (SP) and Rio de Janeiro (RJ), southeastern Brazil.

4. Orcas, Orcinus orca

In a 27-day interval between November and December 2012, a group of 16 orcas travelled approximately 277 km in shallow coastal waters ranging from 20 to 30 m deep. Two cataloged individuals of the monitored group on the coast of Rio de Janeiro (OORJ 003 and 004) were re-sighted in São Paulo coastal waters (OO 002 and 006) (Table 2, Figure 4).

5. Bryde's whales, Balaenoptera edeni

In summer months between 2012 and 2014, three Bryde's whales sighted in waters ranging from 14 to 49 m deep, travelled distances between 218 and 327 km (Table 2, Figure 4). All movements occurred northwards from São Paulo to Rio de Janeiro coastal waters in late spring and summer. One adult individual (BE 001), sighted in December 2012 on the northern coast of São Paulo state, was re-sighted in the same summer season in February 2013 on the Rio de Janeiro state coast (RJ 002), travelling an estimated distance of 250 km after the first sighting. This adult individual was also re-sighted in February 2014 close to the same spot on the coast of Rio de Janeiro. The longest travelled distance was reported for the adult individual BE 004 (=RJ 005), which covered around 327 km between November 2013 and February 2014. Another re-sighting on 04 April 2014 was reported at an intermediate location almost at the same place. The last of the re-sighted individuals was an adult female followed by a young calf, seen in January 2014 on the northern coast of São Paulo state (BE 005), both re-sighted on three occasions from February to March 2014 on the coast of Rio de Janeiro state (RJ 008), after travelling *ca*. 218 km in 28 days.

Discussion

1. Photo-identification technique and gathered results

To evaluate cetacean movements in coastal waters of southeast Brazil, the authors relied on photo-identification. Although it seems to be cheaper when compared to the use of telemetry to gather information on individual movements, the cost involved in regular cruises conducted in time and space should be considered if the aim is to conduct longer-term studies. The time spent organizing and examining photographs, updating catalogs and looking for matches after seasons of field campaigns is considerably higher when compared, for instance, to analyzing satellite tagging location data (see Hammond et al. 1990, Hussey et al. 2015). However, photo-identification provides data on movements over a longer window of time when compared to tagging, and allows investigators to gather additional information on social organization, population abundance, calving intervals, etc. (see Hammond et al. 1990). One of the drawbacks of photo-identification is because it is not always guaranteed that all individuals in a group are precisely photographed, which adds to bias on results imposed by the selection and manipulation of images (Urian et al. 2015). Even with several drawbacks, photo-identification is the *modus operandi* to investigate cetaceans in their element in developing countries. However, it is not always guaranteed that research groups will be able to gather financial support to invest in longitudinal studies in those countries. This is the main reason why research efforts are usually unevenly distributed in time and space, as shown in this study. Notwithstanding, the results presented here are highly valuable based on the persistence of a few cetacean research groups working in a long stretch of coastal waters (*ca*. 700 km) in Latin America over the past 20 years.

To date, the presented data and results are the outcome of the longest-term and largest-range photo-identification cetacean multi-species survey along the coast of Brazil. The authors analyzed 278,000 photographs to identify and follow 1,315 Guiana dolphins: possibly the largest numbers throughout the whole distribution of this shy and relatively small cetacean species. Movements of individuals belonging to four poorly known cetacean species in coastal waters of the SAO were detected, providing important clues to how they use the area. Added to the observations of Guiana dolphins, the analysis of 43,765 photographs helped to identify 578 individuals of 11 cetacean species, including rough-toothed dolphins, orcas and Bryde's whales, which were the other targets of the present study. Considering the whole study, authors analyzed 333,919 photographs to identify a total of 1,893 individuals.

2. Guiana dolphins, Sotalia guianensis

Even though the Guiana dolphin is the most common cetacean species dwelling along the coast of Brazil, the knowledge on individual movements is restricted to several bays and estuaries. In the present study, not only were visits to coastal waters by dolphins considered resident to bays and estuaries observed, but also longer-range movements between protected sites. The observed switches of individuals using two of the surveyed estuarine basins (CE and PEC) possibly occurred through coastal waters, as Guiana dolphins have never been reported in the man-made inner connective waters between the two larger monitored estuarine sites. The same pattern of coastal water movement was observed for three individuals that switched between the two surveyed protected bays in Rio de Janeiro (SEP and GB). Longer travel distances ranged from 86 to 135 km, when individuals left one surveyed estuary/bay and entered another, representing the longest reported travelled distances by Guiana dolphins to date.

This leads to whether these long-range movements observed for several Guiana dolphins should be considered dispersal, defined as a permanent displacement of an individual from one area to another (Stern 2009). The most striking difference between dispersal and foraging movements lies in spatiotemporal scales, where the latter is performed within a home range and several times per day (Jeltsch et al. 2013). Dispersal is closely related to the metapopulation conceptual framework, which is defined as "populations connected by the dispersal of several individuals between them" (Hanski & Gaggiotti, 2004). Dispersal reduces the risk of population extinction and maintains genetic heterozygozy (Stern 2009), which, in turn, is important in changing environments facing anthropogenic influences and/or climate change. All the reported seven individuals that moved between two estuaries/bays have, so far, made permanent switches. The observed dispersal movements are likely to be the first data leading to an understanding of Guiana dolphins in a metapopulation context, providing better understanding of their distribution and area utilization in part of their geographic range.

3. Rough-toothed dolphins, Steno bredanensis

In the present study, a longer-range movement (270 km) on a larger time scale (3 years) was observed, showing that researchers chose reliable natural marks to catalog and follow individuals in time and space. Both sightings and re-sightings occurred in shallow depths (16 to 52.7 m), reinforcing the use of shallower waters by *S. bredanensis* in southeastern Brazil. The three monitored individuals were seen in the same group on three occasions in five years, interesting evidence of close associations among them. The first support for strong bonds among individually marked rough-toothed dolphins was found in a survey conducted around Utila Island, Honduras (Kuczaj & Yeater, 2007). In that study, a total of 12 individuals monitored through photo-identification showed strong site fidelity to local waters, as well as high association indices among themselves. Rough-toothed dolphins have been sighted in all seasons in the surveyed area. They may use a wider area, ranging from coastal to neritic waters, to cope with their biological and ecological requirements to survive. The movement of two rough-toothed dolphins of around 480 km was observed in Hawaii using photo-identification (Baird et al. 2008), showing clues that these dolphins are likely to have large home ranges.

4. *Orcas, Orcinus orca*

In this study, in a 27-day interval, a group of 16 orcas travelled around 277 km after sightings in shallow coastal waters (20 to 30 m). To date, this is the only available information on movement of a group of orcas along the Brazilian coast. Orcas are social mammals and usually form social units containing a few to 20 or more individuals (Ford 2009). Thus, information on movements should be properly associated with these social units when the aim is to evaluate the importance of these movements to specific populations/stocks. Caution should thus be taken when considering the movement of *ca*. 600 km by a lone individual male reported in southeast Brazil (Santos & Netto, 2005, Lodi & Farias-Junior 2011), where it was witnessed killing and feeding on a La Plata dolphin (Santos & Da Silva 2009). The feeding habits of orcas usually drive the range of their movement patterns, with fish specialists generally showing relatively shorter ranges when compared to mammal-eating orcas (Bigg et al. 1990, Baird & Dill 1995, Ford & Ellis 1999). The available data on feeding items, identified from both washed ashore individuals and live individuals, witnessed foraging and feeding activities along the Brazilian southern/southeastern coast to include cephalopod, fish (*e.g.*, stingrays, weakfish, tuna and swordfish) and cetacean species (see a review in Santos & Netto, 2005). A more detailed evaluation on the feeding habits of orcas, based on the use of additional tools such as stable isotopes and fatty acids, may possibly drive the investigators to the description of the existence of different social units/stocks in the coast of Brazil. Although orcas were witnessed attacking and killing Bryde's whales elsewhere (Silber et al. 1990, Alava et al. 2013), and both species have been commonly found in late spring and summer in the surveyed waters, this kind of interaction was still not reported in the studied area.

5. *Bryde's whales, Balaenoptera edeni*

Three adult Bryde's whales travelled distances ranging from 218 to 327 km in a two-year period, sighted in shallow coastal waters from 14 to 49 m. These are the largest known ranges reported for *B. edeni* in the SAO. A previous investigation witnessed shorter-range movements of three individually marked Bryde's whales over a maximum distance of 126 km along the coast of Rio de Janeiro (Figueiredo et al. 2014). In all three sightings reported here, individuals were first sighted on the São Paulo state coast at the end of spring and early summer, then moved northwards to the coast of Rio de Janeiro where they were sighted from mid to the end of summer. The considerably small sample size drives us to avoid assessing seasonal patterns. A general pattern of migration towards the Equator in winter and to higher latitudes in summer was described elsewhere for other ocean basins (see Kato & Perrin 2009). Their destination in colder months in southeast Brazil remains a mystery. However, the influence of a cold and highly productive water mass, known as the South Atlantic Central Water (Sverdrup et al. 1942, Castro et al. 2006), may be responsible for the aggregation of their food supply in southeast Brazil in late spring and summer months, which is likely to represent the main trigger for their seasonal higher sighting rates.

6. *Final outcomes*

In conclusion, with the support of photo-identification, the first insights on long-range movements of four cetacean species were described for a poorly known area of the SAO. The reported observations regarding 15 individuals of four species represent important insights on cetacean movements in southeast Brazil. So far, these are the longest-range movement estimates reported for *S. guianensis*, *S. bredanensis*, *O. orca* and *B. edeni* in the surveyed area. Except for Guiana dolphins in a few estuarine basins, for the three other cetacean species presented here, there is no available information on their abundance, seasonality of local coastal water utilization, or even data on life history traits in the SAO. As a consequence, the clues presented here begin to pave the next steps to better understand the dynamics related to cetacean movements in local coastal waters, as well as to better investigate further aspects of their biology and ecology. Other cetacean species have also been found in the surveyed area (see Santos et al. 2017). Their shorter-range movements are under investigation and should provide clues on the role they play in spatiotemporal cetacean biodiversity patterns.

For the four cetacean species studied here, two are categorized as "data deficient" by the IUCN (*O. orca* and *B. edeni*), one as "near threatened" (*Sotalia guianensis*) and, although still poorly known, *S. bredanensis* is listed in the category "least concern" (see Hammond et al. 2012, Reilly et al. 2012, Taylor et al. 2013, Secchi et al., 2018). That makes absolutely clear that studies on the dynamics of cetacean movements are still in need in most ocean basins.

Acknowledgments

The Yatch Club Ilhabela (YCI) kindly provided a safe and comfortable port when surveys were conducted along the northern coast of São Paulo state. We thank the Faculdade de Oceanografia (UERJ), Iate Clube Jardim Guanabara and the administration of Guapimirim Environmental Protection Area for their logistical support. Linda Waters reviewed the final version of this manuscript to adjust grammar and English flow.

Financial support

The Guiana dolphin longitudinal study in the Cananéia Estuary was funded by the Cetacean Society International (CSI), the Whale and Dolphin Conservation Society, the Earthwatch Institute (EWI) and the

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; Process 01/05128-8; 2005/59439-5; 2005/54149-9). In the Paranaguá Estuarine Complex, the financial support came from the CSI, the EWI and FAPESP (Process 2005/59439-5; 2005/54149-9). Júlia Emi de Faria Oshima received grants from Programa de Recursos Humanos da ANP para o setor de petróleo e gás - PRH 05-ANP/MCT/FINEP (Process 48610002667/99-16) and grants and a fellowship from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) (Process 2008/56543-4). Coastal surveys along São Paulo state were funded by FAPESP (Process 2011/51543-9). The survey at the Parque Estadual Marinho da Laje de Santos conducted between 2013 and 2015 was funded through an environmental license process issued by the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) to regulate the natural gas production and outflow system from the Campo de Merluza at Santos Basin under Petrobras (Process 02022.010930/2002-19). The Guiana dolphin longitudinal study in Sepetiba Bay was funded by TELBRAX, MBR.SA, VALE and sponsored by PETROBRAS through Petrobras Socioambiental. We are grateful to the MAQUA team for field assistance and insightful advice. Rio de Janeiro State Government Research Agency (FAPERJ) and Cetacean Society International have supported research developed by MAQUA in Guanabara Bay. AFA and JL-B have research grant from CNPq (PQ-1D), FAPERJ (JCNE) and UERJ (Prociência).

Author Contributions

Marcos C. de O. Santos: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

José Laílson-Brito: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Leonardo Flach: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Júlia E. F. Oshima: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Giovanna C. Figueiredo: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Rafael R. Carvalho: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Elisa S. Ventura: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Julia M. B. Molina: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Alexandre F. Azevedo: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual contente.

Conflict of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALAVA J.J., SMITH K.J., O'HERN J., ALARCÓN D., MERLEN G. & DENKINGER J. 2013. Observations of killer whale (*Orcinus orca*) attacks on Bryde's Whales (*Balaenoptera edeni*) in the Galápagos islands. *Aquatic Mammals* 39, 196–201.

ANDRIOLO A., DA ROCHA J.M., ZERBINI A.N., SIMÕES-LOPES P.C., MORENO I.B., LUCENA A., DANILEWICZ D. & BASSOI M. 2010. Distribution and relative abundance of large whales in a former whaling ground off eastern South America. *Zoology* 27, 741–750.

AZEVEDO, A.F., LAILSON-BRITO, J.JR., CUNHA, H.A. & SLUYS, M.V. 2004. A note on site fidelity of marine tucuxis (*Sotalia fluviatilis*) in Guanabara Bay, southeastern Brazil. *Journal of Cetacean Research and Management* 6, 265-268.

AZEVEDO A.F., OLIVEIRA A.M., VIANA S.C. & VAN SLUYS M. 2007. Habitat use by marine tucuxis (*Sotalia guianensis*) (Cetacea: Delphinidae) in Guanabara Bay, south-eastern Brazil. *Journal of the Marine Biological Association* 87, 201–205.

BAIRD R.W. & DILL L.M. 1995. Occurrence and behaviour of transient killer whales: seasonal and pod-specific variability, foraging behaviour, and prey handling. *Canadian Journal of Zoology* 73, 1300−1311.

BAIRD R.W., WEBSTER D.L., MAHAFFY S.D., MCSWEENEY D.J., SCHORR G.S. & LIGON A.D. 2008. Site fidelity and association patterns in a deep-water dolphin: rough-toothed dolphins (*Steno bredanensis*) in the Hawaiian Archipelago. *Marine Mammal Science* 24, 535–553.

BIGG M.A., OLESIUK P.F., ELLIS G.M., FORD J.K.B. & BALCOMB K. C. 1990. Social organization and genealogy of resident killer whales (*Orcinus orca*) in the coastal waters of British Columbia and Washington State. *Report of the International Whaling Commission* 12, 383−405.

CASTRO B.M., LORENZZETTI J.A., DA SILVEIRA I.C.A. & MIRANDA L.B. 2006. Estrutura termohalina e circulação na região entre o Cabo de São Tomé (RJ) e o Chuí (RS). In C.L.D.B. Rossi-Wongtschowski and L.S. Madureira (eds.), *O ambiente oceanográfico da plataforma continental e do talude na região sudeste-sul do Brasil.* Editora da Universidade de São Paulo, SP, pp. 11−20.

COMMITTEE ON TAXONOMY. 2018. List of marine mammal species and subspecies. Society for Marine Mammalogy. www.marinemammalscience.org (consulted on 25 May 2018).

COSTA, A.F., SICILIANO, S., EMIN-LIMA, R., MARTINS, B.M.L., SOUSA. M.E.M., GIARRIZZO, T. & SILVA-JUNIOR, J.S. 2017. Stranding survey as a framework to investigate rare cetacean records of the north and north-eastern Brazilian coasts. *ZooKeys* 688, 111–134.

CYPRIANO-SOUZA, A.L., MEIRELLES, A.C.O., CARVALHO, V.L. & BONATTO, S.L. 2017. Rare or cryptic? The first report of an Omura's whale (*Balaenoptera omurai*) in the South Atlantic Ocean. *Marine Mammal Science* 33, 80-95.

DAHLHEIM M.E., SCHULMAN-JANIGER A., BLACK N., TERNULLO R., ELLIFRIT D. & BALCOMB K.C. 2008. Eastern temperate North Pacific offshore killer whales (*Orcinus orca*): occurrence, movements, and insights into feeding ecology. *Marine Mammal Science* 24, 719−729.

DA SILVA, V.M.F., FETTUCCIA, D., RODRIGUES, E.S., EDWARDS, H., MORENO, I.B., MOURA, J.F., WEDEKIN, L.L., BAZZALO, M., EMIN-LIMA, N.R., CARMO, N.A.S., SICILIANO, S. & UTRERAS, V.B. 2010. Report on the working group on distribution, habitat characteristics and preferences, and group size. *Latin American Journal of Aquatic Mammals* 8, 31−38.

DIAS L.A., HERZING D. & FLACH L. 2009. Aggregations of Guiana dolphins (*Sotalia guianensis*) in Sepetiba bay, Rio de Janeiro, southeast Brazil: distribution patterns. *Journal of the Marine Biological Association* 89, 967–973.

DI TULLIO, J.C., GANDRA, T., ZERBINI, A.N. & SECCHI, E.R. 2016. Diversity and distribution patterns of cetaceans in the subtropical Southwestern Atlantic outer continental shelf and slope. *Plos One* 11, p. e0155841.

DURBAN J.W. & PITMAN R. L. 2012. Antarctic killer whales make rapid, round-trip movements to subtropical waters: evidence for physiological maintenance migrations? *Biology Letters* 8, 274−277.

FEARNBACH H., DURBAN J.W., ELLIFRIT D.K., WAITE J.M., MATKIN C.O., LUNSFORD C.R., PETERSON M.J., BARLOW J. & WADE P. R. 2014. Spatial and social connectivity of fish-eating "resident" killer whales (*Orcinus orca*) in the northern North Pacific. *Marine Biology* 161, 459−472.

FIGUEIREDO L.D., TARDIN R.H., LODI L., MACIEL I.S., ALVES M.A.S. & SIMÃO S. M. 2014. Site fidelity of Bryde's whales (*Balaenoptera edeni*) in Cabo Frio region, southeastern Brazil, through photoidentification technique. *Brazilian Journal of Aquatic Science and Technology* 18, 59−64.

FLACH L., FLACH P.A. & CHIARELLO, A.G. 2008. Aspects of behavioral ecology of *Sotalia guianensis* in Sepetiba bay, southeast Brazil. *Marine Mammal Science* 24, 503–515.

FLORES, P.A.C. 1999. Preliminary results of a photoidentification study of the marine tucuxi *Sotalia fluviatilis* in southern Brazil. *Marine Mammal Science* 15, 840-847.

FLORES P.A.C. & BAZZALO M. 2004. Home ranges and movement patterns of the marine tucuxi dolphin *Sotalia fluviatilis*, in Baía Norte, Southern Brazil. *Latin American Journal of Aquatic Mammals* 3, 37–52.

FORD J.K.B. 2009. Killer whale *Orcinus orca*. In W.F.Perrin, B., Würsig, and J.G. M. Thewissen, (eds.), *Encyclopedia of marine mammals.* Academic Press, San Diego, CA, pp. 650–657.

FORD J.K.B. & ELLIS G.M. (Eds.) 1999. *Transients: mammal-hunting orcas of British Columbia, Washington, and Southeastern Alaska.* UBC Press and University of Washington Press, Vancouver, BC and Seattle, WA.

FRUET, P.F., SECCHI, E.R., DI TULLIO, J.C. & KINAS, P.G. 2011. Abundance estimation of bottlenose dolphins, *Tursiops truncatus* (Cetacea, Delphinidae), inhabiting the Patos Lagoon estuary, southern Brazil: implications for conservation. *Zoologia* 28, 23–30.

FRUET, P.F., GENOVES, R.C., MOLLER, L.M., BOTTA, S. & SECCHI, E.R. 2015. Using mark-recapture and stranding data to estimate reproductive traits in female bottlenose dolphins (*Tursiops truncatus*) of the Southwestern Atlantic Ocean. *Marine Biology* 162, 661–673.

GREIG, A.B., SECCHI, E.R., ZERBINI, A.N. & DALLA ROSA, L. 2001. Stranding events of southern right whales, *Eubalaena australis*, in southern Brazil. *Journal of Cetacean Research and Management* Special Issue 2, 157–160.

HAMMOND P.S., MIZROCH S.A. & DONOVAN G.P. (Eds.) 1990. *Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters*. Report of the International Whaling Commission, special issue 12. Cambridge, UK.

HAMMOND P.S., BEARZI G., BJØRGE A., FORNEY K.A., KARKZMARSKI L., KASUYA T., PERRIN W.F., SCOTT M.D., WANG J.Y., WELLS R.S. & WILSON B. 2012. *Steno bredanensis*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Downloaded on 28 May 2018.

HANSKI I. & GAGGIOTTI, O.E**.** 2004. Metapopulation biology: past, present and future. In I. Hanski, and O.E. Gaggiotti (eds.) *Ecology, genetics and evolution of metapopulations.* Elsevier Academic Press, pp. 3–22.

HEYNING J.E. & DAHLHEIM M.E. 1988. *Orcinus orca*. *Mammalian Species* 304, 1−9.

HUSSEY N.E., KESSEL S.T., AARESTRUP K., COOKE S.J., COWLEY P.D., FISH A.T., HARCOURT R.G., HOLLAND K.N., IVERSON S.J., KOCIK J.F., FLEMMING J.E.M. & WHORISKEY F.G. 2015. Aquatic animal telemetry: a panoramic window into the underwater world. *Science* 348. DOI 10.1126/science.1255642.

JELTSCH F., BONTE D., PE'ER G., REINEKING B., LEIMGRUBER P., BALKENHOL N., SCHÖDER B., BUCHMANN C.M., MUELLER T., BLAUM N., ZURELL D., BÖHNING-GAESE K., WIEGAND T., ECCARD J.A., HOFER H., REEG J., EGGERS U. & BAUER S. 2013. Integrating movement ecology with biodiversity research – exploring new avenues to address spatiotemporal biodiversity dynamics. *Movement Ecology* 1,1–13.

KATO H. & PERRIN W.F. 2009. Bryde's whales *Balaenoptera edeni*/*brydei.* In W.F. Perrin, B., Würsig, and J.G.M. Thewissen, (eds.), *Encyclopedia of marine mammals*. Academic Press, San Diego, CA, pp. 158–163.

KJERFVE B., RIBEIRO C.H.A., DIAS G.T.M., FILIPPO A.M. & QUARESMA V.S. 1997. Oceanographic characteristics of an impacted coastal bay: Baía de Guanabara, Rio de Janeiro, Brazil. *Continental Shelf Research* 17, 1609–1643.

KUCZAJ S.A. & YEATER D.B. 2007. Observations of rough-toothed dolphins (*Steno bredanensis*) off the coast of Utila, Honduras. *Journal of the Marine Biological Association* 87, 141–148.

LODI L. 1992. Epimeletic behavior of free-ranging rough-toothed dolphins, *Steno bredanensis*, from Brazil. *Marine Mammal Science* 8, 284–287.

LODI L. & HETZEL, B. 1999. Rough-toothed dolphin, *Steno bredanensis*, feeding behaviors in Ilha Grande Bay, Brazil. *Biociências* 7, 29–42.

LODI L. & FARIAS-JUNIOR S. 2011. Movements of a solitary adult male killer whale, *Orcinus orca* (Cetacea, Delphinidae), along the coast of south-eastern Brazil. *Panamerican Journal of Aquatic Sciences* 6, 325–328.

LODI L., OLIVEIRA R.H.T., FIGUEIREDO L.D. & SIMÃO S. M. 2012. Movements of the rough-toothed dolphin (*Steno bredanensis*) in Rio de Janeiro state, south-eastern Brazil. *Marine Biodiversity Records* 5, e47 (4 pages), Published online.

MATTHEWS C.J.D., LUQUE S.P., PETERSEN S.D., ANDREWS R.D. & FERGUSON S.H. 2011. Satellite tracking of a killer whale (*Orcinus orca*) in the eastern Canadian Arctic documents ice avoidance and rapid, long-distance movement into the North Atlantic. *Polar Biology* 34, 1091–1096.

MOURA J.F. & SICILIANO S. 2012. Stranding pattern of Bryde's whales along the south-eastern coast of Brazil. *Marine Biodiversity Records* 5, e73 (7 pages), Published online.

NATHAN R., GETZ W.M., REVILLA E., HOLYOAK M., KADMON R., SALTZ D. & SMOUSE P.E. 2008. A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences* 105, 19052–19059.

NOERNBERG, M.A., LAUTERT L.F.C., ARAÚJO A.D., MARONE E., ANGELOTTI R., NETTO JR J.P.B. & KRUG L.A. 2006. Remote sensing and GIS integration for modelling the Paranaguá estuarine complex–Brazil. *Journal of Coastal Research* 39, 1627−1631.

OSHIMA J.E.F., SANTOS M.C.O., BAZZALO M., FLORES, P.A.C. & PUPIM F.N. 2010. Home ranges of Guiana dolphins (*Sotalia guianensis*) (Cetacea: Delphinidae) in the Cananéia estuary, Brazil. *Journal of the Marine Biological Association* 90, 1641–1647.

OSHIMA J.E.F. & SANTOS, M.C.O. 2016. Guiana dolphin home range analysis based on 11 years of photo-identification research in a tropical estuary. *Journal of Mammalogy* 97, 599–610.

RAMOS, R.; SICILIANO, S.; BOROBIA, M.; ZERBINI, A.; PIZZORNO, J.L.; FRAGOSO, B.; BRITO JR, J.L.; AZEVEDO, A. DE F.; LOPES, P.C.S. & SANTOS, M.C.O. 2001. A note on strandings and age of sperm whales (*Physeter macrocephalus*) on the Brazilian coast. *Journal of Cetacean Research and Management* 3, 321–327.

REILLY S.B., BANNISTER J.L., BEST P.B., BROWN M., BROWNELL JR R.L., BUTTERWORTH D.S., CLAPHAM P.J., COOKE J., DONOVAN G.P., URBÁN J., & ZERBINI A.N. 2008. *Balaenoptera edeni*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Downloaded on 28 May 2018.

SANTOS, M.C.O., ROSSO, S., SICILIANO, S., ZERBINI, A., ZAMPIROLLI, E., VICENTE, A.F.C. & ALVARENGA, F. 2000. Behavioral observations on the marine tucuxi dolphin (*Sotalia fluviatilis*) in São Paulo estuarine waters, Southeastern Brazil. *Aquatic Mammals* 23, 260–267.

SANTOS, M.C.O.; ACUÑA, L. & ROSSO, S. 2001. Insights on site fidelity and calving intervals of the marine tucuxi dolphin (*Sotalia fluviatilis*) in south-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom* 81, 1049–1052

SANTOS M.C.O. & NETTO D.F. 2005. Killer whale (*Orcinus orca*) predation on a franciscana dolphin (*Pontoporia blainvillei*) in Brazilian waters. *Latin American Journal of Aquatic Mammals* 4, 69−72.

SANTOS M.C.O. & DA SILVA E. 2009. Records of a male killer whale (*Orcinus Orca*) off southeastern Brazil. *Brazilian Journal of Oceanography* 57, 65−68.

SANTOS M.C.O., SICILIANO S., VICENTE A.F.C., ALVARENGA F.S., ZAMPIROLLI E., SOUZA S.P. & MARANHO A. 2010a. Cetacean records along São Paulo state coast, southeastern Brazil. *Brazilian Journal of Oceanography* 57, 123−142.

SANTOS M.C.O., OSHIMA J.E.F., PACÍFICO E.S. & DA SILVA E. 2010b. Guiana dolphins, *Sotalia guianensis* (Cetacea: Delphinidae) in the Paranaguá estuarine complex: insights on the use of area based on the photo-identification technique. *Zoology* 27, 324−330.

SANTOS, M.C.O., FIGUEIREDO, G.C. & VAN BRESSEM, M.F. 2017. Cetaceans using the marine protected area of "Parque Estadual Marinho da Laje de Santos", southeastern Brazil. *Brazilian Journal of Oceanography* 65, 605–613.

SECCHI, E.R., SANTOS, M.C. de O. & REEVES, R.R. 2018. *Sotalia guianensis*. *The IUCN Red List of Threatened Species* 2018: e.T181359A50386256. http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T181359A50386256. en. Downloaded on 23 February 2019.

SICILIANO, S. 1994. Review of small cetaceans and fishery interactions in coastal waters of Brazil. *Reports of the International Whaling Commission* 15*,* 241–250.

SICILIANO S., LAÍLSON-BRITO J. & AZEVEDO A.F. 1999. Seasonal occurrence of killer whales (*Orcinus orca*) in waters of Rio de Janeiro, Brazil. *Zeitschrift Fur Saugetierkunde* (*Mammalian Biology*) 64, 251−255.

SICILIANO S., SANTOS, M.C.O., VICENTE, A.F.C., ALVARENGA F.S., ZAMPIROLLI E., LAÍLSON-BRITO J., AZEVEDO A.F. & PIZZORNO J.L. 2004. Strandings and feeding records of Bryde's whales (*Balaenoptera edeni*) in south-eastern Brazil. *Journal of the Marine Biological Association* 84, 857–859.

SILBER G.K., NEWCOMER M.W. & PEREZ CORTES, M.H. 1990. Killer whales (*Orcinus orca*) attack and kill a Bryde's whale (*Balaenoptera edeni*). *Canadian Journal of Zoology* 68, 1603–1606.

SIMÕES-LOPES, P.C. & FABIAN, M.E. 1999. Residence patterns and site fidelity in bottlenose dolphins, *Tursiops truncatus* (Montagu) (Cetacea, Delphinidae) off Southern Brazil. *Revista Brasileira de Zoologia* 16, 1017-1024.

SMA 1991. *Macrozoneamento do complexo estuarino-lagunar de Iguape e Cananéia*. Secretaria do Meio Ambiente (SMA), Programa Estadual de Gerenciamento Costeiro, São Paulo, SP, Brasil.

STERN S.J. 2009. Migration and movement patterns. In W.F. Perrin, B., Würsig, and J.G.M. Thewissen (eds.), *Encyclopedia of marine mammals.* Academic Press, San Diego, CA, pp. 726–730.

STEWMAN J., DEBURE K., HALE S. & RUSSEL A. 2006. Interative 3-D pose correction and content-based image retrieval for dorsal fin recognition. In J. Stewman, K. Debure, S. Hale and A. Russel, A. (eds.). *Image analysis and recognition: lecture notes in computer science.* Springer-Verlag, pp. 648–660.

SVERDRUP H.U., JONHSON M.W. & FLEMING R.H. (Eds.) 1942. *The Oceans: their physics, chemistry, and general biology*. New York, Prentice Hall.

TAYLOR B.L., BAIRD R., BARLOW J., DAWSON S.M., FORD J., MEAD J.G., NOTARBARTOLO DI SCIARA G., WADE P. & PITMAN R. L. 2013. *Orcinus orca*. The IUCN Red List of Threatened Species. Version 2015.2. <www.iucnredlist.org>. Downloaded on 18 May 2018.

URIAN K., GORGONE, A., READ A., BALMER, B., WELLS, R.S., BERGGREN, P., DURBAN, J., EGUCHI, T., RAYMENT W. & HAMMOND P. S. 2015. Recommendations for photo-identification methods used in capture-recapture models with cetaceans. *Marine Mammal Science* 31, 298−321.

WEST K.L., MEAD J.G. & WHITE W. 2011. *Steno bredanensis* (Cetacea: Delphinidae). *Mammalian Species* 43, 177−189.

WURSIG, B. & JEFFERSON, T.A. 1990. Methodology of photo-identification for small cetaceans. In P.S. Hammond, S.A. Mizroch and G. P. Donovan (eds.), *Individual recognition of cetaceans: use of photo-identification and other techniques to estimate population parameters.* International Whaling Commission, Special Issue, no. 12, pp. 43−52.

ZERBINI, A.N., SECCHI, E.R., SICILIANO, S. & SIMÕES-LOPES, P.C. 1997. A review on the occurrence and distribution of whales of the genus *Balaenoptera* along the Brazilian coast. *Reports of the International Whaling Commission* 47, 407-417.

Received: 16/10/2018
Revised: 28/12/2018
Accepted: 19/02/2019

Biota Neotropica 19(2): e20180602, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Treehopper (Membracidae, Auchenorrhyncha) assemblages in four semi-arid areas of caatinga of Northeast Brazil

Rembrandt Romano Andrade Dantas Rotheá[1,2], *Aline Lourenço*[1,2], *Alexandre Vasconcellos*[1,2]* &

Antonio José Creão-Duarte[1,2]

[1]*Universidade Federal da Paraíba, Centro de Ciências Exatas da Natureza, Departamento de Sistemática e Ecologia, CEP 58051-970, João Pessoa, PB, Brasil*
[2]*Universidade Federal da Paraíba, Programa de Pós-Graduação em Ciências Biológicas, CEP 58051-970, João Pessoa, PB, Brasil*
**Corresponding author: Alexandre Vasconcellos, e-mail: alextermites@gmail.com*

ROTHEÁ, R.R.A.D., LOURENÇO, A., VASCONCELLOS, A., CREÃO-DUARTE, A.J. **Treehopper (Membracidae, Auchenorrhyncha) assemblages in four semi-arid areas of caatinga of Northeast Brazil**. Biota Neotropica. 19(2): e20180602. http://dx.doi.org/10.1590/1676-0611-BN-2018-0602

Abstract: Treehopper assemblages were sampled in four semi-arid areas of caatinga in Northeast Brazil during the rainy seasons of 2016 and 2017, using three different capture methods. The areas are located in two Ecoregions of the Caatinga: "Depressão Sertaneja Setentrional" (Northern Sertaneja Depression) and "Planalto da Borborema" (Borborema Plateau). A total of 2,394 individuals of 43 species were sampled, corresponding to approximately 87% of the mean estimated species richness for all four areas. The species richness varied among the areas from 14 to 21, while the abundance of individuals ranged from 129 to 1860. Eighteen species were shared among areas, of which only *Enchenopa eunicea*, *Sundarion* sp. 1, *Ceresa vitulus* and *Erosne parvula* occurred in all four due to the general behavior of the first three and the wide distribution of host plant of *Erosne parvula* - *Mimosa tenuiflora*. Twenty-five species occurred only in one area, of which 23 were categorized as rare with a maximum of eight individuals sampled, and seven with only one individual collected (singletons). Considering that the Caatinga Domain possesses at least nine Ecoregions with different climates, geomorphology and vegetation, along with marked endemism of plants, it is expected that the species richness of treehoppers will increase substantially on a regional scale.

Keywords: *biodiversity, Neotropical Region, host plants, treehoppers.*

Taxocenoses de membracídeos (Membracidae, Auchenorrhyncha) de quatro áreas de caatinga do semi-árido do nordeste do Brasil

Resumo: As taxocenoses de membracídeos foram amostradas em quatro áreas de Caatinga do semiárido do Nordeste brasileiro, durante a estação chuvosa de 2016 e 2017, utilizando três métodos distintos de captura. Essas áreas estão inseridas em duas Ecorregiões da Caatinga, Depressão Sertaneja Setentrional e Planalto da Borborema. Um total de 2.394 indivíduos pertencentes a 43 espécies foram amostrados, correspondendo a aproximadamente 87% da riqueza de espécies média estimada para o conjunto das quatro áreas. A riqueza de espécies por área variou de 14 a 21, enquanto a abundância de indivíduos variou de 129 a 1860. Dezoito espécies foram compartilhadas, mas apenas *Enchenopa eunicea*, *Sundarion* sp. 1, *Ceresa vitulus* e *Erosne parvula* ocorreram em todas as áreas, em decorrência do comportamento generalista das três primeiras espécies e da distribuição ampla da planta hospedeira de *Erosne parvula* - *Mimosa tenuiflora*. Vinte cinco espécies foram exclusivas, ocorrendo em apenas uma das áreas, sendo 23 dessas categorizadas como raras, com no máximo oito indivíduos amostrados, e sete com apenas um único indivíduo coletado (*singletons*). Tendo em vista que o Domínio da Caatinga possui, pelo menos, nove Ecorregiões, com diferentes características de clima, geomorfologia e vegetação, inclusive com uma marcante taxa de endemismo de plantas, há expectativa que a riqueza de espécies de membracídeos possa aumentar substancialmente numa escala regional.

Palavras-chave: *biodiversidade, Região Neotropical, plantas hospedeiras, membracideos.*

Introduction

Treehoppers (Membracidae) are phytophagous hemiptera of the suborder Auchenorrhyncha. They frequently go unnoticed by most people because the elaborated form of the pronotum often resembles parts of the plant son which they occur Deitz 1975. They are mostly found on the most sun-exposed parts of their host plants (Funkhouser 1951), such as new branches, inflorescences and infructescences (Haviland 1925), where they suck sap and, as result, excrete *honeydew*, which attracts mainly bees and ants with which they establish mutualistic relationships (Loye 1987, Del Claro & Oliveira 1999, Delabie 2001).

The treehopper fauna, and their geographic distributions, were initially recorded systematically by Funkhouser (1927). Supplements to this first catalog have since been published (Metcalf & Wade 1965, McKamey 1998). The last supplement included about 3,300 species distributed in six zoogeographic regions, the richest being the Neotropical Region with about 43% of the total species. Currently, 686 species have been recorded in Brazil (Evangelista et al. 2018), but the real richness is likely much higher considering the low number of researchers in the country devoted to the group, numbers gaps in sampling of the main phytogeographic domains of Brazil and difficulties accessing canopy fauna, where most of the species occur.

The Caatinga encompasses an area of 912,529 km^2, representing 10.7% of the area of Brazil, and is composed of Seasonally Dry Tropical Forests (SDTFs) of the Neotropical Region (Silva et al. 2017). A significant proportion of the country's population lives in this region, but since no public policy for maintaining ecosystems was in effect during their occupation, less than 2% of the area is protected as full protection Conservation Areas (Tabarelli et al. 2000). The rhetoric that prevailed in the past, that the Caatinga harbored few species and therefore had low conservation priority, no longer finds support among the scientific community. The high number of endemic species described each year is a testament to their differentiated diversity and, at the same time, to the lack of knowledge about them (Bellini 2014, Mariano & Costa 2014, Carvalho & Bravo 2014, Lecci et al. 2014, Vasconcellos & Moura 2014, Creão-Duarte et al. 2017, Ernesto et al. 2018).

The climate of the Caatinga is marked by two well-defined seasons (drought and rain) that affect the dynamics of all the biotic components of the ecosystem. Precipitation and relative humidity are the main climatic variables that predict an increase in plant biomass, which in turn substantially alter the life cycle of animals and, consequently, all the biological activities performed by these organisms (Vasconcellos et al. 2010). The rainy season determines significant changes in the abundance of insects (Gusmão & Creão Duarte 2004, Iannuzzi et al. 2006, Vasconcellos et al. 2010, Liberal et al. 2011) and, consequently, increases in reproduction, predation, parasitism (Fogden 1972, Wolda 1978) and foraging (Janzen & Schoener 1968).

Research on treehoppers (Membracidae) of the Caatinga has had the aim of describing new species (Creão-Duarte & Rothéa 2006, Rothéa & Creão Duarte 2007) or emphasizing aspects of their ecology, such as insect/host plant relationships (Creão-Duarte et al. 2012, 2016). Strictly speaking, the present study represents the first systematized effort to inventory the fauna of Membracidae in the Caatinga by describing its abundance and species richness in four areas of located in two Ecoregions of Northeast Brazil.

Material and Methods

1. *Study areas*

The study was developed in four areas of caatinga in the state Paraíba, Brazil. (i) RPPN Fazenda Almas (RPFA) (centered approximately at 7°28'12"S, 36°53'52"W): this area is located in the municipalities of São José dos Cordeiros and Sumé, has an area of 3,505 ha and a maximum elevation of 720 m. The mean annual precipitation is 600 mm, and the climate is classified as BSh (Köppen 1948). The vegetation is open arboreal, with low levels of anthropic disturbance (Lima & Barbosa 2014, Barbosa et al. 2015). (ii) Serra de Santa Catarina (SESC) (centered at approximately 7°00'46"S, 38°11'12"W): this area is located in the municipalities of Nazarezinho and São José da Lagoa Tapada, has an area of 11,210 ha, and a maximum elevation of 830 m. The mean annual precipitation is 892 mm, and the climate is classified as *4ath*, that is, hot tropical and markedly dry (Bagnolds. & Gaussen 1963). Seasonal deciduous caatinga occurs in areas at higher elevations, and possess high aerial biomass and great potential for conservation of Caatinga biodiversity (Giulietti et al. 2003, Ernesto et al. 2018). (iii) Parque Ecológico Engenheiro Ávidos (PEEA) (6°59'12"S, 38°27'22"W): this area is located in the municipality of Cajazeiras, has area of 181.98 ha and a maximum elevation of 290 m. The mean annual precipitation is 870 mm, and the climate is classified as *Aw* (Köppen). The vegetation is shrubby and open, and exhibits clear signs of recent anthropic impacts (Feitosa et al. 2002). (iv) Parque Estadual da Pedra da Boca (PEPB) (6°27'32"S, 35°40'38"W): this area is located in the municipality of Araruna, has an area of 157 ha and a maximum elevation of 400 m. The mean annual precipitation is 950mm, and the climate is classified as BSh (Köppen). The vegetation is shrubby with high levels of anthropic disturbance. The first three study areas are located in the "Depressão Sertaneja Setentrional" (Northern Sertaneja Depression) and the last in "Planalto da Borborema" (Barborema Plateau), according to the classification of Caatinga Ecoregions proposed by Velloso et al. (2002).

2. *Collection protocol*

Treehoppers were captured in each area during four expeditions during the rainy season of 2015 (PEEA and PEPB) and 2016 (RPFA and SESC), using three methods: yellow adhesive cards, light traps and active collection. The two-sided (11 x 21cm) adhesive cards (PROMIP®) were arranged in the study areas at two heights — up to 1.5m and the tree canopy — separated from each other by at least 20m and from the edge by 50m. The cards were thrown into the canopy with the help of a slingshot, (Durepox®) spheres and throwing lines. Thirty cards were placed at each height for a total of 60 cards per area. Each card was considered a sample unit and remained in the field for five days. The insects collected with adhesive cards were removed from the glue using Varsol® solvent for 24h followed by acetone.

The light trap was equipped with a 250w and 220v mixed mercury light, powered by a 60Hz and ~700W portable gasoline generator, against a white fabric background (2.0 x 2.0m). For this method, 90 minutes of collection was considered a sample unit, with 10 sample units being performed in each area. The distance between traps was 100m.

Active collection was performed along a transect of about 1,200m, distributed along trails and in the edges of the forest, using an entomological net, plastic bags and killing-jars to capture insects seen up to the reach height of the entomological net (about 4m). Active collection sampled a total of 30 sampling units of 30m each spaced at 10m intervals along the transect.

The entire collection protocol employed four people experienced in collecting treehoppers over the course of five days at a cost $200 per area, including food, adhesive cards and fuel. After mounting and drying, the specimens were incorporated into the Coleção (Entomológica) do Departamento de Sistemática e Ecologia (DSEC)/UFPB.

3. *Data analysis*

EstimateS 9.1 software (Colwell 2000) was used to estimate species richness through the Chao1 estimator and to construct the collector curve. In this case, as the abundances are very different between the areas, we chose to relate the richness to the number of samples, which had the same strength per area, one hundred samples. In all cases the data were submitted to 1000 randomizations without replacement (Walther & Moore 2005, Colwell 2006). The amount of additional effort, in terms of individuals required to reach 95% (g = 0.95) and 100% (g = 1) of the estimated richness was calculated by Chao et al. (2009). Finally, differences in species composition between areas were illustrated using a Venn diagram constructed with Venny 2.1 software (Oliveros 2015).

Results

A total of 2,394 treehoppers, belonging to 43 species and 24 genera, were collected in the four caatinga areas (Table 1). The total species richness sampled (43 spp.) corresponded to 93,26% of total estimated richness (46.11 spp.) (Table 2) and the additional effort to reach 95% (g = 0.95) and 100% (g = 1) of the species richness in the areas would require an increase in the collection effort to 1,300 and 4,391 individuals, respectively.

The observed species richness varied among areas, ranging from 14 to 21, with estimates ranging from 14.0±0.1 to 26.0±5.5 (Chao1) (Table 1 and 2). The trend-lines produced (the species accumulation curves) suggest that increased sampling effort in SESC and PEPB would have a high likelihood increasing significantly the species richness of these areas (Figure 1).

The number of individuals per area ranged from 129 (RPFA) to 1,860 (SESC), with *Melusinella nervosa* being the most abundant in two of the areas. More broadly, dominance of the assemblages was such that only three species, *Melusinella nervosa*, *Erosne parvula* and *Sundarion* sp. 1, were responsible for approximately 79% of all the individuals (Table 1). In the set of assemblages, seven species had only one individual collected (singletons), with areas possessing 1 (PEEA), 2 (PEPB) and 4 (SESC).

Eighteen species were shared between two, three or four areas, with *Erosne parvula*, *Sundarion* sp. 1, *Enchenopa eunicea* and *Ceresa vitulus* being the species that were collected in all areas or, in other words, 9.3% of the species. However, the number of exclusive species was 25, with SESC being the area with the highest number of species (12) in this category (Figure 2).

Discussion

When the species richness of the present study (43 spp.) is combined with other pre-existing records in DSEC Entomological Collection, the total treehopper species richness for the Caatinga in Paraíba reaches 64. Furthermore, 30 (46,8%) of these species represent exclusive records for the Caatinga Domain of this state and so have a high likelihood of being endemic species. In addition, 20 species of this total may be new to science, although only two Ecoregions of the Caatinga have been explored: "Depressão Sertaneja Setentrional" (RPFA, SESC and PEEA) and "Planalto da Borborema" (PEPB).

Treehoppers are phytophagous insects and the dominance of these species in an environment is a function of an equally abundant occurrence of plants (resources) that hosts these Hemiptera (Wallace, 2008). *Melusinella nervosa* was the most abundant species in the present study, having been recorded in three of the four areas and with 1,509 individuals having been captured in RPFA alone, where its most important host plant, *Sida galheirensis* Ulbr. (Malvaceae), occurs in abundance as well. In a study of niche overlap in the Caatinga, using only active collection, *Enchenopa concolor*, *E. eunicea* and *E. minuta* were found to be the most abundant species, and were collected in association with eight, nine and four species of host plants, respectively (Creão-Duarte et al. 2012).

The number of species shared by two or more areas (18) was less than the number of exclusive species (25). We believe that the sharing of species between areas may be more likely for generalist species or when a species-specific host is widely distributed. *Enchenopa eunicea, Erosne parvula*, *Sundarion* sp. 1, and *Ceresa vitulus*, were the only species shared by all four areas of the present study. Of these, *E. eunicea* is a generalist species, *Sundarion sp.* and *Ceresa vitulus* have been recorded for four host plants, and *Erosne parvula* was collected on *Mimosa tenuiflora* (Wild.) Poiret (Fabaceae), a common Caatinga plant, with eight species of treehoppers already recorded using it as a host in this domain (Creão-Duarte et al. 2012).

Among the exclusive species (25), 23 were represented by eight or fewer individuals; that is, they were rare species in the assemblage (Table 1), but host specificity among treehoppers is more relevant to species of temperate regions, since tropical species tend to be polyphagous (Wood 1984, 1993). Lopes (1995) listed 26 species of treehoppers for the Cerrado, which occurred on 40 species of host plants, about 27% of which were recorded for a single host plant. The scope of this study, however, did not seek to identify host plants, although a study of Membracidae niche overlap in the Caatinga found that only about 15% of the species were associated with a single host; thus, the generalization of Wood (1984, 1993) also applies to Caatinga treehoppers.

Comparing the 26 species collected in a locality of the Cerrado by Lopes (1995) with the 43 species collected in the Caatinga reveals that only three species are common to both biomes: *Enchenopa concolor*, *Enchenopa gracilis* and *Prorcyrta pectoralis*. The two species of *Enchenopa* have broad distributions, ranging from Central America to southern Brazil (McKamey 1998). In the Cerrado, *E. concolor* was only recorded on *Bauhinia holophylla* Steud (Leguminosae), while *E. gracilis* was recorded on six host plants from six different botanical families. In the Caatinga, *E. concolor* was recorded on eight different

Table 1. Species richness and abundance of Membracidae in four areas of caatinga in the state of Paraíba, Brazil.

Species	RPFA	SESC	PEEA	PEPB	N	N (%)	N ac (%)
Melusinella nervosa (Fairmaire)	1509	0	14	53	1576	65.83	65.83
Erosne parvula Evangelista & Sakakibara	145	1	69	4	219	9.15	74.98
Sundarion sp.1	68	10	4	7	89	3.72	78.7
Paraceresa sp.1	0	23	55	0	78	3.26	81.96
Tolania sp. 5	27	0	31	11	69	2.88	84.84
Stictopelta sp.	29	0	18	11	58	2.42	87.26
Enchenopa eunicea Rothéa & Creão-Duarte	16	1	9	29	55	2.3	89.56
Procyrta pectoralis (Fabricius)	3	37	0	1	41	1.71	91.27
Notocera sakakibarai Creão-Duarte & Rothéa	12	0	2	9	23	0.96	92.23
Hygris beckeri Sakakibara	3	0	1	18	22	0.92	93.15
Micrutalis diminuta Sakakibara	0	19	0	0	19	0.79	93.94
Ceresa vitulus (Fabricius)	1	2	13	1	17	0.71	94.65
Peltosticta yonkei Sakakibara	15	0	0	2	17	0.71	95.36
Enchenopa gracilis (Germar)	4	0	0	11	15	0.63	95.99
Tolania sp. 4	15	0	0	0	15	0.63	96.62
Enchenopa minuta Creão-Duarte & Rothéa	0	0	8	0	8	0.33	96.95
Amastris sp. 1	2	0	0	4	6	0.25	97.2
Ceresa ustulata Fairmaire	1	5	0	0	6	0.25	97.45
Enchenopa concolor (Fairmaire)	0	5	0	0	5	0.21	97.66
Euwalkeria sp.	0	5	0	0	5	0.21	97.87
Heteronotus sp. 1	0	4	0	0	4	0.17	98.04
Sundarion sp. 2	1	0	2	1	4	0.17	98.21
Tolania sp. 2	4	0	0	0	4	0.17	98.38
Tolania sp. 1	1	0	0	3	4	0.17	98.55
Cymbomorpha sp. 1	0	3	0	0	3	0.13	98.68
Heteronotus sp. 2	0	3	0	0	3	0.13	98.81
Notocera colavitei Creão-Duarte & Rothéa	0	3	0	0	3	0.13	98.94
Tolania sp. 3	1	2	0	0	3	0.13	99.07
Amastrini sp.	0	0	0	2	2	0.08	99.15
Amastris sp. 2	0	0	2	0	2	0.08	99.23
Amblyophallus sp.	2	0	0	0	2	0.08	99.31
Cymbomorpha sp. 2	0	0	0	2	2	0.08	99.39
Darnis sp.	0	0	2	0	2	0.08	99.47
Micrutalis sp.	0	0	0	2	2	0.08	99.55
Paraceresa sp. 2	0	2	0	0	2	0.08	99.63
Philya longicauda Sakakibara	0	0	0	2	2	0.08	99.71
Cymbomorpha nigrofasciata (Fairmaire)	0	0	0	1	1	0.04	99.75
Lycoderides olivae Sakakibara	0	1	0	0	1	0.04	99.79
Micrutalis binaria Fairmaire	1	0	0	0	1	0.04	99.83
Ramedia pauperata Creão-Duarte & Sakakibara	0	1	0	0	1	0.04	99.87
Smiliorachis sp. 2	0	1	0	0	1	0.04	99.91
Smiliorachis sp. 3	0	1	0	0	1	0.04	99.95
Tynelia longula Burmeister	0	0	0	1	1	0.04	99.99
Abundance	1860	129	230	175	2394	100	
Species richness	21	20	14	21	43		

Table 2. Values of richness observed and estimated.

	RPFA	SESC	PEEA	PEPB	TOTAL
Species Richness	21	20	14	21	43
Singletons	6	6	1	5	7
Doubletons	2	3	4	5	8
Chao 1 ± SD	26.0±5.5	23.7±4.2	14.0±0.1	22.7±2.2	46.11±3.1
Sampled richness %	80.77	84.39	100.00	90.09	93.26

The column "TOTAL" represents the combining of the four areas into 400 sample units.

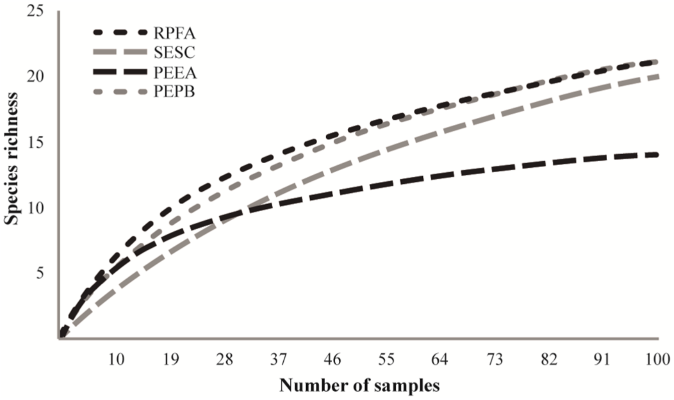


Figure 1. Species accumulation curves for four areas of caatinga in the state of Paraíba, Brazil.

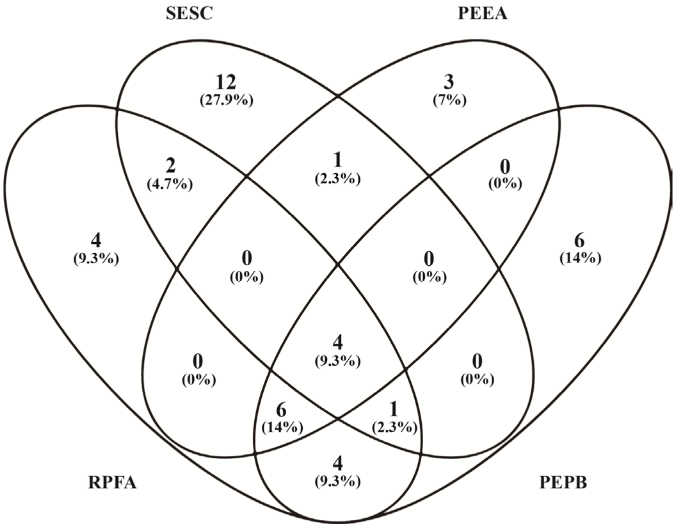


Figure 2. Venn diagram for species of Membracidae, exclusive and shared, in four areas of caatinga in the state of Paraíba, Brazil.

plant species, the most common being *Indigofera suffruticosa* Mill. (Fabaceae) (Creão Duarte et al. 2012). The low proportion of common species found between the domains may be historical and indicate that the Caatinga and Cerrado are not the result of a single ancestral area, as stated by Zanella (2010).

The species accumulation curves for each area translate the richness estimates obtained with the Chao1 estimator. The PEEA curve was the only one that approached the asymptote, but the stopping rule was not met due to the occurrence of a singleton, *Hygris beckeri*. In the other three areas it was not possible to identify any stability trend and the curves indicate that additional sampling efforts should be directed to them in order to reach 95% and 100% of the estimated richness.

The Caatinga Domain makes up the richest area of Dry Seasonal Tropical Forest in the New World, where more than 3,000 plant species are found with an estimated endemism of 23% (Queiroz et al*.* 2017). In this domain, which corresponds to 10.7% of the national territory, there are at least 13 compositions of different vegetal physiognomies distributed among nine distinct ecoregions (Veloso et al. 2002, Silva et al. 2017). The heterogeneity of the Caatinga, the low similarity between the sampled assemblages of treehoppers in different areas and the large sampling gaps in this domain suggest that the biodiversity of these insects will increase substantially with increased sampling effort, especially in ecoregions not yet explored.

Acknowledgments

The authors would like to thank Josimar Bezerra (SESC) for the fieldwork assistance and José Roberto (RPFA). RRADR and AL thanks CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nivel Superior) for the scholarship. AV thanks CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for the research grant (proc. 304210/2017-0).

Authors Contributions

Rembrandt Romano Andrade Dantas Rothéa: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation and critical revision, adding intellectual content.

Aline Lourenço: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation and critical revision, adding intellectual content.

Alexandre Vasconcellos: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation and critical revision, adding intellectual content.

Antonio José Creão-Duarte: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation and critical revision, adding intellectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

BAGNOLDS, F. & GAUSSEN, H. 1963. Os Climas Biológicos e sua Classificação. Boletim Geográfico, Rio de Janeiro, 22(176): 545-566.

BELLINI, B.C. 2014. Fauna de Collembola (Arthropoda) em áreas úmidas do semiárido. In Artrópodes do Semiárido. Biodiversidade e Conservação (F. Bravo & A. Calor, eds.). Printmídia, Feira de Santana, p.57-68.

CARVALHO, J. DE & BRAVO, F. 2014. Odonata do semiárido. In Artrópodes do Semiárido. Biodiversidade e Conservação (F. Bravo & A. Calor, eds.). Printmídia, Feira de Santana, p.83-90.

CHAO, A., COLWELL, R.K, CHIH-WEI L., & GOTELLI, N.J. 2009. Sufficient sampling for asymptotic minimum species richness estimators. Ecology. 90(4): 1125–1133.

COLWELL, R.K. 2000. Statistical estimation of species richness and shared species from samples. Version 9 and earlier. User's Guide and application. University of Connecticut.

COLWELL, R.K. 2006. User's guide to Estimates statistical. Estimation of species richness and shared species from samples. http://viceroy.eeb.uconn.edu/estimates (last access in 15/jun/2017).

CREÃO-DUARTE, A.J. & ROTHÉA, R.R.A.D. 2006. A new species of *Calloconophora* Dietrich (Hemiptera, Membracidae) from the semi-arid region of northeastern Brazil. Rev. Bras. Entomol. 50(4): 473-474.

CREÃO-DUARTE, A.J., ANJOS, U.U. & SANTOS, W.E. 2012. Diversidade de membracídeos (Hemiptera, Membracidae) e sobreposição de recursos tróficos em área do semi-árido. Iheringia Ser. Zool. 102(4): 355–467.

CREÃO-DUARTE, A.J., HERNÁNDEZ, M., ROTHÉA, R.R.A.D. & SANTOS, W. 2016. Temporal variation of Membracidae (Hemiptera: Auchenorrhyncha) composition in areas of caatinga with different vegetation structures. Sociobiology. 63: 826-830.

CREÃO-DUARTE, A.J., ROTHÉA, R.R.A.D., LOURENÇO, A., CABRAL, V.A. & EVANGELISTA O. 2017. Two remarkable new species of *Notocera* Amyot and Serville, 1843 (Hemiptera, Membracidae, Hypsoprorini) from the Brazilian Caatinga. Zootaxa. 4281(1): 77–89.

DEITZ, L.L. 1975. Classification of the higher categories of the New World treehoppers (Homoptera: Membracidae). North Carolina Agricultural Experiment Station, Technical Bulletin. 225:1–177.

DEL-CLARO, K. & OLIVEIRA, P.S. 1999. Ant-Homoptera interactions in a neotropical savana: the honeydew-producing treehopper Guayaquila xiphias (Membracidae) and is associated ant fauna on Didymopanax vinosum (Araliaceae). Biotropica. 31:135-144.

DELABIE, J.H.C. 2001. Trophobiosis between Formicidae and Hemiptera (Sternorrhyncha and Auchenorrhyncha): an overview. Neotropical Entomology. 30: 501-516.

ERNESTO, M.V., LIBERAL, C.N., FERREIRA, A.F., ALVES, A.C.F., ZEPPELINI, D., MARTINS, C.F., PEREIRA-COLAVITE, A., CREÃO-DUARTE, A.J. & VASCONCELLOS, A. 2018. Hexapod decomposers of Serra de Santa Catarina, Paraíba, Brazil: an area with high potential for conservation of Caatinga biodiversity. Biota Neotropica. 18(2): e20170410.

EVANGELISTA, O., SAKAKIBARA, A. & TAKIYA, D.M. 2018. Membracidae in Catálogo Taxonômico da Fauna do Brasil. PNUD. Disponível em: <http://fauna.jbrj.gov.br/fauna/faunadobrasil/1304>. Acesso em: 01. Out. 2018.

FEITOSA, A.F.M.A., WATABABE, T. & MENEZES, M.A. DE. 2002. Unidades de Conservação no Semi-Árido Nordestino: O caso do Parque Ecológico Engenheiro Ávidos. Raízes. 21(1): 101-113.

FOGDEN, M.P.L. 1972. The seasonality and population dynamics of equatorial forest birds in Sarawak. Ibis. 114(3): 307-343.

FUNKHOUSER, W.D. 1951. Homoptera Family Membracidae. Genera Insectorum. 208:1–383.

FUNKHOUSER, W.D. 1927. General Catalogue of the Hemiptera. Fascicle I: Membracidae. Smith College Press. 581 pp.

GIULIETTI, A.M., BOCAGE NETA, A.L. DU, CASTRO, A.A.J.F., GAMARRA-ROJAS, C.F.L., SAMPAIO, E.V.S.B., VIRGÍNIO, J.F., QUEIROZ, L.P., FIGUEIREDO, M.A., RODAL, M.J.N., BARBOSA, M.R.V. & HARLEY, R.M. 2003. Diagnóstico da vegetação nativa do bioma Caatinga. In Biodiversidade da Caatinga: áreas e ações prioritárias para a conservação (J.M.C. Silva, M. Tabarelli, M.T. Fonseca & L.V. Lins orgs.). Ministério do Meio Ambiente, Brasília.

GUSMÃO, M.A.B. & CREÃO-DUARTE, A.J. 2004. Diversidade e análise faunística de Sphingidae (Lepidoptera) em área de brejo e Caatinga no estado da Paraíba, Brasil. Revista Brasileira de Zoologia. 21(3):491–498.

HAVILAND, M.D. 1925. The Membracidae of Kartabo, Bartica District, British Guiana, with description of new species and bionomical notes. Zoologica. 6:229–290.

IANNUZZI, L., MAIA, A.C.D. & VASCONCELOS, S.D. 2006. Ocorrência e sazonalidade de coleópteros buprestídeos em uma região de caatinga nordestina. Biociências. 14(2): 174−179.

JANZEN, D.H. & SCHOENER, T.W. 1968. Differences in insect abundance and diversity between wetter and drier sites during a tropical dry season. Ecology. 49(1): 96−110.

KÖPPEN, W. 1948. Climatologia: con un estudio de los climas de la Tierra. Fondo de Cultura Economica, Ciudad de México.

LECCI, L.S., SIMÕES, T.V.D. & CALOR, A.R. 2014. Plecoptera do Semiárido: conhecimento atual e desafios. In: Artrópodes do Semiárido. Biodiversidade e Conservação (F. Bravo & A. Calor, eds.). Printmídia, Feira de Santana, p.91-98.

LIBERAL, C.N., FARIAS, A.M.I. DE, MEIADO, M.V., FILGUEIRAS, B.K.C. & IANNUZZI, L. 2011. How habitat change and raisfall affect dung beetle diversity in Caatinga, a Brazilian semi-arid ecosystem. J. Insect. Sci. 11(114): 1-11.

LOYE, J.E. 1987. Behavior of a Solitary Treehopper Microcentrus perditus (Membracidae: Homoptera). Journal of Kansas Entomological Society. 60(3):403-407.

LOPES, B.C. 1995. Treehoppers (Homoptera, Membracidae) in Southeastern Brazil: use of host plants. Revista Brasileira de Zoologia. 12:595-608.

MARIANO, R. & COSTA, S.S. 2014. Ephemeroptera do Semiárido. In: Artrópodes do Semiárido. Biodiversidade e Conservação (F. Bravo & A. Calor, eds.). Printmídia, Feira de Santana, p.69-81.

MCKAMEY, S.H. 1998. Taxonomic catalogue of the Membracoidea (exclusive of leafhoppers): second supplement to fascicle 1– Membracidae of the general catalogue of the Hemiptera. Memoirs of the American Entomological Institute. 60:1–377.

OLIVEROS, J.C. 2015. Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html (last access in 15/mar/2018).

QUEIROZ, L.P., CARDOSO, D., FERNANDES, M.F. & MORO, M.F. 2017. Diversity and Evolution of Flowering Plants of the Caatinga Domain. 23-63 pp. In: Silva J.M.C., Leal I.R., Tabarelli M. (eds) Caatinga: The Largest Tropical Dry Forest Region in South America. Springer, Dordrech. 482 pp.

ROTHÉA, R.R.A. D. & CREÃO-DUARTE, A.J. 2007. Duas novas espécies de *Enchenopa* Amyot & Serville (Hemiptera, Membracidae) do semi-árido do Estado da Paraíba, Brasil. Rev. Bras. Entomol. 51(1): 35-37.

SILVA, J.M.C., BARBOSA, L.C.F., LEAL, I.R. & TABARELLI, M. 2017. The Caatinga: Understanding the Challenges. 3-19. In: Silva J.M.C., Leal I.R., Tabarelli M. (eds) Caatinga: The Largest Tropical Dry Forest Region in South America. Springer, Dordrech. 482 pp.

TABARELLI, M., SILVA, J.M.C., SANTOS, A.M.M. & VICENTE, A. 2000. Análise de representatividade das unidades de conservação de uso direto e indireto na Caatinga: análise preliminar. In: Silva, J.M.C. & Tabarelli, M. (coord.) Workshop Avaliação e identificação de ações prioritárias para a conservação, utilização sustentável e repartição de benefícios da biodiversidade do bioma Caatinga. Petrolina, Pernambuco. www.biodiversitas.org.br./caatinga.

VASCONCELLOS, A., ANDREAZZE, R., ALMEIDA. A.M., ARAUJO, H.F.P., OLIVEIRA, E.S. & OLIVEIRA, U. 2010. Seasonality of insects in the semi-arid Caatinga of northeastern Brazil. Rev. Bras. Entomol. 54 (3): 471-476.

VASCONCELLOS, A. & MOURA, F.M.S. 2014. Térmitas de oito ecossistemas inseridos no Domínio do Semiárido Brasileiro. In Artrópodes do Semiárido. Biodiversidade e Conservação (F. Bravo & A. Calor, eds.). Printmídia, Feira de Santana, p.99-109.

VELLOSO, A.L., SAMPAIO, E.V.S.B. & PAREYN, F.G.C. 2002. Ecorregiões: Propostas Para O Bioma Caatinga. Associação Plantas Do Nordeste, Instituto De Conservação Ambiental The Nature Conservancy Do Brasil, Recife. 76p.

WALLACE, M. S. 2008. Occurrence of treehoppers (Hemiptera: Membracidae: Smiliinae) on oaks in Delaware Water Gap National Recreation Area, 2004-2006. Journal of Insect Science. 8:59.

WALTHER, B.A. & MOORE, J. L. 2005. The concepts of bias, precision and accuracy, and their use in testing the performance of species richness estimators, with a literature review of estimator performance. Ecography. 28(6): 815-829.

WOLDA, H. 1978. Seasonal fluctuations in rainfall, food, and abundance of tropical insects. Journal Animal Ecology. 47:369-81.

WOOD, T.K. 1984. Life history patterns of tropical membracids (Homoptera: Membracidae). Sociobiology. 8:299–344.

WOOD, T.K. 1993. Diversity in the New World Membracidae. Annual Review of Entomology. 38:409-433.

ZANELLA, F.C.V. 2011. Evolução da biota da diagonal de formações abertas secas da América do Sul. 198-220 pp. In: CARVALHO, C.J.B. & ALMEIDA, E.A.B. Biogeografia da América do Sul: Padrões e Processos. 306 pp.

Received: 19/06/2018
Revised: 05/10/2018
Accepted: 14/01/2019

Biota Neotropica 19(2): e20180580, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)

Article

The influence of a large reservoir on the reproductive activity of the white piranha, *Serrasalmus brandtii* (Lütken, 1875) in Southeast Brazil

Nilo Bazzoli[1]*, *Viviane Elizabeth de Souza Silva*[1], *Lucas Marcon*[1], *Kleber Biana Santiago*[2],

José Enemir dos Santos[1] *& Elizete Rizzo*[3]

[1]*Pontificia Universidade Católica de Minas Gerais, Belo Horizonte, Brasil*
[2]*Centro Integrado de Recursos Pesqueiros e Aquicultura de Três Marias, Três Marias, MG, Brasil*
[3]*Universidade Federal de Minas Gerais, Departamento de Morfologia, Belo Horizonte, MG, Brasil*
**Corresponding author: Nilo Bazzoli, e-mail: bazzoli@pucminas.br*

BAZZOLI, N., SILVA, V. E. S., MARCON, L., SANTIAGO, K. B., SANTOS, J. E., RIZZO, E. **The influence of a large reservoir on the reproductive activity of the white piranha, *Serrasalmus brandtii* (Lütken, 1875) in Southeast Brazil**. Biota Neotropica. 19(2): e20180580. http://dx.doi.org/10.1590/1676-0611-BN-2018-0580

Abstract: In order to assess the influence of the Três Marias dam on the reproduction of the white piranha, *Serrasalmus brandtii*, 1569 specimens captured from June 2011 to May 2012 in three sections of the São Francisco River basin were analyzed: section 1 = Três Marias reservoir (TMR); section 2 = São Francisco River (SFR) segment immediately downstream of TMR; and section 3 = SFR segment at the confluence with the Abaeté River. Total length (TL), body weight (BW), gonadosomatic index (GSI), and gonadal maturation stages were determined for each specimen, while vitellogenic follicle diameter, and absolute (AF) and relative (RF) fecundities were also determined for females. Reproductive activity, including spawned females and spermed males, was recorded throughout the year in the three river segments. Higher frequencies of females and males at the maturing/mature stage were found in the November/December and January/February, which coincided with the period of higher temperature, rainfall, and dissolved oxygen concentration. Mean GSI and vitellogenic follicle diameter were higher in sections 1 and 3 than in section 2 ($P < 0.05$), while AF and RF were higher in section 1 ($P < 0.05$) than in sections 2 and 3. The results of the present study showed that *S. brandtii* had lower reproductive performance in section 2, probably due to thermal disturbances caused by the dam, thus confirming the influence of dams on the reproduction of fish downstream from hydroelectric plant reservoirs.

Keywords: *environmental impact, thermal disturbances, gonadal maturation.*

A influência de um grande reservatório na atividade reprodutiva da piranha branca, *Serrasalmus brandtii* (Lütken, 1875) no sudeste do Brasil

Resumo: Com o objetivo de avaliar a influência da barragem de Três Marias na reprodução da piranha branca, *Serrasalmus brandtii*, foram analisados 1569 exemplares capturados de junho de 2011 a maio de 2012 em três trechos da bacia do rio São Francisco: seção 1 = reservatório de Três Marias (RTM); seção 2 = segmento do rio São Francisco (SRF) imediatamente a jusante do RTM; e seção 3 = segmento RSF na confluência com o rio Abaeté. O comprimento total (CT), peso corpóreo (PC), índice de gonadossomático (IGS) e estágio de maturação gonadal foram determinados para cada amostra, enquanto o diâmetro do folículo vitelogênico, fecundidade absoluta (FA) e relativa (FR) foram determinadas para fêmeas. Nas três seções estudadas, a atividade reprodutiva foi registrada ao longo do ano, incluindo fêmeas desovadas e machos espermiados. Maiores frequências de fêmeas e machos na fase de maturação/maduro foram encontradas nos meses de novembro/dezembro e janeiro/fevereiro, coincidindo com o período de maior temperatura, precipitação e concentração de oxigênio dissolvido. O IGS médio e o diâmetro do folículo vitelogênico foram maiores nas seções 1 e 3 do que na seção 2 ($P < 0{,}05$), enquanto FA e FR foram maiores na seção 1 ($P < 0{,}05$) do que nas seções 2 e 3. Os resultados do presente estudo mostraram que *S. brandtii* teve menor desempenho reprodutivo na seção 2, provavelmente devido a perturbações térmicas causadas pela barragem, confirmando a influência das barragens na reprodução de peixes a jusante de reservatórios de usinas hidrelétricas.

Palavras-chave: *impacto ambiental, distúrbios térmicos, maturação gonadal.*

Introduction

Most of the world's major rivers have some kind of damming that causes negative impacts to entire fish communities (Olden & Naiman 2010). Although important for economic development, dams cause serious and irreversible impacts to the natural hydrological regime of rivers including altering the downstream flow (Agostinho et al. 2010, Nunes et al. 2015). Such impacts have been detected downstream of several hydroelectric plants in different parts of the world (Clakson & Childs 2000, Todd et al. 2005, Olden & Naiman 2010). In the São Francisco River in the Southeast Region of Brazil, negative impacts of the Três Marias dam on the reproductive activity of the ichthyofauna have been detected in migratory species, such as *Prochilodus argenteus* (Arantes et al. 2010), *Leporinus reinhardti* (Weber et al. 2013) and *Brycon orthotaenia* (Nunes et al. 2015), and foraging species, such as *Astyanax fasciatus* and *A. bimaculatus* (Normando et al. 2014), but there have been no studies evaluating impacts on typically sedentary species or species pre-adapted to lentic environments such as *Serrasalmus brandtii*.

Among Brazilian carnivorous fish, piranhas and pirambebas exhibit a higher degree of agility in catching prey than other species due to their voracity, especially when they inhabit lentic waters (Braga 1981). The white piranha, *S. brandtii*, is native to Brazil and endemic to the São Francisco River basin. It belongs to the Serrassalmidae family (Mirande 2010), which includes piranhas and pirambebas, which are voracious carnivorous freshwater fish that are restricted to South America and occur in Brazilian reservoirs (Jégu 2003). Males of the species perform uni-parental care and the species is pre-adapted to lentic environments (Braga 1981, Agostinho 2003).

Considering that studies related to the reproduction of *S. brandtii* have only been conducted in lentic environments (Honorato-Sampaio et al. 2015), the objective of the present study was to comparatively analyze the reproduction of *S. brandtii* in three sections of the São Francisco River basin — the Três Marias reservoir, a lentic environment, and two lotic sections of the São Francisco River — and to evaluate the impact of the dam on the reproduction of the species.

Materials and methods

1. Study area

A total of 1569 specimens of *S. brandtii* were captured in three sections of the São Francisco River (SFR) basin: section 1 = Três Marias reservoir (TMR) (18º23'27" S, 45º13'12" W); section 2 = SFR in the first 34 km downstream of the TMR (18º07'59" S, 45º14'01" W); and section 3 = 34 to 54 km downstream of the TMR, after the confluence of the SFR with the Abaeté River (18º00'49" S, 45°10'51" W) (Figure 1 and Table 1). Fish were sampled bimonthly from June 2011 to May 2012 using gill nets with meshes ranging from 3.0 to 7.0 cm between opposite knots. The fish, if still alive, were killed by cross-sectioning the cervical cord, following the ethical principles for animal handling established by the Brazilian College of Animal Experimentation - COBEA (www.cobea.org.br).

2. Sampling, biological indices, and biometry

All fish were dissected and measured for total length (TL), body weight (BW), and gonad weight (GW). These biometric data were used to calculate the gonadosomatic index (GSI = GWx100/BW) and the Fulton condition factor (K = BWx100/TL^3) for each of the three river sections.

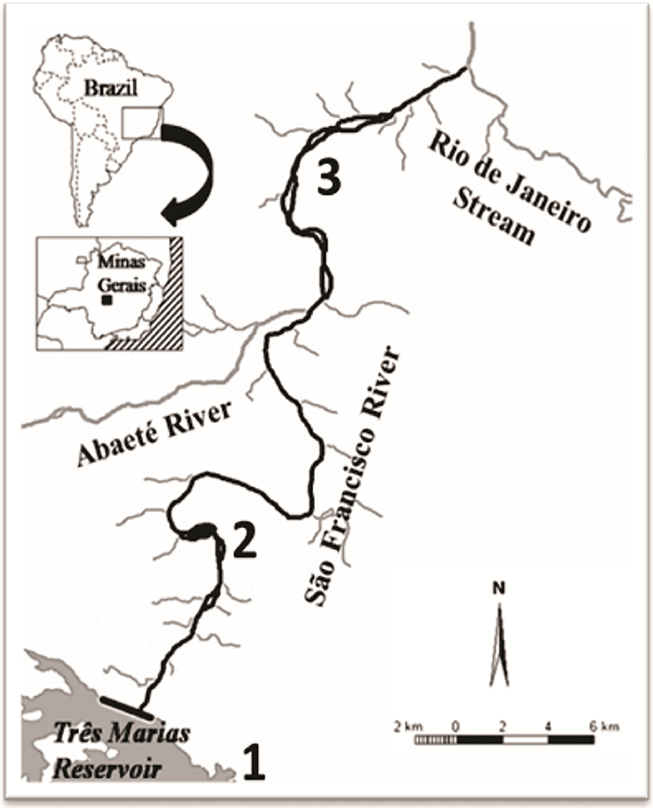


Figure 1. Study area in the São Francisco River (SFR) basin, showing the locations of the studied sections: section 1 = Três Marias Reservoir; section 2 = section of the SFR above the confluence with the Abaeté River, extending from the dam 34 km downstream to the confluence with the Abaeté River; and section 3 = section below the confluence with the Abaete River, extending from 34 to 54 km downstream from the dam.

3. Histology, gonadal maturation stage and spawning type

For histological analysis, fragments from the middle region of the ovaries and testes were fixed in Bouin's fluid for 24 hr, embedded in paraffin, sectioned at 5 µm thickness, and stained with haematoxylin-eosin (HE). Stages of gonadal maturation, spawning type, and the frequency distribution were established based on the macro- and microscopic characteristics of the gonads and on variation in the gonadosomatic index (Honorato-Sampaio et al. 2009, Weber et al. 2013, Normando et al. 2014, Nunes et al. 2015, Brandao et al. 2017).

Size at first gonadal maturation was determined as the smallest total length of females and males with gonads in the maturing/mature stage from each section (Boncompagni-Júnior et al. 2013, Brandão et al. 2017).

4. Histometry, fecundity and sex ratio

The diameter of 50 vitellogenic follicles, with little shrinkage and intact spherical shape, at the maturating/mature stage of development was measured from histological slides using an Olympus BX 50 light microscope with Olympus CellSens Standard 1.9 software.

http://dx.doi.org/10.1590/1676-0611-BN-2018-0580

Table 1. Water temperature, dissolved oxygen concentration and pH for the three studied sections of the São Francisco River Basin (SFR) from June 2011 to May 2012: section 1 = Três Marias dam; section 2 = SFR immediately downstream of the Três Marias dam; and section 3 = confluence between SFR and Abaeté River.

	Section 1	Section 2	Section 3
	Mean ± SD	Mean ± SD	Mean ± SD
Temperature (ºC)	28.83 ± 0.01	23.21 ± 0.57	24.31 ± 0.71
Oxygen (mg L^{-1})	9.65 ± 0.01	5.55 ± 1.67	7.97 ± 0.55
pH	6.58 ± 0.06	5.83 ± 0.46	6.35 ± 0.51
Flow (m^3 s)	-	570.2 ± 61.2	630.8 ± 60.4

In order to determine fecundity, sub-samples of mature ovaries (n = 10 from for each section) were collected. Samples from the middle region of the ovaries were fixed in a modified Gilson solution (100 ml of 60% ethanol, 880 ml of distilled water, 15 ml of 80% nitric acid, 18 ml of glacial acetic acid, and 20 g of mercuric chloride). Dissociated vitellogenic follicles were separated and counted under a stereoscopic microscope. The number obtained in the sub-sample was extrapolated for the total weight of the ovaries through the simple rule of three. Absolute fecundity (AF) was calculated using the equation: AF = OVA × GW, where OVA is the number of follicles per gram of ovary. Relative fecundity (RF) was calculated using the equations TL (AF/TL) and GW (AF/GW).

The sex ratio in the three sections of the basin was determined as the ratio of the absolute frequency of females to that of males. The chi-square test (x^2; $p < 0.05$) was applied to detect possible differences in the proportions between the sexes.

5. *Statistical analysis*

The variables of the three sections were tested for normality (Shapiro-Wilk) and subjected to an analysis of variance to test for significant differences in mean values of TL, BW, GSI, K, DF, AF, AF/TL and AF/GW. The parametric Duncan test was used to compare mean values. When the assumption of normality was not met, even after appropriate transformations, the data were subjected to the nonparametric Kruskal-Wallis test. A significance level of $P < 0.05$ was employed for all tests.

Results

A total of 992 specimens was collected in section 1 (522 females, 470 males), 349 in section 2 (216 females, 133 males), and 228 in section 3 (118 females, 110 males). There were slightly more females than males in sections 1 and 3, but not significantly so (section 1 = 2.72, section 3 = 0.28), whereas females predominated in section 2 (section 2 = 19.74).

Females and males had statistically higher values for TL, BW, and GSI in the section immediately downstream from the Três Marias reservoir (section 1), than in sections 2 and 3 (Table 2).

Three stages of gonadal maturation were established for adult females (F) and males (M): F1 (16.87 ± 2.80)/M1 (16.43 ± 5.23 cm) = resting; F2 (22.75 ± 8.80 cm)/M2 (18.19± 6.60 cm) = maturing/mature; and F3 (20.79 ± 6.23 cm)/M3 (19.90 ± 6.80 cm) = spawned for females and spent for males (Figures 2 and 3).

Fish in reproductive activity, including spawned females and spermed males, were recorded throughout the year in all three sections. The peak for spawned females and spermed males in the three sections occurred in the bimonthly periods of November/December and January/February. A long reproductive period (Figure 4) and the occurrence of spawning females with follicles in all stages of development, including post-ovulatory folicles, confirm that *S. Brandtii* is a partial spawner.

Sections 1 and 3 had greater vitellogenic follicle diameters ($p < 0.05$) than section 2 (Table 2).

The size at first gonadal maturation for each section was: section 1 = females 14.2 cm and males 12.5 cm; section 2 = females 12.1 cm and males 11.8 cm; and section 3 = females 12.8 cm and males 11.6 cm. Absolute fecundity (AF) and relative fecundity (RF), calculated using TL and GW, were greater in section 1 ($P < 0.05$) than in sections 2 and 3 (Table 2).

Discussion

The results of the present study showed that *S. brandtii* reproduces in all three of the analyzed sections of the São Francisco River basin, although with lower performance in section 2 immediately downstream of the Três Marias dam. The physical and chemical conditions of the water in this section are known to be unfavourable for the reproduction of several fish species (Sato et al. 2005). The lower reproductive performance of *S. brandtii* in this section may be related to the low dissolved oxygen and temperature of the water due to thermal stratification of the reservoir during the summer and to water of the hypolimnion entering the turbines, as well as the anthropic impact of flow control (Santos et al. 2012). Impacts caused by the release of cooler water with low dissolved oxygen have been detected downstream of several other dams in different locations throughout the world (Clarkson & Childs 2000, Donaldson et al. 2008).

Fish from sections 2 and 3 had significantly lower lengths and weights than those from section 1, which may also be due to the negative environmental impacts (Nikolsky 1963) immediately downstream of the dam, as was also observed for *Schizodon knerii* (Brandão et al. 2016). Larger size at first gonadal maturation was observed for females in section 1, which may be related to differences in environmental conditions and food availability (Nikolsky 1963, Pawson et al. 2000). It could also be related to the production of sex hormones, such as 17-β oestradiol, which is responsible for both the somatic growth and gonadal development (Blázquez et al. 1998, Arantes et al. 2010).

The morphological characteristics of the ovaries and testes of *S. brandtii* we found to be similar to those of other species of the family Serrassalmidae (Honorato-Samapio et al. 2009). As has been reported for other piranhas, the mature ovaries of *S. brandtii* exhibited asynchronous development with follicles in different growth stages (i.e. perinucleolar, cortical alveolar and vitellogenic follicles; Honorato-Samapio et al. 2009, Marcon et al. 2017). Three gonadal maturation stages for females and males were established in the present study, which are similar

Table 2. Biological variables for females and males of *S.brandtii* captured in three sections of the São Francisco River basin (SFR) from June 2011 to May 2012. Section 1 = Três Marias dam; section 2 = SFR immediately downstream of the Três Marias dam; and section 3 = confluence of the SFR with the Abaeté River. N = number of fish caught; TL = total length; BW = body weight; GSI = gonadosomatic index at maturing/mature stage; K = Fulton condition factor; DF = diameter of the vitellogenic follicle; AF = absolute fecundity; relative fecundity RF = AF/TL and AF/gonadal weight (GW).

	Females					
	Section 1 (n=522)		Section 2 (n=216)		Section 3 (n=118)	
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
TL	10.2 - 34.7	18.7 ± 4.9^{a}	14.0 - 22.5	15.4 ± 2.6^{b}	13.8 -29.0	15.4 ± 4.2^{b}
BW	30.0 - 480.0	143.0 ± 104.0^{a}	23.0 - 219.7	83.7 ± 42.5^{b}	26.0 - 432.0	85.0 ± 70.8^{b}
GSI	0.7 - 3.6	2.1 ± 0.9^{a}	0.6 - 2.6	1.40 ± 0.6^{b}	0.5 - 1.6	1.42 ± 0.3^{b}
K	1,2 - 3,2	1.90 ± 0.3^{a}	0.3 - 2.3	1.70 ± 0.4^{b}	0.3 - 4.6	1.90 ± 0.6^{ab}
DF	622.9 - 1124.7	866.7 ± 156.3^{a}	544.6 - 1031.3	729.2 ± 112.4^{b}	619.3 - 984.4	859.9 ± 112.5^{a}
AF	6011.0 - 8700.0	7041.6 ± 1105.5^{a}	3277.0 - 4436.0	3856.5 ± 579.5^{b}	5393.0 - 5822.0	5607.5 ± 303.3^{ab}
AF/TL	248.3 - 330.6	302.4 ± 46.9^{a}	192.7 - 208.2	200.4 ± 10.9^{b}	190.8 - 199.0	194.9 ± 5.8^{b}
AF/GW	464.7 - 542.0	503.3 ± 54.6^{a}	272.3 - 360.6	316.4 ± 62.4^{b}	373.3 - 377.2	375.2 ± 2.7^{ab}
	Males					
	Section 1 (n=470)		Section 2 (n=133)		Section 3 (n=110)	
TL	10.0 - 31.5	17.4 ± 4.2^{a}	13.0 - 22.0	14.9 ± 2.8^{b}	13.0 - 26.0	15.5 ± 2.7^{b}
BW	25.1 – 312.0	103.2 ± 66.3^{a}	22.4 - 216.0	77.2 ± 42.7^{b}	25.0 - 182.0	85.0 ± 40.8^{b}
GSI	0.4 - 2.9	1.3 ± 0.9^{a}	0.3 - 0.9	0.4 ± 2^{b}	0.3 - 1.0	0.5 ± 0.2^{b}
K	0.4 - 2.9	2.0 ± 0.4^{a}	0.4 - 3.2	1.8 ± 0.6^{a}	1.0 - 3.5	1.9 ± 0.3^{a}

Data expressed as mean ± standard deviation (SD); different letters in the same row indicate statistically significant differences between sections ($p < 0.05$).

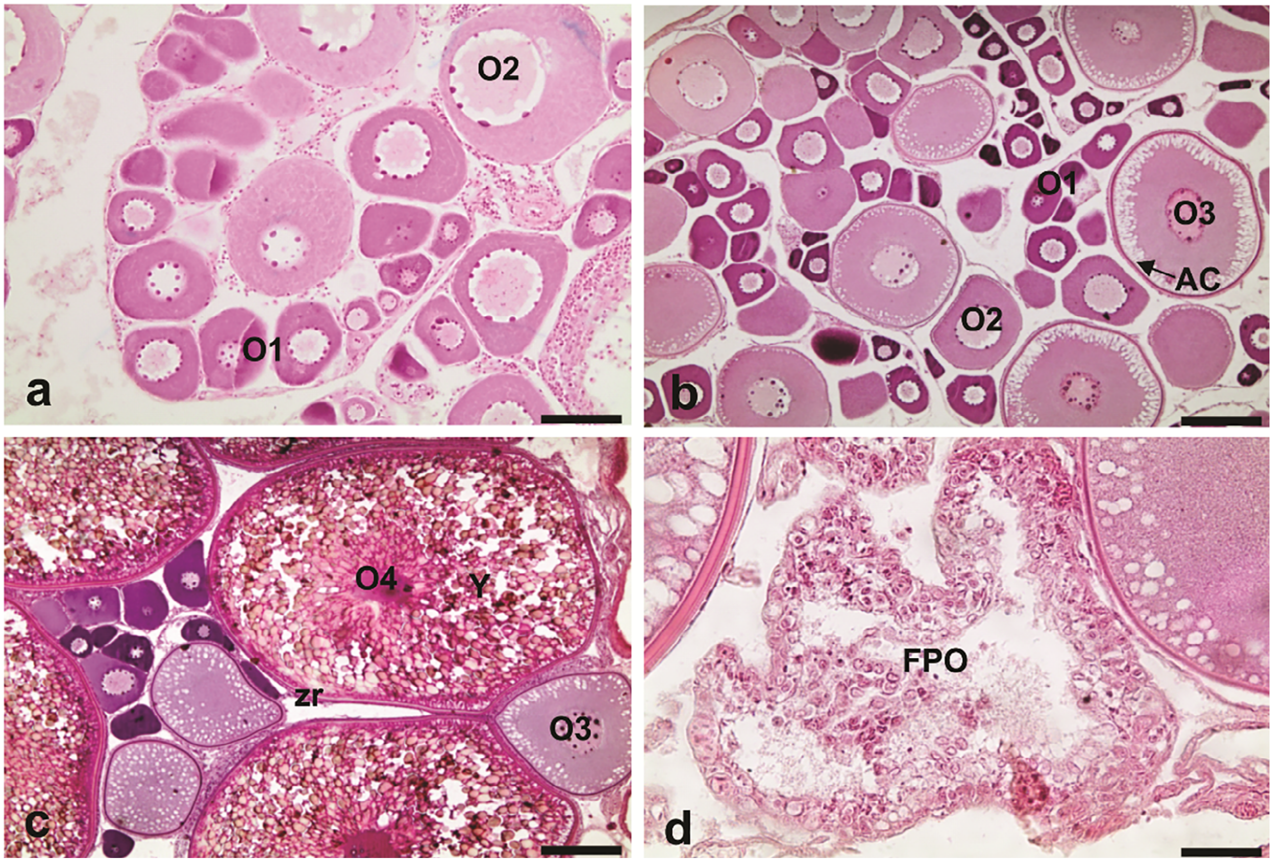


Figure 2. Histological sections of ovaries of *S. brandtii* in different gonadal maturation stages and stained with HE. **a** F1 = resting stage: ovary containing initial (O1) and advanced (O2) perinucleolar follicles. **b** presence of pre-vitellogenic follicles (O3) with cortical alveoli (CA) in a moment of rapid development, which is rare to find in ovarian tissue prior to F2. **c** F2 = maturing/mature stage: ovaries with pre-vitellogenic (O3) and vitellogenic (O4) follicles. **d** F3 = spawned stage: with post-ovulatory follicles (POF). Legend: Vitellogenic follicles (O4) filled with yolk globules (Y), and zona radiata (ZR). **d** F3 = spawned stage: with post-ovulatory follicles (POF). Bars: a, b and c = 200 µm; d = 50 µm.

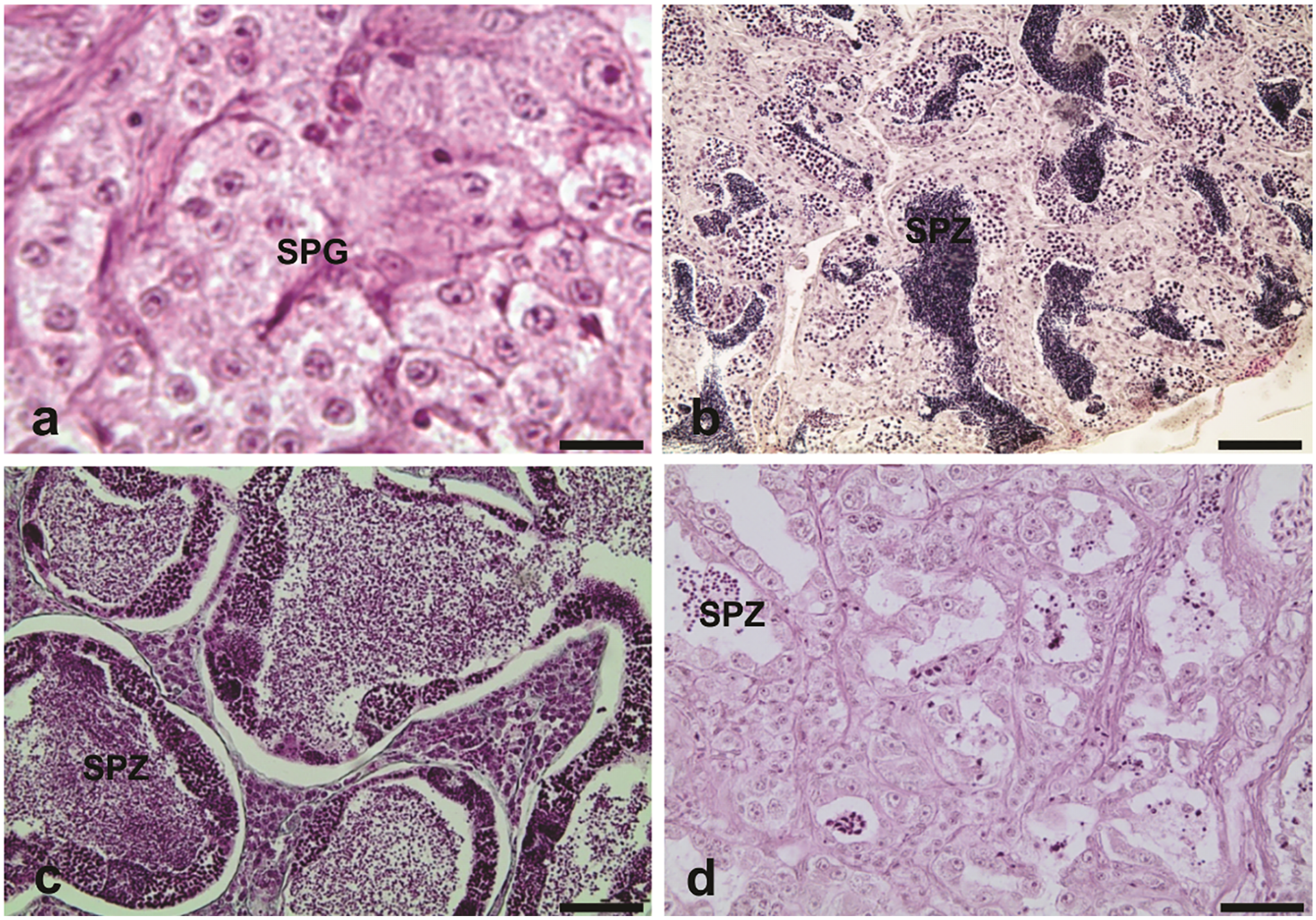


Figure 3. Histological sections of testes of *S. brandtii* in different gonadal maturation stages and stained with HE. **a** M1 = resting stage: containing only spermatogonia (SPG). **b** small amount of spermatozoa (SPZ) in the lumen of the seminiferous tubules in a moment of rapid development, which is rare to find in testis tissue prior to M2. **c** M2 = maturing/mature stage: with seminiferous tubules filled with spermatozoa (SPZ). **d** M3 = spent stage: empty lumen of the seminiferous tubules or with a small amount of residual spermatozoa. Bars: a = 20 µm; b and c = 80 µm; d = 40 µm.

to those that have been reported for other species of Serrassalmidae (Honorato-Samapio et al. 2009) and for fish of other groups (Weber et al., 2013, Normando et al. 2014, Nunes et al. 2015, Brandao et al. 2017).

The GSI can reflect environmental quality since it is directly linked to energy reserves available for breeding (Le Cren, 1951, Yoda & Yoneda 2009, Thomé et al. 2012). The GSI for three sections followed gonad development, with higher values in section 1, thus confirming that *S. brandtii* preferentially favors lentic environments (Braga 1981). The higher GSI values for females caught above the Três Marias dam may be a consequence of the greater number of vitellogenic follicles produced (fecundity) and/or the larger vitellogenic follicles (diameter) for the females of this section.

The highest frequency of spawned females and spent males for the three sections occurred in the bimonthly periods of November/December and January/February, which coincide with high temperatures and rainfall, and longer photoperiods. Indeed, high values for these environmental factors are known to favor the reproductive activity of fish in the Neotropical Region (Lowe McConnel 1987).

The Fulton condition factor (K) provides important information on the physiological state of fish, assuming that individuals with higher K values are in better health (Nikolsky 1963, Vazzoler 1996, Froese 2006). In the present study, the highest K values for females and males were recorded in section 1 where water temperature, and oxygen quality were more favorable.

Histometric analysis of maturing/mature ovaries showed four classes of vitellogenic follicle diameter, indicating asynchronous folliculogenesis with distinct populations of vitellogenic follicles that will be spawned in batches. These findings are consistent with that observed for other teleosts that have fractioned spawning and batch fecundity (Melo et al. 2011, Armstrong & Witthames 2012). Asynchronous folliculogenesis is characteristic of species with long reproductive periods, multiple or fractioned spawning or batch fecundity. As are histological characteristics of spawned females, frequency of maturation stages and variation in the gonadosomatic index (Nikolsky 1963, Núñez & Duponchelle 2009; Lubzens et al. 2010), as is the case for *S. brandtii* of the present study.

The present study found batch fecundity and statistically higher fecundity relative to total length and gonadal weight in section 1, followed by section 3, showing that, as with GSI and vitellogenic follicle diameter, fecundity may be influenced by more favorable reproductive conditions for *S. brandtii*, particularly in section 1. Physical and chemical conditions of water are known to be the main factors influencing reproductive potential, as evidenced by vitellogenic follicle number and diameter (Yoda & Yoneda 2009, Armstrong & Witthames

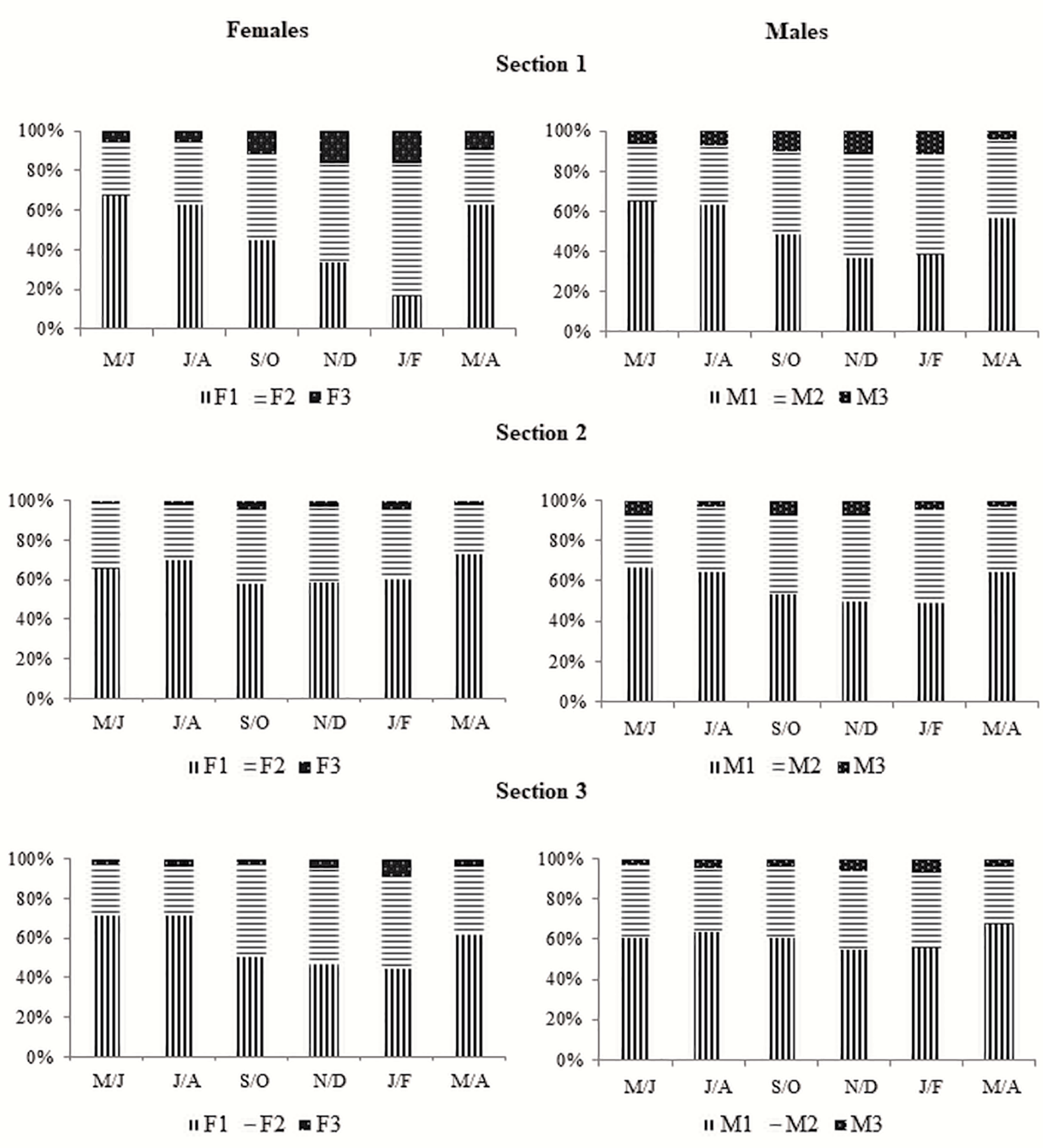


Figure 4. Bimonthly (May-June = M/J; July-August = J/A; September-October = S/O; November-December = N/D; January-February = J/F; March-April = M/A) relative frequency of gonadal maturation stages of female and male *S. brandtii* captured June 2011 to May 2012 in the Três Marias reservoir (section 1), São Francisco River immediately downstream of the Três Marias reservoir (section 2), and after the confluence of the São Francisco River with the Abaeté River (section 3). F1 = resting, F2 = maturation/mature, F3 = spawned, M1 = resting, M2 = maturing/mature, and M3 = spermiated.

2012). In addition to the known effects of the hypothalamic-pituitary axis on reproduction, Jensen et al. (2004), Chakrabarty et al. (2012), and Marcon et al. (2017) found differences in the concentrations of vitellogenin, estradiol and aromatase, and in vitellogenic follicle diameter, in impacted environments, indicating impairment of follicular development. The present results showed that fish in section 2 may be experiencing endocrine alteration in the advanced secondary follicles; however, further investigation into the pathways leading to this change are needed.

Sex ratio may be related to differences between the sexes, selectivity of the sampling apparatus, food availability, and/or population stratification (Carvalho et al. 2009). Furthermore, male parental care of eggs and larvae, a behavior peculiar to piranhas and pirambebas (Braga 1981, Agostinho 2003), make them less readily captured by gill nets. On the other hand, a 1:1 sex ratio has been reported for the piranha *Pygocentrus piraya* in the Três Marias Reservoir (Cruz et al., 1996, Ferreira et al., 1996). In general, the present study sampled slightly more females than males in the three studied sections of the São Francisco River Basin.

The results of the present study showed that *S. brandtii*, a sedentary species, reproduces in all three of the studied sections of the São Francisco River basin, although with lower reproductive performance in section 2, which is immediately downstream of the Três Marias dam and where fish had lower values for TL, BW, GSI, K, FD, AF, and RF. These findings are probably related to the thermal disturbances caused by the release of cooler water from the hypolimnion of the reservoir since tropical fish decrease their feeding activity under low temperatures, which negatively affects their health (Lowe McConnel 1987). Indeed fish are able to find favorable conditions for reproduction by moving away from dams, such as in section 3, thus confirming the negative impact of dams on fish reproduction downstream of reservoirs of hydroelectric power plants.

Acknowledgments

The authors thank the partnership of Companhia Energética de Minas Gerais (CEMIG-GT)/Companhia de Desenvolvimento dos Vales do São Francisco e do Parnaíba (CODEVASF) and the Brazilian research foundations of Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação de Amparo a Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Author Contributions

Nilo Bazzoli: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation.

Viviane Elizabeth de Souza Silva: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation.

Lucas Marcon: Substantial contribution in the concept and design of the study. Contribution to data analysis and interpretation. Contribution to manuscript preparation.

Kleber Biana Santiago: contribution to fishes collection in the three sections.

José Enemir dos Santos: Contribution to data collection.

Elizete Rizzo: Contribution to critical revision, adding intelectual content.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

AGOSTINHO, C.S. 2003. Reproductive aspects of piranhas *Serrasalmus spilopeura* and *Serrasalmus marginatus* into the upper Paraná River. Braz. J. Biol. 63: 1-6. http://dx.doi.org/10.1590/S1519-69842003000100002.

AGOSTINHO, A.A., PELICICE, F.M. & GOMES, L.C. 2008. Dams and the fish fauna of the Neotropical region: impacts and management related to diversity and fisheries. Braz. J. Biol. 68: 1119-1132. http://dx.doi.org/10.1590/S1519-69842008000500019.

ARANTES, F.P., SANTOS H.B., RIZZO, E., SATO, Y. & BAZZOLI, N. 2010. Profiles of sex steroids, fecundity, and spawning of the curimatã-pacu *Prochilodus argenteus* in the São Francisco River, downstream from the Três Marias Dam, Southeastern Brazil. Anim. Reprod. Sci. 118: 330-336. doi: 10.1016/j.anireprosci.2009.07.004.

ARMSTRONG, M.J. & WITTHAMES, P.R. 2012. Developments in understanding of fecundity of fish stocks in relation to egg production methods for estimating spawning stock biomass. Fish. Res. 118: 35-47. https://doi.org/10.1016/j.fishres.2010.12.028.

BLÁZQUEZ, M., BOSMA, P.T., FRASER, E.J., VAN LOOK, K.J. & TRUDEAU, V.L. 1998. Fish as models for the neuroendocrine regulation of reproduction and growth. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.119: 345-364. https://doi.org/10.1016/S0742-8413(98)00023-1.

BONCOMPAGNI-JÚNIOR, O., NORMANDO, F.T., BRITO, M.F.G. & BAZZOLI, N. 2013. Reproductive biology of *Prochilodus argenteus* Agassiz., 1829 (Pisces: Prochilodontidae) in São Francisco River, Brazil. J. Appl. Ichthyol. 29: 132-138. https://doi.org/10.1111/jai.12018.

BRAGA, A. R. 1981. Ecologia e etologia das piranhas do nordeste do Brasil (Pisces – Serrasalmus Lacépede, 1803). Fortaleza, p. 268.

BRANDÃO, D.E.L., VIRTUDE, A.N., MARCON, L., SANTOS, J.E., SANTIAGO, K.B., RIZZO, E. & BAZZOLI, N. 2017. Comparative analyses of reproductive activity in *Schizodon knerii* (Steindachner, 1875) (Characiformes: Anostomidae) in three sections of the São Francisco River basin. J. Appl. Ichthyol. 33: 1118-1124. https://doi.org/10.1111/jai.13457.

CHAKRABARTY, S., RAJAKUMAR, A., RAGHUVEER, K., SRIDEVI, P., MOHANACHARY, A., PRATHIBHA, Y., BASHYAM, L., DUTTA-GUPTA, A. & SENTHILKUMARAN, B. 2012. Endosulfan and flutamide, alone and in combination, target ovarian growth in juvenile catfish, *Clarias batrachus*. Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol. 155: 491-497. doi: 10.1016/j.cbpc.2011.12.007.

CLARKSON, R.W. & CHILDS, M.R. 2000. Temperature Effects of Hypolimnial-Release Dams on Early Life Stages of Colorado River Basin Big-River Fishes. Copeia. 2000: 402-412. http://www.jstor.org/stable/1448187.

CRUZ, A.M.G., SATO, Y., RIZZO, E., SANTOS, G.B. & BAZZOLI, N. 1996. Sexual maturation of piranha *Pygocentrus piraya* (Pisces, Characidae) from Três Marias Reservoir, Minas Gerais. Bios 4: 17-21.

DONALDSON, M.R., COOKE, S.J., PATTERSON, D.A. & MACDONALD, J.S. 2008. Cold shock and fish. J. Fish Biol. 73: 1491-1530. https://doi.org/10.1111/j.1095-8649.2008.02061.x.

FERREIRA, R.M.A., BAZZOLI, N., RIZZO, E. & SATO, Y. 1996. Aspectos reprodutivos da piranha, *Pygocentrus piraya* (Teleostei, Characiformes), espécie nativa da bacia do Rio São Francisco. Arq. Bras. Med. Vet. Zoo. 48: 71-76.

FROESE, B.R. 2006. Cube law, condition factor and weight – length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22: 241-253. https://doi.org/10.1111/j.1439-0426.2006.00805.x.

JÉGU, M. 2003. Subfamilia Serrasalmidae. In: Reis, R.E., Kullander, S.V., Ferraris Jr, C.J. (Eds). Check list of the freshwater fishes of South and Central America. Porto Alegre: EDIPUCRS.

JENSEN, K.M., KAHL, M.D., MAKYNEN, E.A., KORTE, J.J., LEINO, R.L., BUTTERWORTH, B.C. & ANKLEY, G.T. 2004. Characterization of responses to the antiandrogen flutamide in a short-term reproduction assay with the fathead minnow. Aquat. Toxicol. 70: 99-110. https://doi: 10.1016/j.aquatox.2004.06.012.

HONORATO-SAMPAIO, K., PRADO, P.S., SATO, Y. & BAZZOLI, N. 2015. Comparative morphology of the oocyte surface and early development in four characiformes from the São Francisco River, Brazil. J. Morphol. 276: 1258-1278. doi: 10.1002/jmor.20416.

HONORATO-SAMPAIO, K., SANTOS, G.B., BAZZOLI, N. & RIZZO, E. 2009. Observations on the seasonal breeding biology and fine structure of the egg surface in the white piranha *Serrasalmus brandtii* from the São Francisco River basin, Brazil. J. Fish Biol. 75(7): 1874-1882. doi: 10.1111/j.1095-8649.2009.02422.x.

LE CREN, E.D. 1951: The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). J. Anim. Ecol. 20:201-219.

LOWE-MCCONNELL, R.H. 1987. Respostas dos Peixes às Condições em Águas Tropicais. In: Lowe-McConnell RH. Ecological Studies in Tropical. Cambridge University Press, London.

LUBZENS, E., YOUNG, G., BOBE, J., & CERDÀ, J. 2010. Oogenesis in teleosts: how eggs are formed. Gen. Comp. Endocrinol. 165: 367-89. doi: 10.1016/j.ygcen.2009.05.022.

MARCON, L., THOMÉ, R.G., MOUNTEER, A.H., BAZZOLI, N., RIZZO, E. & BENJAMIN, L.A. 2017. Immunohistochemical, morphological and histometrical analyses of follicular development in *Astyanax bimaculatus* (Teleostei: Characidae) exposed to an organochlorine insecticide. Ecotoxicol. Environ. Saf. 143: 249-258. doi: 10.1016/j.ecoenv.2017.05.029.

MELO, R.M.C., FERREIRA, C.M., LUZ, R.K., SATO, Y., RIZZO, E. & BAZZOLI, N. 2011. Comparative oocyte morphology and fecundity of five characid species from São Francisco River basin, Brazil. J. Appl. Ichthyol. 27: 1332-1336. https://doi.org/10.1111/j.1439-0426.2011.01876.x.

MIRANDE, J.M. 2010. Phylogeny of the family characidae (Teleostei: Characiformes): from characters to taxonomy. Neotrop. Ichthyol. 8: 385-568. http://dx.doi.org/10.1590/S1679-62252010000300001.

NIKOLSKY, G.V. 1963. The Ecology of Fishes. Academic Press, London, 352 pp.

NORMANDO, F.T., SANTIAGO, K.B., GOMES, M. V. T., RIZZO, E. & BAZZOLI, N. 2014. Impact of the Três Marias dam on the reproduction of the forage fish *Astyanax bimaculatu* and *A. fasciatus* from the São Francisco River, downstream from the dam, southeastern Brazil. Environ. Biol. Fish. 97: 309-319. https://doi.org/10.1007/s10641-013-0153-3.

NUNES, D.M.F., MAGALHÃES, A.L.B., WEBER, A.A., GOMES, R.Z., NORMANDO, F.T., SANTIAGO, K.B., RIZZO, E. & BAZZOLI, N. 2015. Influence of a large dam and importance of an undammed tributary on the reproductive ecology of the threatened fish matrinxã *Brycon orthotaenia* Günther, 1864 (Characiformes: Bryconidae) in southeastern Brazil. Neotrop. Ichthyol. 13: 317-324. doi: 10.1590/1982-0224-20140084.

NÚÑEZ, J. & DUPONCHELLE, F. 2009. Towards a universal scale to assess sexual maturation and related life history traits in oviparous teleost fishes. Fish Physiol. Biochem. 35: 167-80. doi: 10.1007/s10695-008-9241-2.

OLDEN, J.D. & NAIMAN, R.J. 2010. Incorporating thermal regimes into environmental flows assessments: modifying dam operations to restore freshwater ecosystem integrity. Freshw. Biol. 55: 86-107. https://doi.org/10.1111/j.1365-2427.2009.02179.x.

PAWSON, M.G., PICKETT, G.D. & WITTHAMES, P.R. 2000. The influence of temperature onset of first maturity in sea bass. J. Fish Biol. 56: 319-327. https://doi.org/10.1111/j.1095-8649.2000.tb02109.x.

SANTOS, H.A., POMPEU, P.S. & KENJI, D.O.L. 2012. Changes in the flood regime of São Francisco River (Brazil) from 1940 to 2006. Eg. Environ. Change. 12: 123-132. https://doi.org/10.1007/s10113-011-0240-y.

SATO, Y., BAZZOLI, N., RIZZO, E., BOSCHI, M.B. & MIRANDA, M.O.T. 2005. Influence of the Abaeté River on the reproductive success of the neotropical migratory teleost *Prochilodus argenteus* in the São Francisco River, downstream from the Três Marias Dam, southeastern Brazil. River Res. Appl. 21: 939-950. https://doi.org/10.1002/rra.859.

THOMÉ, R.G., DOMINGOS, F.F.T., SANTOS, H.B., MARTINELLI, P.M., SATO, Y., RIZZO, E. & BAZZOLI, N. 2012. Apoptosis, cell proliferation and vitellogenesis during the folliculogenesis and follicular growth in teleost fish. Tissue Cell. 44: 54-62. doi: 10.1016/j.tice.2011.11.002.

TODD, C. R., RYAN, T., NICOL, S. J. & BEARLIN, R. A. 2005. The impact of cold water releases on the critical period of post-spawning survival and its implications for Murray cod (*Maccullochella peelii peelii*): a case study of the Mitta River, southeastern Australia. River Res. Appl. 21: 1035-1052. https://doi.org/10.1002/rra.873.

VAZZOLER, A.E. 1996. Biologia da reprodução de peixes teleósteos: teoria e prática. EDUEM, Maringá, 169 pp. (in Portuguese).

WEBER, A.A., NUNES, D.M.F., GOMES, R.Z., RIZZO, E., SANTIAGO, K.B. & BAZZOLI, N. 2013. Downstream impacts of a dam and influence of a tributary on the reproductive success of *Leporinus reinhardti* in São Francisco River. Aquat. Biol. 19: 195-200. https://doi.org/10.3354/ab00531.

YODA, M. & YONEDA, M. 2009. Assessment of reproductive potential in multiple-spawning fish with indeterminate fecundity: a case study of yellow sea bream *Dentex hypselosomus* in the East China Sea. J. Fish Biol. 74: 2338-2354. doi: 10.1111/j.1095-8649.2009.02246.x.

Received: 07/05/2018
Revised: 16/01/2019
Accepted: 11/02/2019

Biota Neotropica 19(2): e20180685, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Stream macroalgal flora from Parnaíba River Basin, Brazil: reducing Wallacean shortfall

Marina Ramos Auricchio[1]*, Richard Wilander Lambrecht*[2] *& Cleto Kaveski Peres*[1]*

[1]*Universidade Federal da Integração Latino-Americana, Instituto Latino-Americano de Ciências da Vida e da Natureza, Foz do Iguaçu, PR, Brasil.*
[2]*Instituto de Botânica, Núcleo de Pesquisa em Ecologia, São Paulo, SP, Brasil.*
**Corresponding author: Cleto Kaveski Peres, e-mail: cletoperes@yahoo.com.br*

AURICCHIO, M. R., LAMBRECHT, R. W., PERES, C. K. **Stream macroalgal flora from Parnaíba River Basin, Brazil: reducing Wallacean shortfall**. Biota Neotropica. 19(2): e20180685. http://dx.doi.org/10.1590/1676-0611-BN-2018-0685

Abstract: The global biodiversity loss is a consensus. The biodiversity conservations shortfalls make conservation of biological diversity even more challenging. For many taxa, the knowledge about their distributions is deficient, and this is called the Wallacean shortfall. This situation is no different within algae biodiversity, especially in Brazil. There is still an enormous inequity of sample effort, as is the case of the Parnaíba River Basin (Northeast region), which had only 10 algal species in published (the lowest number of algal species reported among the main Brazilian basins). The present work had the objective of increasing the knowledge of algal flora in Brazil by conducting a taxonomic study of the stream macroalgal species of the Parnaíba River Basin. The sampling of macroalgae was carried out in 21 segments of streams from the Middle and Lower Parnaíba Basin, in the Piauí and Maranhão states. Macroalgae were manually removed and preserved in 4% formaldehyde. Environmental characteristics of each segment were measured in order to describe the sampling sites. The taxonomic survey of the macroalgal communities resulted in the identification of 38 taxa in total, of which 32 at a specific level; three vegetative groups; two sporophytic stages of red algae and one unidentified species. Among the 38 species recorded, 37 are new records for the Parnaíba River Basin. Following the same pattern, 23 species are new records for the Brazilian Northeast region, and *Microcoleus lacustris* represented the first report in Brazil. Despite the fact that it remains the Brazilian basin with the lowest number of algal species documented, this study contributed to the increase of almost five times the number of species sampled in the Parnaíba River Basin (from only 10 to 47 species). These data reinforce that the differences presented in algal diversity in the Brazilian regions are more related to the sampling effort than other factors (e.g. environmental characteristics, geographic distribution, biomes, among others). In addition, this survey illustrates not only the group's lack of information in the region but also shows the importance of this type of study as a tool for expanding the knowledge about biodiversity and its conservation.
Keywords: *Northeast region, Piauí, Maranhão, filamentous algae, taxonomy.*

Flora de macroalgas de riachos da Bacia Hidrográfica do Rio Parnaíba, Brasil: reduzindo o déficit Wallaceano

Resumo: A perda de biodiversidade é um consenso. Os déficits de conservação tornam a conservação da diversidade biológica ainda mais desafiadora. Para muitos táxons, o conhecimento sobre suas distribuições é escasso, o que é chamado de Déficit Wallaceano. Essa situação não é diferente quando tratamos da biodiversidade de algas, especialmente no Brasil. Ainda há uma enorme desigualdade no esforço amostral, como é o caso da Bacia Hidrográfica do Rio Parnaíba (região Nordeste), a qual possui apenas 10 espécies de algas (o menor número de espécies de algas dentre as principais bacias brasileiras). O presente trabalho teve como objetivo aumentar o conhecimento da flora algal no Brasil, através da realização de um estudo taxonômico das espécies de macroalgas de riachos da Bacia do Rio Parnaíba. A amostragem das macroalgas foi realizada em 21 segmentos de riachos das Bacias do Médio e Baixo Parnaíba, nos estados do Piauí e Maranhão. As macroalgas foram removidas manualmente e preservadas em formaldeído 4%. Características ambientais de cada segmento foram tomadas com o intuito de descrever a área de estudo. A pesquisa taxonômica das comunidades de macroalgas resultou na identificação de 38 táxons no total, nos quais 32 em nível específico; três grupos vegetativos; dois estágios esporofíticos de algas vermelhas e uma espécie não identificada. Dentre as 38 espécies, 37 delas são novos registros para a Bacia do

Rio Parnaíba. Seguindo o mesmo padrão, 23 espécies são novos registros para a região Nordeste, e *Microcoleus lacustris* representou o primeiro registro para o Brasil. Apesar do fato de permanecer como a bacia brasileira com o menor número de espécies de algas documentadas, este trabalho contribuiu para o aumento de quase cinco vezes o número de espécies amostradas na Bacia do Rio Parnaíba (de apenas 10 para 47 espécies). Estes dados reforçam que as diferenças apresentadas na diversidade algal nas regiões brasileiras estão mais relacionadas com o esforço amostral do que com outros fatores (e.g. características ambientais, distribuição geográfica, biomas, entre outros). Além disso, este trabalho ilustra não só a falta de informação do grupo na região, mas também mostra a importância deste tipo de estudo como ferramenta para expandir o conhecimento sobre a biodiversidade e conservação.

Palavras-chave: *região Nordeste, Piauí, Maranhão, algas filamentosas, taxonomia.*

Introduction

It is a consensus that global biodiversity is threatened and the species loss is taking place at an accelerated level (Whittaker et al. 2005). The main factors associated with this loss of biodiversity have an anthropogenic origin (Brown & Lomolino 1998). In addition to the anthropic impacts, the Biodiversity Conservations Shortfalls make conservation of biological diversity even more difficult (Mace 2004). For many taxa, the knowledge about their global, regional, or even local distributions is insufficient, a problem that Lomolino (2004) has named the Wallacean shortfall. As a result of this lack of knowledge that many areas of the globe still remain poorly sampled, which for most taxa results in scarce basic information (Whittaker et al. 2005). In contrast, this gap could be circumvented, or at least mitigated, by investing in biodiversity inventories (Whittaker et al. 2005, Bini et al. 2006).

This scenario is no different when the algal biodiversity is approached, especially in Brazil. Despite the total number of described algae for the whole Brazilian territory (4700 species, Flora do Brasil 2020 under construction, 2018) be close to the estimated number of 5600 species in the country (Menezes et al. 2015), no doubt there's still an enormous inequality of sampling effort, with huge regions without any type of surveys. This fact not only makes it impossible for adequate management and the proposal of local conservation strategies but also creates an illusion that different regions present more species richness than others. In terms of Brazilian Hydrographic Basins (sensu Agência Nacional de Águas 2015), most surveys with this algal group cover only two basins: Southeast Atlantic (ASe) and Paraná (Pr). Both basins, according to Menezes et al. (2015), are the richest in number of species in Brazil, reaching values that exceed a thousand species. In contrast, little sampled Hydrographic Basins have a modest number of recorded species, as is the case of the Parnaíba River Basin (Pnb), which has only 10 species of algae (Menezes et al. 2015, Flora of Brazil 2020 under construction, 2018) (Figure 1). Among these species, seven are planktonic microalgae (four euglenoids, two diatoms and one cyanobacteria) and three are filamentous algae (*Chara martiana*, *Cladophora glomerata* and *Hapalosiphon pumilus*).

Particularly for stream macroalgae (sensu Sheath & Cole 1992), the floristic surveys have focused basically only the Southeastern (Branco & Necchi Júnior 1996, Necchi Júnior et al. 1997, Pereira & Branco 2010, Almeida et al. 2011) and Southern regions of Brazil (Krupek et al. 2008, Peres et al. 2008, Branco et al. 2008, 2009, 2011). Although these efforts have contributed profoundly to the knowledge of Brazilian flora, there is a huge gap in the knowledge of the distribution of organisms throughout the territory, thus clearly contributing to the Wallacean shortfall in the group. Studies in different regions and biogeographic provinces are essential, not only providing occurrence information but also allowing an increase of reference material in herbaria. The increase of reference collections constitutes a valuable asset (Pyke and Ehrlich, 2010), also making possible future studies re-evaluating species and taxonomic positions.

In this context, the present investigation had the objective of increasing the knowledge of algal flora in Brazil by conducting a taxonomic study of the stream macroalgae species of the Parnaíba River Basin. This basin comprises a very interesting biogeographic region with the contact of three major Brazilian biomes (Cerrado, Caatinga, Amazônia, and its ecotones) and has a wide land use gradient. Moreover, as this basin presents the lowest number of algal species recorded so far (among the main Brazilian basins), it is considered as of greater relevance and a valuable contribution to the reduction of the Wallacean shortfall.

Material and Methods

The Parnaíba River Basin (Agência Nacional de Águas 2015) is located in the northeastern region of Brazil between latitudes 02º21'S and 11º06'S and longitudes 47º21'W and 39º44'W, encompassing the states of Ceará (4.1% of the total area of the basin), Maranhão (19.8%) and Piauí (75.3%) (MMA 2006). The region is an ecotone between the Cerrado and Caatinga biomes, also influenced by Amazonian and coastal vegetation (MMA 2006). It presents a well defined seasonal water regime (rainy and dry period) with several streams being intermittent (MMA 2006). Most of the selected streams are placed in areas disturbed by the extensive farming use, typical of this region. Nevertheless, some sampled streams are inserted in low disturbed landscapes while others are inserted in very disturbed areas with an urban influence.

The sampling of macroalgae was carried out in 21 segments of streams belonging to Parnaíba River Basin, in the Piauí and Maranhão states (Figure 2, ICMBio License number 58717-3), during a period of drought and after a period of regular precipitation. The selected segments are 1st to 3rd stream order and comprised microbasins in different parts of the Middle and Lower Parnaíba River Basin. The collection and preservation of the algal samples followed the standard procedures described by Necchi Júnior et al. (1991). Each sampling unit consisted of a stream segment of 10 meters long. Using a transparent underwater viewer it was possible to observe and remove macroalgae, which were preserved in 4% formaldehyde. Environmental characteristics were taken in field, such as water temperature (Temp.) using a thermometer Akso™, pH using a pHmeter Akso™, conductivity (Cond.) using a

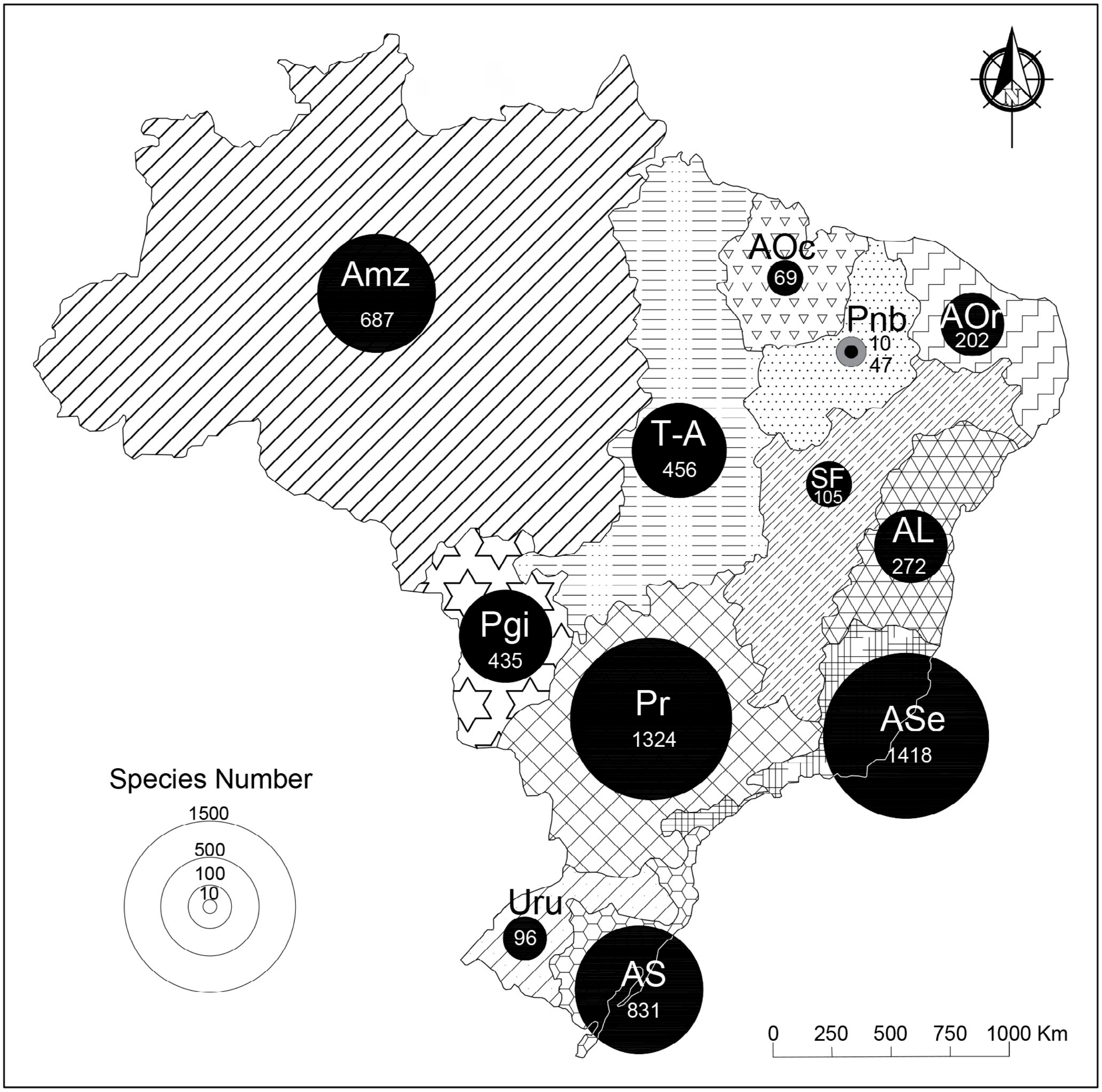


Figure 1. Species richness of epicontinental algae and cyanobacteria for each Brazilian hydrographic regions, with emphasis on the increase of species in the Parnaíba Basin. (Amz - Amazon, AL - East Atlantic, AOc - Occidental Northeast Atlantic, AOr - Oriental Northeast Atlantic, ASe - Southeast Atlantic, AS - South Atlantic, Pgi - Paraguay, Pr - Paraná, Pnb - Parnaíba, SF - São Francisco, T-A - Tocantins-Araguaia, and Uru - Uruguay).

conductivimeter Akso^{TM}, predominant substrate type (Subst.) by visual observation, according Gordon et al. (1992) classification, average current velocity (Vel.) using the floating object method, average width and depth of each segment using a measuring tape. The range and mean of these variables are presented for each species. In addition to these variables, the geographic coordinates and the estimate of incident irradiance (Shading) were taken by canopy photos in each segment, which were analyzed through ImageJ^{TM} (software that allows quantifying pixel area with presence or absence of canopy cover) and expressed as a percentage to describe each sampling site.

The identification of the macroalgae was carried out with a Zeiss trinocular microscope (model AxioLab.A1^{TM}) equipped with an image capture system (AxioCam). Using a software (ZEN), photomicrographs were obtained to perform ten randomized morphometric measurements of each structure of the populations. For the algae with larger structures, the photomicrographs were obtained with a stereomicroscope (model Zeiss Discovery V.12^{TM}). Macroalgae were identified at the specific level whenever possible, and appropriate references were used for each taxonomic group (e.g. Senna & Ferreira 1987, Necchi Júnior 1990, Azevedo et al. 1996, Necchi Junior et al. 2001, Skinner & Entwisle 2001, Branco et al 2002, Kumano 2002, Novis 2004, Bicudo & Menezes 2006,

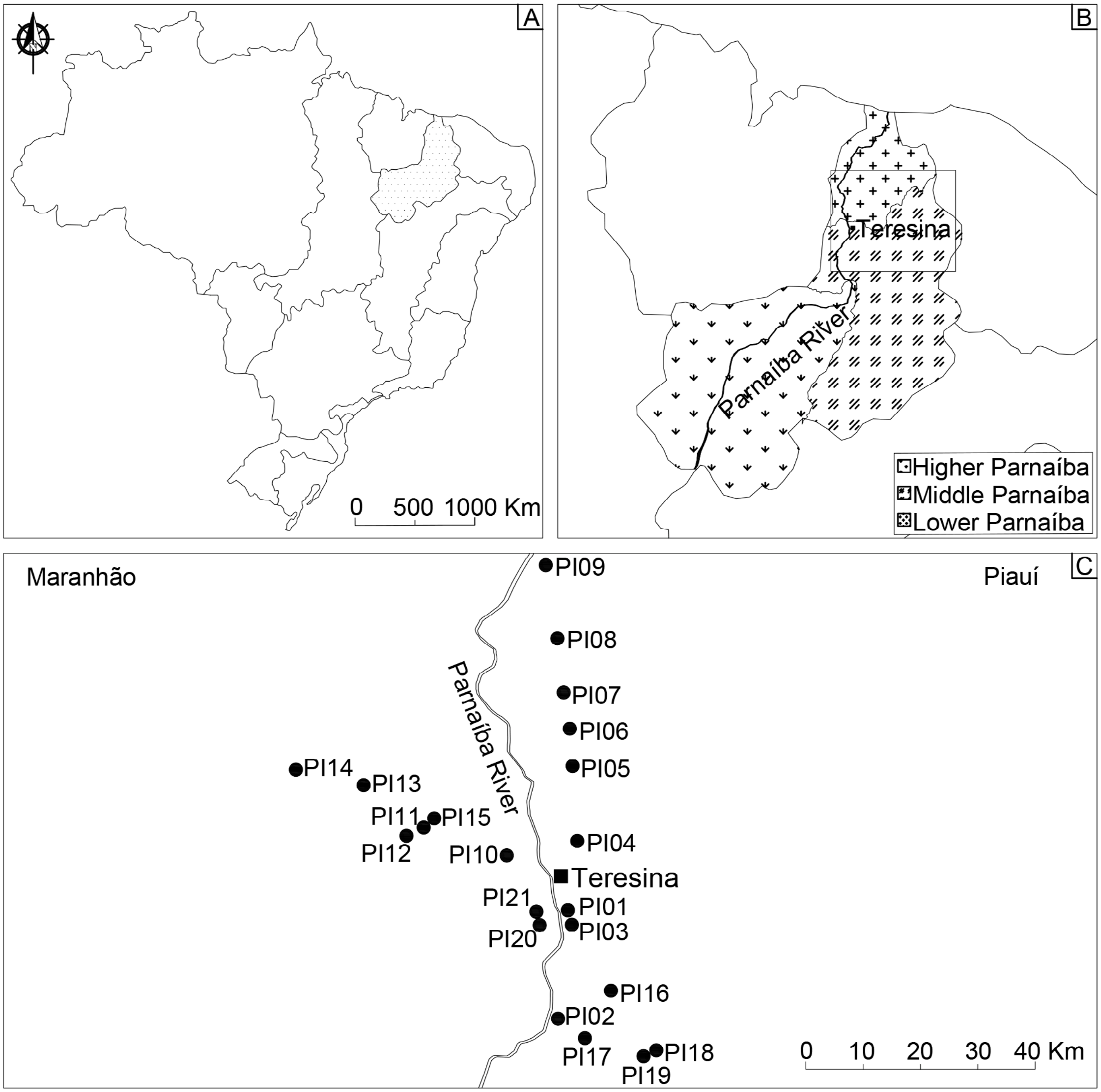


Figure 2. Location map of the Parnaíba basin (A) with its sub-basins and the sampling area (B) showing the distribution of sampling points in relation to Teresina (PI) (C).

Peres & Branco 2014). The samples were kept in formaldehyde 4% and deposited in the Herbarium of the Universidade Estadual do Oeste do Paraná (UNOP). New records of the species are marked before the name of each species, single asterisk (*) represent a new record for the Rio Parnaíba basin, two asterisks (**) mean that the species is a new record for the northeastern region of Brazil, and three asterisks (***) show that the species is a new record for Brazil.

The following informations are presented: i) descriptions of the species; ii) photomicrographs of the main structures; iii) location of the sampling points; iv) environmental information for each taxon and; v) taxonomic comments (when applicable). The classification system was based in Guiry & Guiry (2018).

Results

The taxonomic survey of the macroalgal communities from the Parnaíba River Basin resulted in the identification of 38 taxa in total, of which 32 at a specific level; three vegetative groups of: *Spirogyra*, *Oedogonium* and *Batrachospermum*; two sporophytic stages of red algae: 'Chantransia' *macrospora* and 'Chantransia' *pygmaea* and one unidentified species of the genus *Phormidium*. The best represented taxonomic group was Chlorophyta with 12 taxa (31.6% of the total), followed by Cyanobacteria with 10 taxa (26.3%), Bacillariophyta with nine taxa (23.7%), Rhodophyta with six species (15.8%) and finally Charophyta with only one taxon sampled (2.6%).

PHYLUM BACILLARIOPHYTA

CLASS BACILLARIOPHYCEAE

ORDER EUNOTIALES

1.* *Eunotia didyma* Grunow ex Zimmermann, Broteria, Ser. Bot. 13(2): 51. 1915.

Plate 1. Figure B.

Pseudo-filamentous colonies formed by the union of the frustules by the valvar face; valvar face with biondulate dorsal margin and slightly concave ventral margin, 55.6-75.0 µm long and 10.9-13.4 µm wide; sharp apices and parallel transapical striations; cellular content yellowish-green.

Distribution in the Parnaíba River Basin (n=2): Sites PI-10, PI-15. BRAZIL, MARANHÃO: 05º3’36.68” S and 42º53’38.29” W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5790-1); *idem*, 05º0’36.97” S and 43º1’12.97” W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5801-1).

Environmental conditions (n=2): Temp. 26.9-27.6°C ($\bar{x}$ = 27.2°C); Cond. 49-51 µS.cm^{-1} ($\bar{x}$ = 50 µS.cm^{-1}); pH 6.6-7.3 ($\bar{x}$ = 6.9); Vel. 0.8-1.2 m.s^{-1} ($\bar{x}$ = 1 m.s^{-1}); Depth 11.7-25.2 cm ($\bar{x}$ = 18.4 cm); Width 3.0-12.1 m ($\bar{x}$ = 7.5 m); Subst. bedrock and boulders; Shading 32-86% ($\bar{x}$ = 59%).

Taxonomic comments: the population presented slightly smaller width than described by Costa *et al.* (2017) and Metzeltin & Lange-Bertalot (1998), but polymorphism of the species is widely recognized.

2.** *Eunotia formica* Ehrenberg, Ber. K. Akad. Wiss. Berlin, 414. 1843.

Plate 1. Figure C.

Pseudo-filamentous colonies formed by the union of the frustules by the valvar face; valves slightly arcuate with swollen central region, 51.4-105.0 µm in long and 10.1-11.3 µm wide; cuneate or rounded ends; regularly spaced parallel transapical striae; cellular content yellowish-green.

Distribution in the Parnaíba River Basin (n=3): Sites PI-11, PI-13, PI-15. BRAZIL, MARANHÃO: 05º0’30.99” S and 43º1’2.38” W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5792-1); *idem*, 04º57’4.608” S and 43º7’57.21” W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5794); *idem*, 05º0’36.97” S and 43º1’12.97” W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5801).

Environmental conditions (n=3): Temp. 26.9-27.4°C ($\bar{x}$ = 27.1°C); Cond. 26-49 µS.cm^{-1} ($\bar{x}$ = 40.3 µS.cm^{-1}); pH 5.8-6.9 ($\bar{x}$ = 6.4); Vel. 0.8-1.2 m.s^{-1} ($\bar{x}$ = 0.96 m.s^{-1}); Depth 20.6-26.6 cm ($\bar{x}$ = 24.1 cm); Width 2.4-7.0 m ($\bar{x}$ = 4.1 m); Subst. bedrock and sand; Shading 0-87% ($\bar{x}$ =40%).

3.** *Eunotia meridiana* Metzeltin & Lange-Bertalot, Iconogr. Diatomol., 5: 67-68. 1998.

Plate 1. Figure D.

Pseudo-filamentous colonies formed by the union of the frustules by the valve face; arched valves with convex dorsal margin and slightly concave ventral margin, 22.5-34.6 µm long and 6.1-7.7 µm wide; rounded valvar extremity, non-detached from the valvar body; cellular content olive green.

Distribution in the Parnaíba River Basin (n=1): Site PI-10. BRAZIL, MARANHÃO: 05º3’36.68” S and 42º53’38.29” W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5790).

Environmental conditions (n=1): Temp. 27.6°C; Cond. 51 µS.cm^{-1}; pH 7.3; Vel. 0.8 m.s^{-1}; Depth 11.7 cm; Width 12.1 m; Subst. bedrock; Shading 86%.

4. ***Eunotia transfuga* Metzeltin & Lange-Bertalot, Iconogr. Diatomol. 5: 84-85. 1998.

Plate 1. Figure K.

Pseudo-filamentous colonies of branched chains formed by the union of the ends of the valves; linear, slightly arcuate valves, 175.2-191.1 µm in long and 8.5-11.1 µm in wide, margins with spines; dilated, cuneate-rounded and valvar extremities; parallel transapical striations to slightly irradiated towards the extremities; cellular content yellowish-green, filling approximately the entire cell.

Distribution in the Parnaíba River Basin (n=1): Site PI-13. BRAZIL, MARANHÃO: 04º57’4.608” S and 43º7’57.21” W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5796-1).

Environmental conditions (n=1): Temp. 27.5 °C; Cond. 26 µS.cm^{-1}; pH 5.8; Vel. 0.8 m.s^{-1}; Depth 26.6 cm; Width 7 m; Subst. sand; Shading 87%.

5. ***Eunotia xystriformis* Manguin, Soc. d’Edi. d’Enseig. Supérieur, p. 49. 1952.

Plate 1. Figure L.

Pseudo-filamentous colonies formed by the adhesion of the frustules through the valve face; linear to slightly curved valves, with slightly swollen median portion, 63.9-89.2 µm in long and 9.0-13.4 µm in diam.; sub-capitated rounded or slightly cuneate extremities; parallel transapical striae; cellular content yellowish-green.

Distribution in the Parnaíba River Basin (n=1): Site PI-11. BRAZIL, MARANHÃO: 05º0’30.99” S and 43º1’2.38” W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5792).

Environmental conditions (n=1): Temp. 27.0 °C; Cond. 46 µS.cm^{-1}; pH 6.9; Vel. 0.8 m.s^{-1}; Depth 20.6 cm; Width 2.4 m; Subst. boulders and sand; Shading 0%.

ORDER FRAGILARIALES

1.** *Fragilariforma javanica* (Hustedt) C.E.Wetzel, E.Morales & L.Ector

Plate 1. Figure I-J.

Pseudo-filamentous colonies formed by the diagonal connection of the frustules, forming a zigzag chain, with rare connections by the valve face; linear valves with sub-capitated apices and central region slightly belted, 49.7-62.3 µm long and 5.0-6.9 µm in wide; granulated cell contents yellow-green, filling the whole cell.

Distribution in the Parnaíba River Basin (n=1): Site PI-13. BRAZIL, MARANHÃO: 04º57’4.608” S and 43º7’57.21” W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5796).

Environmental conditions (n=1): Temp. 27.5°C; Cond. 26 µS.cm^{-1}; pH 5.8; Vel. 0.8 m.s^{-1}; Depth 26.6 cm; Width 7.0 m; Subst. sand; Shading 87%.

ORDER NAVICULALES

2.** *Diadesmis confervacea* Kützing, Die Kiesels. Bacil. oder Diatomeen, p. 109. 1844.

Plate 1. Figure E.

Pseudo-filamentous colonies formed by a connection between frustules by the valvar face; lanceolate valves with sharp to slightly rounded ends, 20.2-24.4 µm long and 6.7-9.7 µm in wide; filiform raphe and rounded central nodule; cellular content yellowish-green.

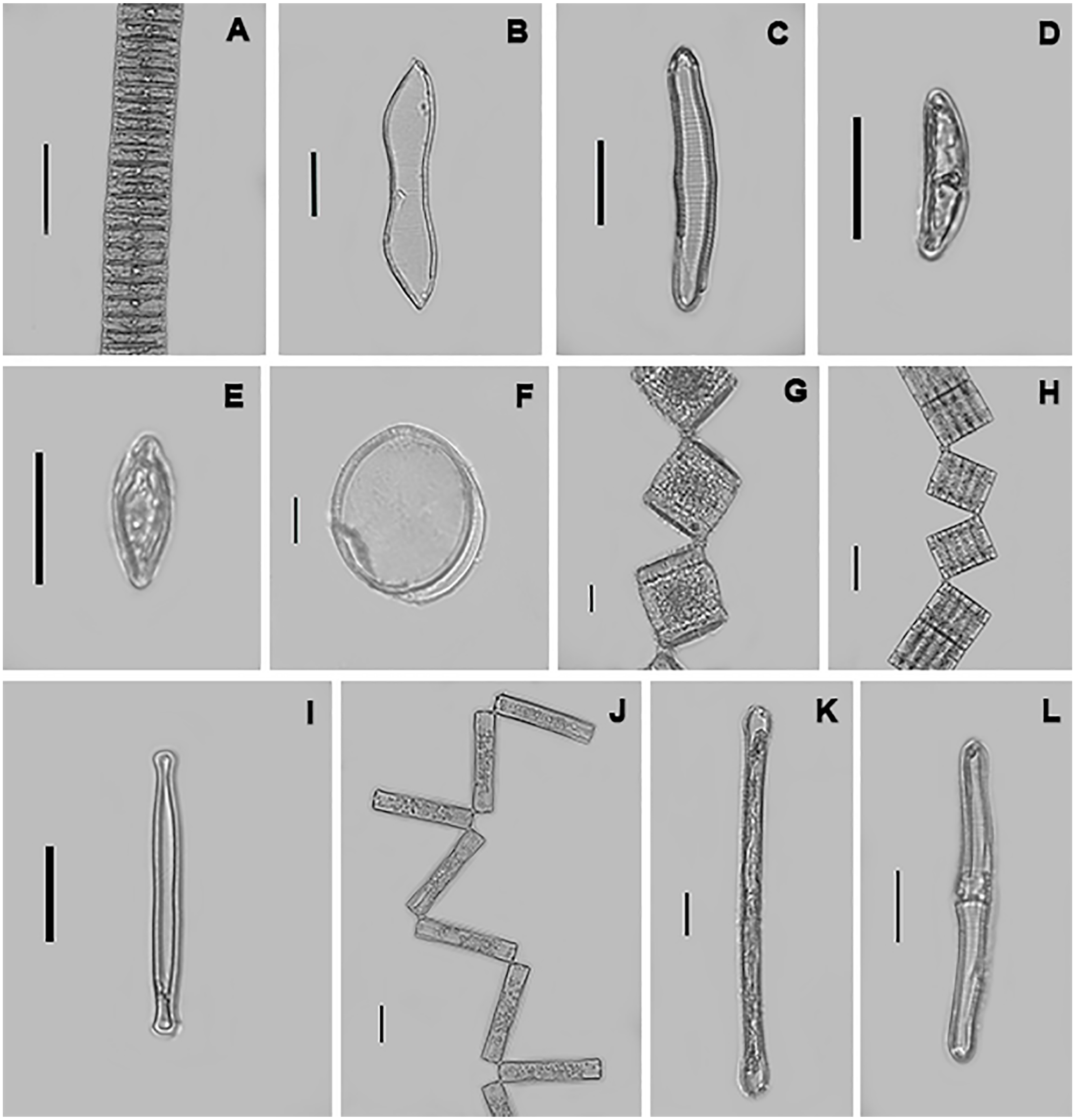


Plate 1. Photomicrographs of the macroalgae species found in the Parnaíba River Basin (PI/MA), Brazil. A. Common view of the *Eunotia* colonies found as macroalgae in this study. B. *Eunotia didyma*; C. *Eunotia formica*; D. *Eunotia meridiana*; E. *Diadesmis cofervacea*; F-G. *Pleurosira laevis*: F. Valvar face showing ocelli; G. General view of the colony; H. *Terpsinoe musica*; I-J. *Fragilariforma javanica*: I. Valvar face; J. General view of the colony; K. *Eunotia transfuga*; L. *Eunotia xystriformis*. Scale bars = 20 µm (Figures B-G, I-L); 100 µm (Figure A, H).

Distribution in the Parnaíba River Basin (n=1): Site PI-02. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5775-2).

Environmental conditions (n=1): Temp. 26.8°; Cond. 321 µS.cm^{-1}; pH 7.8; Vel. 0.5 m.s^{-1}; Depth 5 cm; Width 14.4 m; Subst. bedrock; Shading 63%.

CLASS MEDIOPHYCEAE

ORDER ANAULALES

1.* *Terpsinoe musica* Ehrenberg. Ber. K. Akad. Wiss. Berlin 425. 1843. Plate 1. Figure H.

Pseudo-filamentous colonies forming linear chains by the union of the frustules by the valvar face; valves usually triondulated in valve eyesight, (52.7-) 126.5-136.7 µm long and 28.5-46.7 µm wide; silica bars between the ripples in the form of a musical note; cellular content olive green, filling approximately the entire interior of the cell.

Distribution in the Parnaíba River Basin (n=1): Site PI-02. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5774-1).

Environmental conditions (n=1): Temp. 26.8°; Cond. 321 µS.cm^{-1}; pH 7.8; Vel. 0.5 m.s^{-1}; Depth 5 cm; Width 14.4 m; Subst. bedrock; Shading 63%.

Taxonomic comments: the population showed a great variation in length, being possible the existence of another species of *Terpsinoe* co-occurring in the sample. However, it was not possible to clearly define it using optical microscopy.

ORDER EUPODISCALES

1.* *Pleurosira laevis* (Ehrenberg) Compère, Bacillaria 5: 177. 1982.

Plate 1. Figure F-G.

Basionym: *Biddulphia laevis* Ehrenberg 1843.

Pseudo-filamentous colonies connected by ocelli and forming zigzag chains; cylindrical to subcylindrical frustule; circular to subcircular valves, with a larger diameter of 53.6-76.8 µm and the smaller diameter of 47.6-62.7 µm; cellular content olive green to yellow-green.

Distribution in the Parnaíba River Basin (n=1): Site PI-02. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5774-2).

Environmental conditions (n=1): Temp. 26.8°; Cond. 321 µS.cm^{-1}; pH 7.8; Vel. 0.5 m.s^{-1}; Depth 5 cm; Width 14.4 m; Subst. bedrock; Shading 63%.

PHYLUM CHAROPHYTA
CLASS CHAROPHYCEAE
ORDER CHARALES

1.** *Nitella furcata* (Roxburgh ex Bruzelius) C.Agardh emend., Systema algarum p.124. 1824.

Plate. 2. Figure A-B.

Basionym: *Chara furcata* W.Roxburgh ex A.Bruzelius 1824.

Plants 9-12 cm high; internodes equal to or longer than branchlets, 1-1.6 cm long; 5-6 monomorphic whorled branchlets, 2-4 furcated, 0.9-1.5 cm long; 1-3 dactyls, 1-2 celled, abbreviated or elongated, 241.5-801.7 µm long and 84.4-100.9 µm diam., L/D 4.1-8.5; gametangia sessile, present in first and/or second branchlets furcation; 1-2 nucules, 8 convolutions, 328.6-533.8 µm long and 274.2-407.9 µm diam., L/D 0.9-1.3; globules scarce, 208.9-218.7 µm long and 204.9-214.9 µm diam., L/D 1.0-1.1.

Distribution in the Parnaíba River Basin (n=3): Sites PI-10, PI-14, PI-18. BRAZIL, MARANHÃO: 05º3'36.68" S and 42º53'38.29" W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5786); *idem*, 04º55'41.37" S and 43º14'40.48" W, 98 amsl, 31.V.2017, M.R.Auricchio (UNOP 5798); *idem*, PIAUÍ: 05º22'27.19" S and 42º38'40.05" W, 71 amsl. 01.VI.2017, M.R.Auricchio (UNOP 5815).

Environmental conditions: (n=3): Temp. 27.6-29.4°C ($\bar{x}$ = 28.2°C); Cond. 22-79 µS.cm^{-1} ($\bar{x}$ = 50.6 µS.cm^{-1}); pH 5.8-7.3 ($\bar{x}$ = 6.7); Vel. 0.11-0.8 m.s^{-1} ($\bar{x}$ = 0.3 m.s^{-1}); Depth 11.3-73.5 cm ($\bar{x}$ = 32.1 cm); Width 2.2-12.1 m ($\bar{x}$ = 6.4 m); Subst. bedrock and sand; Shading 62-86% ($\bar{x}$ =78%).

CLASS CONJUGATOPHYCEAE
ORDER ZYGNEMATALES

1.* *Spirogyra* sp.

Plate 3. Figure C.

Free long unbranched filaments, olivaceous, mostly slippery; cylindrical cells, 78.8-242.5 µm long and 31.7-117.4 µm diam., L/D 0.7-7.0; plastids in spiral parietal bands, with two to five bands; zygospores not observed.

Distribution in the Parnaíba River Basin (n=4): Sites PI-02, PI-10, PI-15, PI-17. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5776); *idem*, MARANHÃO: 05º3'36.68" S and 42º53'38.29" W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5789); *idem*, 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5801-2); *idem*, 05º21'20.8" S and 42º45'20.91" W, 59 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5809).

Environmental conditions (n=4): Temp. 26.8-29.1°C ($\bar{x}$ = 27.6°C); Cond. 47-321 µS.cm^{-1} ($\bar{x}$ = 183.5 µS.cm^{-1}); pH 6.6-8.0 ($\bar{x}$ = 7.4); Vel. 0.4-1.2 m.s^{-1} ($\bar{x}$ = 0.7 m.s^{-1}); Depth 5.0-25.2 cm ($\bar{x}$ = 15.6 cm); Width 2.0-14.4 m ($\bar{x}$ = 7.8 m); Subst. bedrock and gravels; Shading 32-83% ($\bar{x}$ = 57.2%).

Taxonomic comments: were found only sterile specimens being impossible to identify them at the specific level.

PHYLUM CHLOROPHYTA
CLASS CHLOROPHYCEAE
ORDER CHAETOPHORALES

1.** *Chaetophora elegans* (Roth) C.Agardh, Dispositio Algarum Sueciae, p. 42. 1812.

Plate: 2. Figure C.

Basionym: *Rivularia elegans* Roth 1802.

Heterotrichous tufts formed by branched filaments immersed in a gelatinous matrix relatively firm, pale green, spherical or hemispherical; basal system composed by prostrate branched filaments with cylindrical cells, producing erect filaments and rhizoids; erect filaments di- or tricotomically branched; lateral branches lax and sparse, fasciculated near the apex; cells of erect system cylindrical, 25.1-63.3 µm long and 4.5-9.1 µm diam., L/D 3.3-9.1; terminal branches densely grouped, tapering at the towards the apex or ending abruptly at an acute apex.

Distribution in the Parnaíba River Basin (n=1): Site PI-14. BRAZIL, PIAUÍ: 04º55'41.37" S and 43º14'40.48" W, 98 amsl, 31.V.2017, M.R.Auricchio (UNOP 5797).

Environmental conditions: (n=1): Temp. 27.8°C; Cond. 22 µS.cm^{-1}; pH 5.8; Vel. 0.1 m.s^{-1}; Depth 73.5 cm; Width 2.2 m; Subst. gravels; Shading 86%.

2.** *Chaetophora pisiformis* (Roth) C.Agardh, Dispositio Algarum Sueciae, p. 43. 1812.

Plate 2. Figure D.

Basionym: *Rivularia pisiformis* Roth 1802.

Heterotrichous tufts formed by branch filaments immersed in a gelatinous matrix relatively firm, pale green, spherical or hemispherical; basal system composed exclusively by rhizoids which arise from basal cells of erect filaments or rarely from median region of filaments; erect filaments di- or tricotomically branched; lateral branches lax and sparse, non fasciculated near the apex; cells of erect system cylindrical, rarely

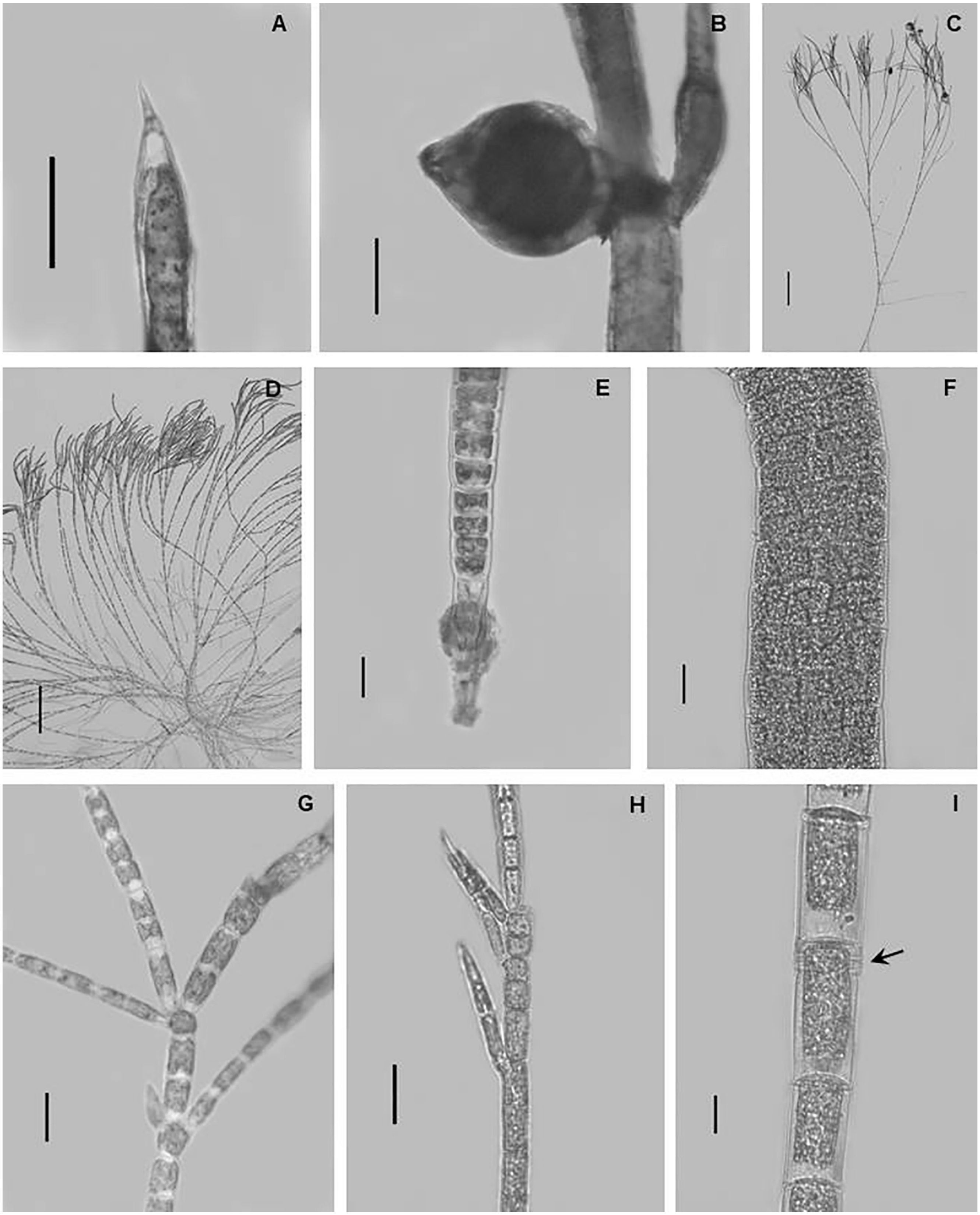


Plate 2. A-B. *Nitella furcata*: A. Branchlet apex (dactyl); B. Gametangia (oogonia); C. *Chaetophora elegans*; D. *Chaetophora pisiformis*; E-F. *Schizomeris leiblenii*: E. Basal cell; F. Mature multiseriate filament; G. *Stigeoclonium amoenum*; H. *Stigeoclonium helveticum*; I. *Oedogonium* sp. with ring-like caps (arrow). Scale bars = 200 µm (Figures A-D); 20 µm (Figures E-I).

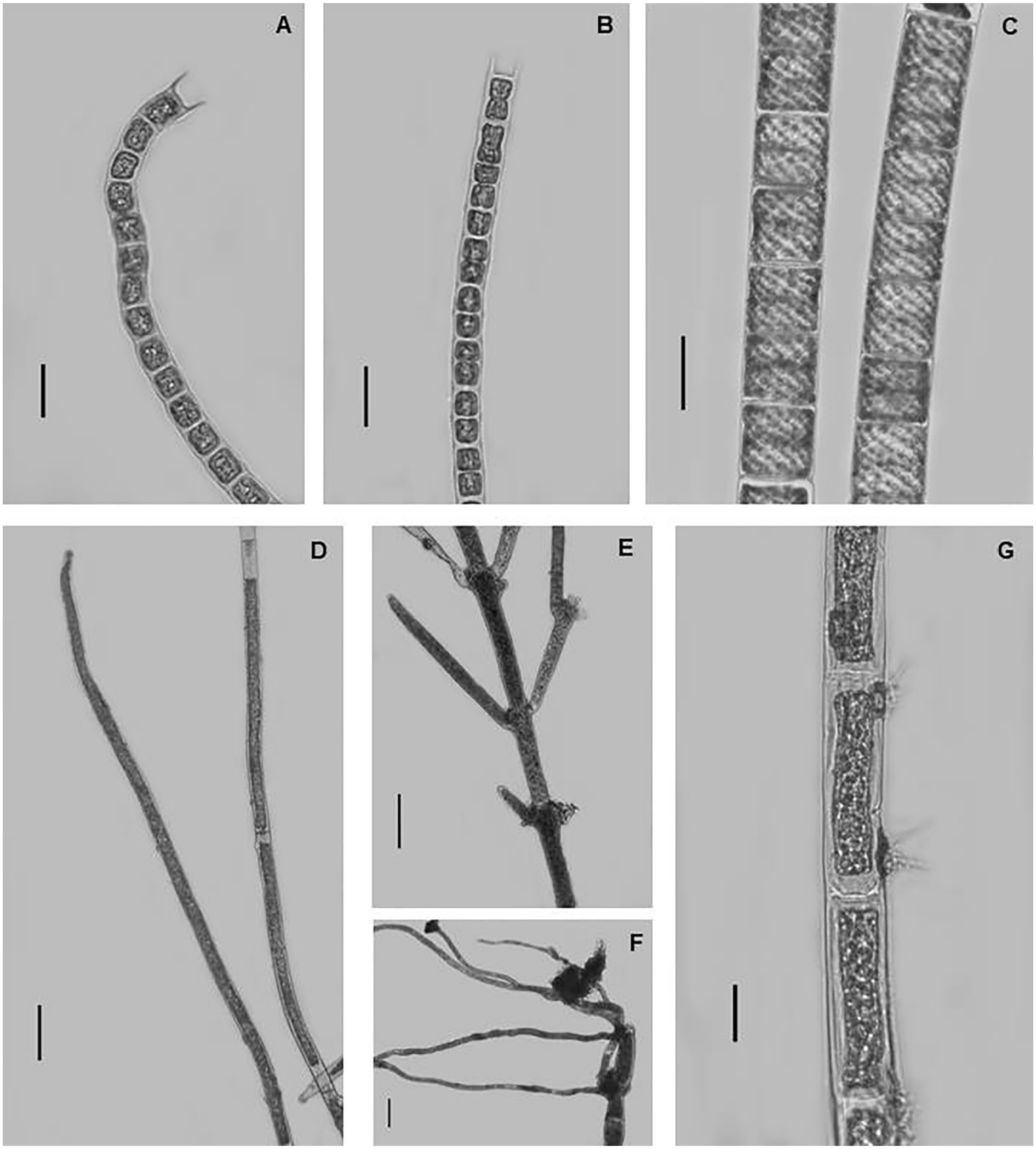


Plate 3. A. *Microspora tumidula*; B. *Microspora willeana*; C. *Spirogyra* sp.; D. *Aegagropilopsis sterrocladia*; E-F. *Cladophora glomerata*: E. Branched filamento; F. Rhizoids; G. *Rhizoclonium hieroglyphicum*. Scale bars = 20 µm (Figures A-B, G); 100 µm (Figure C, E-F); 200µm (Figure D).

inflated 12.9-58.3 µm long and 5.2-11.2 µm diam., L/D 1.9-6.4; branches ending abruptly at an acute or rounded apex.

Distribution in the Parnaíba River Basin (n=3): Sites PI-15, PI-16, PI-18. BRAZIL, MARANHÃO: 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5804); *idem*, PIAUÍ: 05º16'33.73" S and 42º42'53.23" W, 38 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5806); *idem*, 05º22'27.19" and 42º38'40.05" W, 71 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5816).

Environmental conditions: (n=3): Temp. 26.9-29.4°C ($\bar{x}$ = 28°C); Cond. 51-95 µS.cm^{-1} ($\bar{x}$ = 75 µS.cm^{-1}); pH 6.4-7.0 ($\bar{x}$ = 6.7); Vel. 0.1-1.2 m.s^{-1} ($\bar{x}$ = 0.5 m.s^{-1}); Depth 11.3-29.0 cm ($\bar{x}$ = 21.8 cm); Width 2.7-5.1 m ($\bar{x}$ = 3.6 m); Subst. boulders and sand; Shading 32-62% ($\bar{x}$ =47%).

3.** *Schizomeris leibleinii* Kützing, Phycologia Generalis, p. 247. 1843.
Plate: 2. Figures E-F.
Isolated unbranched filaments with slight constrictions in regular interval, olivaceous; basal region attached to substratum by a basal rhizoid-like cell, 28.4-48.3 µm long and 13.4-25.0 µm diam., L/D 1.3-2.8; basal portion uniseriate, cylindrical cells, 5.8-25.9 µm long and 9.9-14.8 µm diam., L/D 0.3-1.0; middle and apical portion multiseriate with quadratic cell in evident rows, 8.3-18.9 µm long and 10.3-31.4 µm diam., L/D 0.4-1.0; apical cell rounded to acute; parietal plastids, forming a band in uniseriate region and perforated in multiseriate region.
Distribution in the Parnaíba River Basin (n=1): Site PI-03. BRAZIL, PIAUÍ: 05º9'2.484"S and 42º48'6.444" W, 59 amsl, 29.V.2017, M.R.Auricchio (UNOP 5777-1).
Environmental conditions (n=1): Temp. 32.3°C; Cond. 432 µS.cm^{-1}; pH 7.3; Vel. 0.4 m.s^{-1}; Depth 6.6 cm; Width 2.1 m; Subst. gravels; Shading 0%.

4.** *Stigeoclonium amoenum* Kützing, Phycologia Germanica, p. 198. 1845.
Plate: 2. Figure G.
Heterotrichous tufts formed by branched filaments, bright green; well developed erect system and reduced prostrate system, composed exclusively of profuse mass of rhizoids emerging from the basal part of erect filaments; main axis differentiated in short and long cells; short cells cylindrical, quadratic to slightly inflated, 4.8-14.0 µm long and 6.5-13.5 µm diam., L/D 0.6-1.2; long cells cylindrical, 6.9-23.8 µm long and 5.4-13.8 µm diam, L/D 0.7-1.5; single, parietal chloroplast; branches alternate or opposite, often with two or three branches from the same short cell, lateral branches formed by cylindrical cells; apex of main branches acuminate with occasional multicellular hyaline hairs.
Distribution in the Parnaíba River Basin (n=1): Site PI-03. BRAZIL, PIAUÍ: 05º9'2.484" S and 42º48'6.444" W, 59 amsl, 29.V.2017, M.R.Auricchio (UNOP 5777).
Environmental conditions (n=1): Temp. 32.3°C; Cond. 432 µS.cm^{-1}; pH 7.3; Vel. 0.4 m.s^{-1}; Depth 6.6 cm; Width 2.1 m; Subst. gravels; Shading 0%.
Taxonomic comments: the morphometric measurements are slightly lower than those described in Branco et al. (2002) to Brazilian streams, however, all morphological characteristics agree with that described for this species.

5.** *Stigeoclonium helveticum* Vischer, Beihefte zum Botanischen Centralblatt 51: 56. 1933.
Plate 2. Figure H.
Heterotrichous tufts formed by branched filaments, bright green; well developed erect system and reduced prostrate system, composed exclusively of profuse mass of short rhizoids emerging from the erect filaments; axial cells cylindrical, similar in shape and size, 5.0-10.6 µm long and 5.8-10.6 µm diam., L/D 0.5-1.3; single parietal chloroplast; alternate branches and rarely opposite or with multiples branches; branch cells, 12.6-28.9 µm long and 4.2-8.0 µm diam., L/D 1.9-6.0; apex of main branches acuminate with occasional multicellular hyaline hairs.
Distribution in the Parnaíba River Basin (n=1): Site PI-21. BRAZIL, MARANHÃO: 05º9'32.76" S and 42º50'16.44" W, 69 amsl, 02.VI.2017, M.R.Auricchio (UNOP 5822).
Environmental conditions (n=1): Temp. 30.0°C; Cond. 48 µS.cm^{-1}; pH 7.3; Vel. 0.05 m.s^{-1}; Depth 21 cm; Width 5.4 m; Subst. sand; Shading 0%.

ORDER OEDOGONIALES
1.** *Oedogonium* sp.
Plate 2. Figure I.
Entangled unbranched filaments, olivaceous to brown; cylindrical cells, slightly constricted in septa, 25.9-174.9 µm long and 10.4-49.3 µm diam., L/D 1.1-4.0; chloroplast parietal, netlike with several pyrenoids; sometimes with ring-like caps near the cross wall; oogonia and antheridia not observed.
Distribution in the Parnaíba River Basin (n=2): Sites PI-10, PI-13. BRAZIL, MARANHÃO: 05º3'36.68" S and 42º53'38.29" W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5790-2); *idem*, 04º57'4.608" S and 43º7'57.21" W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5794-1).
Environmental conditions (n=2): Temp. 27.4-27.6°C ($\bar{x}$ = 27.5°C); Cond. 26-51 µS.cm^{-1} ($\bar{x}$ = 38.5 µS.cm^{-1}); pH 5.8-7.3 ($\bar{x}$ = 6.5); Vel. 0.8 m.s^{-1}; Depth 11.7-26.6 cm ($\bar{x}$ = 19.1 cm); Width 7.0-12.1 m ($\bar{x}$ = 9.5 m); Subst. bedrock and sand; Shading 86-87% ($\bar{x}$ = 86.5%).
Taxonomic comments: were found only sterile specimens, being impossible to identify them at the specific level.

ORDER SPHAEROPLEALES
1.** *Microspora tumidula* Hazen, Memoirs of the Torrey Botanical Club 11: 177. 1902.
Plate 3. Figure A.
Entangled unbranched filaments, constricted in septa with evident H pieces; cylindrical cells, 9.7-15.2 µm long and 8.8-11.2 µm diam., L/D 0.9-1.4; cell wall thin to moderately thick, 0.5-1.0 µm diam.; plastids reticulated, completely surrounding the cell lumen.
Distribution in the Parnaíba River Basin (n=2): Sites PI-12, PI-13. BRAZIL, PIAUÍ: S 05º1'52.82" S and 43º3'35.02" W; 126 amsl. 31.V.2017, M.R.Auricchio (UNOP 5793); *idem*, MARANHÃO: 04º57'4.608" S and 43º7'57.21" W, 82 amsl. 31.V.2017, M.R.Auricchio (UNOP 5795-1).
Environmental conditions (n=2): Temp. 27.4-27.6°C ($\bar{x}$ = 27.5°C); Cond. 24-26 µS.cm^{-1} ($\bar{x}$ = 25 µS.cm^{-1}); pH 5.4-5.8 ($\bar{x}$ = 5.6); Vel. 0.3-0.8 m.s^{-1} ($\bar{x}$ = 0.5 m.s^{-1}); Depth 18.0-26.6 cm ($\bar{x}$ = 22.3 cm); Width 2.3-7.0 m ($\bar{x}$ = 4.6 m); Subst. sand and macrophytes; Shading 84-87% ($\bar{x}$ =85%).

2.** *Microspora willeana* Lagerheim, Berichte der Deutschen Botanischen Gesellschaft 5: 414. 1887.
Plate 3. Figure B.
Entangled unbranched filaments, non or constricted in septa with non-evident H pieces; cylindrical cells, 9.1-178 µm long and 9.3-11.9 µm diam., L/D 0.8-1.8; cell wall thin to moderately thick, 0.5-1.2 µm diam.; plastids in perforated plate, completely surrounding cell lumen.
Distribution in the Parnaíba River Basin (n=4): Sites P-06, PI-07, PI-12, PI-13. BRAZIL, PIAUÍ: 04º50'57.66" S and 42º47'46.89" W, 90 amsl, 30.V.2017, M.R.Auricchio (UNOP 5783); *idem*, 04º47'21.22" S and 42º48'27.18" W, 79 amsl, 30.V.2017, M.R.Auricchio (UNOP 5784); *idem*, MARANHÃO: 05º1'52.82" S and 43º3'35.02" W, 126 amsl, 31.V.2017, M.R.Auricchio (UNOP 5793); *idem*, 04º57'4.608" S and 43º7'57.21" W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5795).
Environmental conditions (n=4): Temp. 27.4-28.7°C ($\bar{x}$ = 28.0 °C); Cond. 24-492 µS.cm^{-1} ($\bar{x}$ = 192 µS.cm^{-1}); pH 5.4-6.1 ($\bar{x}$ = 5.8); Vel.

0.1-0.8 m.s^{-1} ($\bar{x}$ = 0.4 m.s^{-1}); Depth 5.2-26.6 cm ($\bar{x}$ = 14.0 cm); Width 0.4-7.0 m ($\bar{x}$ = 3.5 m); Subst. sand and macrophytes; Shading 18-88% ($\bar{x}$ = 69%).

CLASS ULVOPHYCEAE

ORDER CLADOPHORALES

1.** *Aegagropilopsis sterrocladia* (Skuja) Boedeker, Journal of Phycology 48(3): 822. 2012.

Plate 3. Figure D.

Basionym: *Cladophora sterrocladia* Skuja 1949.

Tufts of long branched filaments, olivaceous; multinucleate cells; fixed in substrate by primary or secondary rhizoids; principal axis poorly branched, only primary branches, elongated, generally unilateral; lateral branches insert in apical or slightly subterminal cellular pole; axis cells cylindrical, 363.3-1194.9 µm long and 49.7-63.1 µm diam., L/D 6.0-18.9; apical cells very longer than axis cells, 1261.2-3289.9 long and 52.3-58.7 diam., L/D 22.7-60.2; zoosporangia not observed.

Distribution in the Parnaíba River Basin (n=1): Site PI-10. BRAZIL, MARANHÃO: 05º3'36.68" S and 42º53'38.29" W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5788).

Environmental conditions (n=1): Temp. 27.6°C; Cond. 51 µS.cm^{-1}; pH 7.3; Vel. 0.8 m.s^{-1}; Depth 11.7 cm; Width 12.1 m; Subst. bedrock; Shading 86%.

Taxonomic comments: the diameter of the cells was slightly narrower and the apical cell slightly larger than that described by Peres & Branco (2014, as *Cladophora sterrocladia*) (axis cell diameter: 61.0-137.7 µm; apical cell length: 778.0-2045.3 µm [-2413.0 µm], apical cell width: 62.6-109.9 µm) for the Brazilian material.

2. *Cladophora glomerata* (Linnaeus) Kützing, Phycologia generalis oder Anatomie, Physiologie und Systemkunde der Tange: 266. 1843.

Plate 3. Figure E-F.

Basionym: *Conferva glomerata* Linnaeus 1753.

Tufts of branched filaments, brownish; multinucleate cells; profusely branched, branches inserted laterally or obliquely in apical portion of cell, alternated, unilateral or opposite; axial cells cylindrical, 164.0-412.8 µm long and 27.7-41.6 µm diam., L/D 5.5-12.8; apical cells longer than axis cells, 87.8–1660.3 µm long and 26.5-38.9 µm diam., L/D 14,3-42,6.

Distribution in the Parnaíba River Basin (n=2): Sites PI-02, PI-17. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5772); *idem*, 05º21'20.8" S and 42º45'20.91" W, 59 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5813).

Environmental conditions (n=2): Temp. 26.8-29.1°C ($\bar{x}$ = 27.9°C); Cond. 312-321 µS.cm^{-1} ($\bar{x}$ = 316 µS.cm^{-1}); pH 7.8-8.0 ($\bar{x}$ = 7.9); Vel. 0.4-0.5 m.s^{-1} ($\bar{x}$ = 0.5 m.s^{-1}); Depth 5.0-20.6 cm ($\bar{x}$ = 12.8 cm); Width 2.0-14.4 m ($\bar{x}$ = 8.2 m); Subst. bedrock and boulders; Shading 48-63% ($\bar{x}$ = 55%).

3.** *Rhizoclonium hieroglyphicum* (C.Agardh) Kützing, Phycologia germanica: 206. 1845.

Plate 3. Figure G.

Basionym: *Conferva hieroglyphica* C.Agardh 1827.

Long unbranched filaments, flexuous; multinucleate cells; cylindrical cells, 150.0-289.0 µm long and 85.3-95.7 µm diam., L/D 1.6-3.1, cell wall narrow to thick and stratified, 2.8-4.0 µm diam.; plastids net-like with several pyrenoids; sporangia not observed.

Distribution in the Parnaíba River Basin (n=1): Site PI-17. BRAZIL, PIAUÍ: 05º21'20.8" S and 42º45'20.91" W, 59 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5810).

Environmental conditions (n=1): Temp. 29.1°C; Cond. 312 µS.cm^{-1}; pH 8.0; Vel. 0.4 m.s^{-1}; Depth 20.6 cm; Width 2 m; Subst. boulders, pebbles and sand; Shading 48%.

PHYLUM CYANOPHYTA

CLASS CYANOPHYCEAE

ORDER OSCILLATORIALES

1.* *Geitlerinema splendidum* (Greville ex Gomont) Anagnostidis, Plant Syst. And Evolut. 164: 43. 1989.

Plate 4. Figure A.

Basionym: *Oscillatoria splendida* Greville ex Gomont 1892.

Mats of filaments, bluish green; trichomes straight or bent, distinctly attenuated at the apex (with proboscis), unconstricted; granulated septa, one or two granules each side; intercalar cells longer than wide, 3.1-4.4 µm long and 1.3-2.6 µm diam., L/D 1.4-2.4; apical cell capitate, (6.9) 9.7-14.5 µm long and 1.0-2.2 µm diam., L/D (3.6) 6.5-11.3; cellular content homogeneous, blue-green.

Distribution in the Parnaíba River Basin (n=4): Sites PI-11, PI-15, PI-16, PI-19. BRAZIL, MARANHÃO: 05º0'30.99" S and 43º1'2.38" W, 82 amsl, 31.V.2017, M.R.Auricchio (UNOP 5791); *idem*, 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5803); *idem*, PIAUÍ: 05º16'33.73" S and 42º42'53.23" W, 38 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5808-2); *idem*, 05º22'45.37" S and 42º39'1.548 W, 68 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5820).

Environmental conditions (n=4): Temp. 26.9-29.4°C ($\bar{x}$ = 27.7°C); Cond. 46-130 µS.cm^{-1} ($\bar{x}$ = 80 µS.cm^{-1}); pH 6.4-6.9 ($\bar{x}$ = 6.6); Vel. 0.2-1.2 m.s^{-1} ($\bar{x}$ = 0.7 m.s^{-1}); Depth 17.6-29.0 cm ($\bar{x}$ = 23.1 cm); Width 2.1-3.0 m ($\bar{x}$ = 2.5 m); Subst. boulders and sand; Shading 0-48% ($\bar{x}$ = 32%).

2.* *Lyngbya* aff. *majuscula* Harvey ex Gomont, Annales des Sciences Naturelles, Botanique, Série 7 16: 131. 1892.

Plate 4. Figure B.

Mats of filaments, dark yellowish green; thick and hyaline sheath, 3.3-5.7 µm; trichomes cylindrical, 46.4-51.4 µm diam., unconstricted in septa; intercalar cells short, discoids, shorter than long, 3.5-8.3 µm long and 36.6-46.7 µm diam., L/D 0.08-0.1; apical cells rounded, without calyptra, 6.9-10.4 µm long and 30.4-39.0 µm diam., L/D 0.1-0.2; cellular content homogeneous, olivaceous to dark reddish-green.

Distribution in the Parnaíba River Basin (n=1): Site PI-17. BRAZIL, PIAUÍ: 05º21'20.8" S and 42º45'20.91" W, 59 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5811).

Environmental conditions (n=1): Temp. 29.1°C; Cond. 316 µS.cm^{-1}; pH 8.0; Vel. 0.4 m.s^{-1}; Depth 20.6 cm; Width 2.0 m; Subst. boulders; Shading 48%.

Taxonomic comments: According to Komárek & Anagnostidis (2005), *L. majuscula* is a typical species of marine environments. However, the identification of this material is consistent with the description proposed by the same authors. In addition, other authors have already described the species in freshwater environments (e.g. Desikachary 1959) and, for this reason, we maintained the identification. In spite of this, we consider that the environmental variation between freshwater

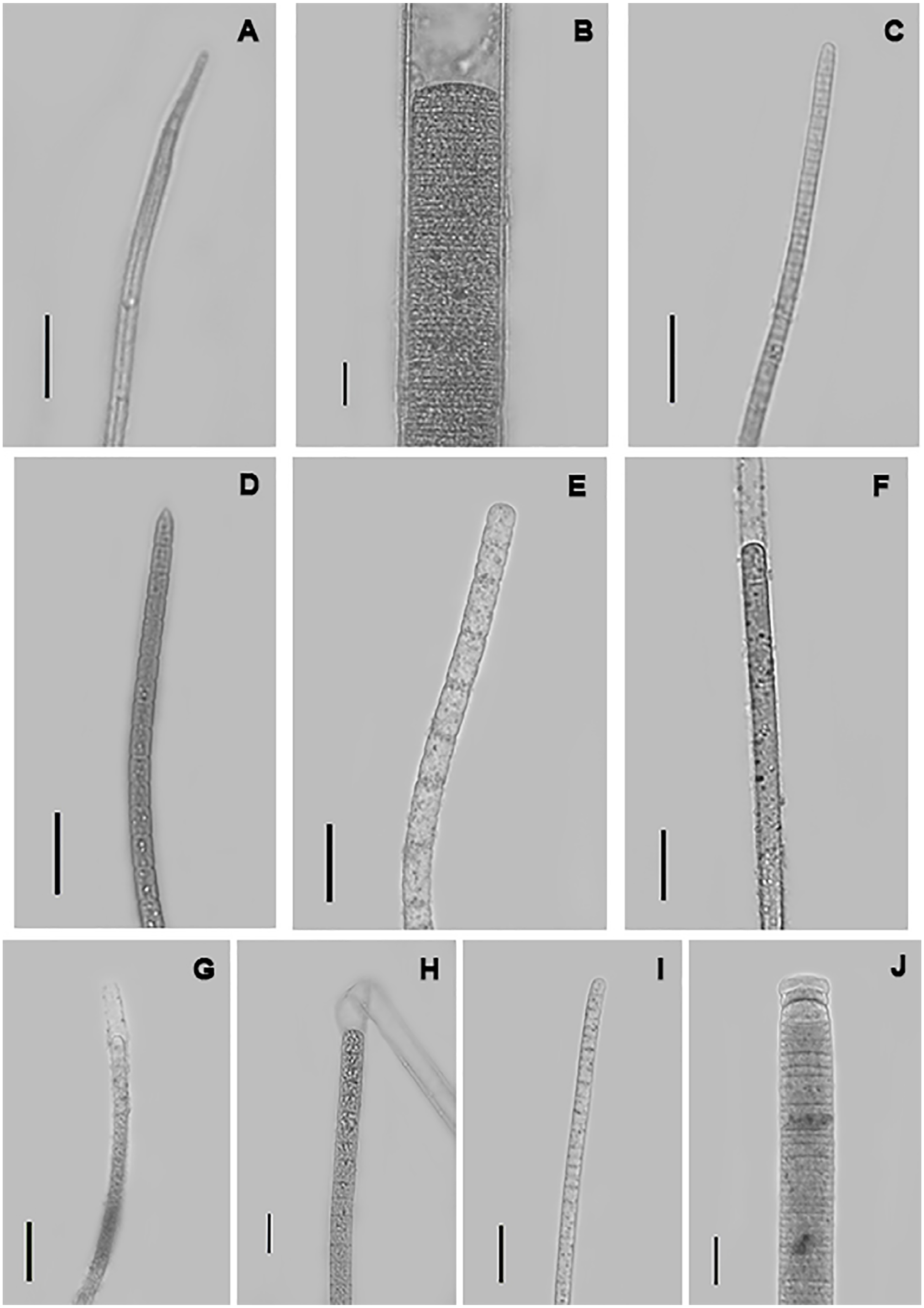


Plate 4. A. *Geitlerinema splendidum*; B. *Lyngbya* aff. *majuscula*; C. *Microcoleus autumnalis*; D. *Microcoleus lacustris*; E. *Phormidium aerugineocaeruleum*; F. *Phormidium retzii*; G. *Phormidium corium*; H. *Phormidium* sp.; I. *Phormidium tergestinum*; J. *Oscillatoria princeps*. Scale bars = 10 µm (Figure A); 20 µm (Figures B-J).

and marine environments probably selects lineages with very different physiological characteristics. Thus, future studies involving molecular biology may clearly support a new taxonomic configuration.

3.** *Microcoleus autumnalis* (Gomont) Strunecky et al., Journal of Phycol. 49(6): 1176. 2013.
Plate 4. Figure C.
Basionym: *Phormidium autumnale* Gomont 1892.
Mats of filaments, greenish blue; trichomes with cellular content homogeneous, unconstricted and granulated septa; intercalar cells wider than long, 1.9-3.8 µm long and 3.4-4.4 µm diam., L/D 0.5-0.9; apical cell rounded to capitate, 3.3-5.9 µm long and 2.4-3.9 µm diam., L/D 0.9-2.0, cellular content homogeneous, blue-green.
Distribution in the Parnaíba River Basin (n=1): Site PI-21. BRAZIL, MARANHÃO: 05º9'32.76" S and 42º50'16.44" W, 69 amsl, 02.VI.2017, M.R.Auricchio (UNOP 5821).
Environmental conditions (n=1): Temp. 30.0°C; Cond. 48 µS.cm^{-1}; pH 7.3; Vel. 0.05 m.s^{-1}; Depth 21.0 cm; Width 5.4 m; Subst. sand; Shading 0%.

4.*** *Microcoleus lacustris* Farlow ex Gomont, Annales des Sciences Naturelles, Botanique, Série 7, 15: 359. 1892.
Plate 4. Figure D.
Mats of filaments, dark greenish blue; filaments formed by one to various trichomes in a wider hyaline sheath; sheath open in apex, with free trichomes; trichomes cylindrical, constricted in septa; intercalar cells granulated, quadratic to longer than wide, 4.1-9.4 µm long and 4.5-6.2 µm diam., L/D 0.8-1.8; apical cells no capitate, conical rounded, 6.2-9.5 µm long and 3.7-5.5 µm diam., L/D 1.4-2.3; cellular content granulated, dark greenish blue.
Distribution in the Parnaíba River Basin (n=1): Site PI-16. BRAZIL, PIAUÍ: 05º16'33.73" and 42º42'53.23" W, 95 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5808).
Environmental conditions (n=1): Temp. 27.8°C; Cond. 95 µS.cm^{-1}; pH 6.4; Vel. 0.1 m.s^{-1}; Depth 29 cm; Width 2,7 m; Subst. gravels and sand; Shading 48%.
Taxonomic comments: the identification is consistent with the one proposed by Komárek & Anagnostidis (2005) for *M. lacustris*, however, in his description, the species has no granulation as reported here.

5.* *Oscillatoria princeps* Vaucher ex Gomont, Annales des Sciences Naturelles, Botanique, Série 7 16: 206. 1892.
Plate 4. Figure J.
Mats of filaments, dark green; sheath absent; trichomes cylindrical, unconstricted; intercalar cells discoids, very wider than long, 2.9-7.5 µm long and 22.2-46.1 µm diam., L/D 0.08-0.2; apical cell truncate, sometimes with a distinct thickening in cell wall, 5.0-10.5 µm long and 20.9-33.4 µm diam., L/D 0.1-0.3; cellular content homogeneous, olivaceous to dark green.
Distribution in the Parnaíba River Basin (n=1): Site PI-04. BRASIL, PIAUÍ: 05º1'58.87" S and 42º46'41.44" W, 43 amsl, 30.V.2017, M.R.Auricchio (UNOP 5780).
Environmental conditions (n=1): Temp. 28.1°C; Cond. 301 µS.cm^{-1}; pH 6.6; Vel. 0.9 m.s^{-1}; Depth 5.4 cm; Width 19.0 m; Subst. bedrock; Shading 31%.

6.** *Phormidium aerugineocaeruleum* (Gomont) Anagnostidis & Komárek, Algolog. Stud., 50-53: 407. 1988.
Plate 4. Figure E.
Basionym: *Lyngbya aerugineo-caerulea* Gomont 1892.
Mats of filaments, greenish blue to dark blue; sheath thin, hyaline, homogeneous, generally absent; trichomes unconstricted in septa or rarely with weak constriction; intercalar cells wider than long, 4.2-6.1 µm long and 5.6-7.4 µm diam., L/D 0.6-0.8; apical cells rounded, 3.3-5.4 µm long and 5.0-6.5 µm diam., L/D 0.5-1.1; cellular content granulated, bright blue-green, granules greenish, small and large.
Distribution in the Parnaíba River Basin (n=1): Site PI-09. BRAZIL, PIAUÍ: 04º34'46.12" S and 42º50'24.72" W, 55 amsl, 30.V.2017, M.R.Auricchio (UNOP 5785).
Environmental conditions (n=1): Temp. 30.2°C; Cond. 54 µS.cm^{-1}; pH 6.7; Vel. 3.3 m.s^{-1}; Depth 15.0 cm; Width 27.7 m; Subst. bedrock; Shading 0%.

7.** *Phormidium corium* Gomont ex Gomont, Annales des Sciences Naturelles, Botanique, Série 7 16: 172. 1892.
Plate 4. Figure G.
Mats of filaments, greenish blue; sheath thin, hyaline, homogeneous, sometimes absent; trichomes unconstricted, non granulated in septa; intercalar cells shorter or longer than wide, 2.3-5.9 µm long and 3.8-4.8 µm diam., L/D 0.6-1.3; apical cells conical rounded, 3.4-4.3 µm long and 3.3-4.8 µm diam., RC/D 0.8-1.6; cellular content homogeneous, greenish blue, with few small granules.
Distribution in the Parnaíba River Basin (n=1): Site PI-04. BRAZIL, PIAUÍ: 05º1'58.87" S and 42º46'41.44" W, 43 amsl, 30.V.2017, M.R.Auricchio (UNOP 5779).
Environmental conditions (n=1): Temp. 28.1°C; Cond. 301 µS.cm^{-1}; pH 6.6; Vel. 0.9 m.s^{-1}; Depth 5.4 cm; Width 19.0 m; Subst. bedrock; Shading 31%.
Taxonomic comments: this population was collected in a transitional environment at the stream border, which confers with the description proposed by Komárek & Anagnostidis (2005), in which *P. corium* is common in subaerophytic environments.

8.** *Phormidium retzii* Kützing ex Gomont, Annales des Sciences Naturelles, Botanique, series 7 15: 175. 1892.
Plate 4. Figure F.
Mats of filaments, greenish blue to dark green; sheath thin, hyaline, sometimes absent; trichomes unconstricted and not granulated in septa; intercalar cells shorter or longer than wide, 3.8-8.1 µm long and 5.2-11.4 µm diam., L/D 0.4-1.4; apical cell truncate or rounded, 3.7-11.2 µm long and 5.1-11.4 µm diam., L/D 0.6-1.4; cellular content homogeneous, blue-green, with few small granules.
Distribution in the Parnaíba River Basin (n=3): Sites PI-02, PI-15, PI-16. BRAZIL, PIAUÍ: 05º19'16.35" S and 42º48'29.01" W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5773); *idem*, MARANHÃO: 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5800); *idem*, PIAUÍ 05º16'33.73" S and 42º42'53.23" W, 38 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5807, UNOP 5805).
Environmental conditions (n=3): Temp. 26.8-27.8°C ($\bar{x}$ = 27.1°C); Cond. 51-321 µS.cm^{-1} ($\bar{x}$ = 156 µS.cm^{-1}); pH 6.4-7.8 ($\bar{x}$ = 6.9); Vel. 0.1-1.2 m.s^{-1} ($\bar{x}$ = 0.6 m.s^{-1}); Depth 5.0-29.0 cm ($\bar{x}$ = 19.7 cm); Width

2.7-14.4 m ($\bar{x}$ = 6.7 m); Subst. bedrock and boulders; Shading 32-63% ($\bar{x}$ = 48%).

9.** *Phormidium tergestinum* (Rabenhorst ex Gomont) Anagnostidis & Komárek, Archiv für Hydrobiologie, Supplement 80: 406. 1988.
Plate 4. Figure I.
Basionym: *Oscillatoria tenuis* var. *tergestina* Rabenhorst ex Gomont 1892.
Mats of filaments, dark blue; sheath thin, hyaline, homogeneous, generally absent; trichomes unconstricted, granulated in septa; intercalar cells wider than long, 2.2-4.6 µm long and 4.6-6.3 µm diam., L/D 0.3-0.8; apical cells rounded, 2.5-5.5 µm long and 4.2-5.9 µm diam., L/D 0.5-1.0; cellular content homogeneous, bright greenish blue.
Distribution in the Parnaíba River Basin (n=2): Sites PI-04, PI-06. BRASIL, PIAUÍ: 05º1’58.87” S and 42º46’41.44” W, 43 amsl, 30.V.2017, M.R.Auricchio (UNOP 5778); *idem*, MARANHÃO: 04º50’57.66” S and 42º47’46.89” W, 90 amsl, 30.V.2017, M.R.Auricchio (UNOP 5781, UNOP 5782).
Environmental conditions (n=2): Temp. 28.1-28.5°C ($\bar{x}$ = 28.3°C); Cond. 301-492 µS.cm^{-1} ($\bar{x}$ = 396 µS.cm^{-1}); pH 5.9-6.6 ($\bar{x}$ = 6.2); Vel. 0.3-0.9 m.s^{-1} ($\bar{x}$ = 0.6 m.s^{-1}); Depth 5.4-6.3 cm ($\bar{x}$ = 5.8 cm); Width 4.3-19.0 m ($\bar{x}$ = 11.6 m); Subst. bedrock and sand; Shading 31-88% ($\bar{x}$ = 59.5%).
Taxonomic comments: the identification agrees with the description of *P. tergestinum* by Komárek & Anagnostidis (2005), however, according to these authors, this concept is broad, being likely to correspond to a set of species.

10.*** *Phormidium* sp.
Plate 4. Figure H.
Mats of filaments, dark green; sheath thin, hyaline, generally absent, not lamellated; trichomes unconstricted, not granulated in septa; intercalar cells wider than long, 4.4-8.9 µm long and 9.6-11.8 µm diam., L/D 0.4-0.8; apical cell truncate or rounded, 5.9-11.0 µm long and 10.0-11.9 µm diam., L/D 0.6-1.0; cellular content homogeneous, blue-green, with few small granules.
Distribution in the Parnaíba River Basin (n=2): Sites PI-10, PI-18. BRAZIL, MARANHÃO: 05º3’36.68” S and 42º53’38.29” W, 67 amsl, 31.V.2017, M.R.Auricchio (UNOP 5787); idem, PIAUÍ: 05º22’27.19” S and 42º38’40.05” W, 71 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5818).
Environmental conditions (n=2): Temp. 27.6-29.4°C ($\bar{x}$ = 28.5°C); Cond. 51-79 µS.cm^{-1} ($\bar{x}$ = 65 µS.cm^{-1}); pH 7.0-7.3 ($\bar{x}$ = 7.1); Vel. 0.11-0.8 m.s^{-1} ($\bar{x}$ = 0.45 m.s^{-1}); Depth 11.3-11.7 cm ($\bar{x}$ = 11.5 cm); Width 5.1-12.1 m ($\bar{x}$ = 8.6 m); Subst. bedrock; Shading 62-86% ($\bar{x}$ = 74%).
Taxonomic comments: although the cellular dimensions are within the predicted in the description of *P. retzii* proposed by Komárek & Anagnostidis (2005), the other characters (e.g. coloration and granulation) present considerable differences with the populations of *P. retzii* identified here. On the other hand, the characteristics of the material do not allow identification in other species. Thus, it is quite likely that this is a new species to be confirmed by future studies including ultrastructural or molecular characters.

DIVISION RHODOPHYTA
CLASS COMPSOPOGONOPHYCEAE
ORDER COMPSOPOGONALES

1.* *Compsopogon caeruleus* (Balbis ex C. Agardh) Montagne, Sciences physiques. Botanique. Cryptogamie 1: 154. 1846.
Plate 5. Figure A.
Basionym: *Conferva caerulea* Balbis ex C.Agardh 1824.
Branched filamentous, dark blue to reddish brown; apical filament uniaxial, uncorticated and branched, older mid and basal filaments corticated, without rhizoidal filaments; axial cells doliform or sub-spherical, 53.5-190.0 µm long and 132.0-335.1 µm diam., L/D 0.4-0.6; cortication formed by regular divisions of the axial cells; external cortical cells generally polygonal, 17.8-27.6 µm long and 12.9-21.5 µm diam., L/D 1.0-1.7.
Distribution in the Parnaíba River Basin (n=2): Sites PI-02, PI-17. BRAZIL, PIAUÍ: 05º19’16.35” S and 42º48’29.01” W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5774); *idem*, 05º21’20.8” S and 42º45’20.91” W, 59 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5812).
Environmental conditions (n=2): Temp. 26.8-29.1°C ($\bar{x}$ = 27.9°C); Cond. 316-321 µS.cm^{-1} ($\bar{x}$ = 318 µS.cm^{-1}); pH 7.8-8.0 ($\bar{x}$ = 7.9); Vel. 0.4-0.5 m.s^{-1} ($\bar{x}$ = 0.5 m.s^{-1}); Depth 5.0-20.6 cm ($\bar{x}$ = 12.8 cm); Width 2.0-14.4 m ($\bar{x}$ = 8.2 m); Subst. bedrock and boulders; Shading 48-63% ($\bar{x}$ = 55%).

CLASS FLORIDEOPHYCEAE
ORDER BATRACHOSPERMALES

1.* ‘Chantransia’ *macrospora*
Plate 5. Figure B.
Uniseriate branched filaments forming tufts. Branches with angles greater than 25°; generally greyish green to reddish brown; cells of the main filaments cylindrical with 25.2-85.0 µm long and 9.2-20.1 µm diam., L/D 2.2-4.7; monosporangia spherical, or in a few cases, obovoidal, 22.5-34.5 µm in diameter.
Distribution in the Parnaíba River Basin (n=2): Sites PI-15, PI-19. BRAZIL, MARANHÃO: 05º0’36.97” S and 43º1’12.97” W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5799); *idem*, PIAUÍ: 05º22’45.37” S and 42º39’1.548” W, 68 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5819).
Environmental conditions (n=2): Temp. 26.9-29.4°C ($\bar{x}$ = 28.1°C); Cond. 51-130 µS.cm^{-1} ($\bar{x}$ = 90 µS.cm^{-1}); pH 6.6; Vel. 0.7-1.2 m.s^{-1} ($\bar{x}$ = 0.9 m.s^{-1}); Depth 17.6-25.2 cm ($\bar{x}$ = 21.4 cm); Width 2.1-3.0 m ($\bar{x}$ = 2.5 m); Subst. boulders; Shading 32-48% ($\bar{x}$ = 40%).

2.* ‘Chantransia’ *pygmaea*
Plate 5. Figure C.
Uniseriate branched filaments forming tufts. Branches with angles lower than 25°; generally greyish green to reddish brown; cells of the main filaments cylindrical with 24.8-48.4 µm long and 5.2-10.9 µm diam., L/D 3.5-5.7; monosporangia obovoidal to spherical, 11.7-25.8 µm in diameter.
Distribution in the Parnaíba River Basin (n=2): Sites PI-02, PI-19. BRAZIL, PIAUÍ: 05º19’16.35” S and 42º48’29.01” W, 64 amsl, 29.V.2017, M.R.Auricchio (UNOP 5771); *idem*, 05º22’45.37” S and 42º39’1.548” W, 68 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5819).

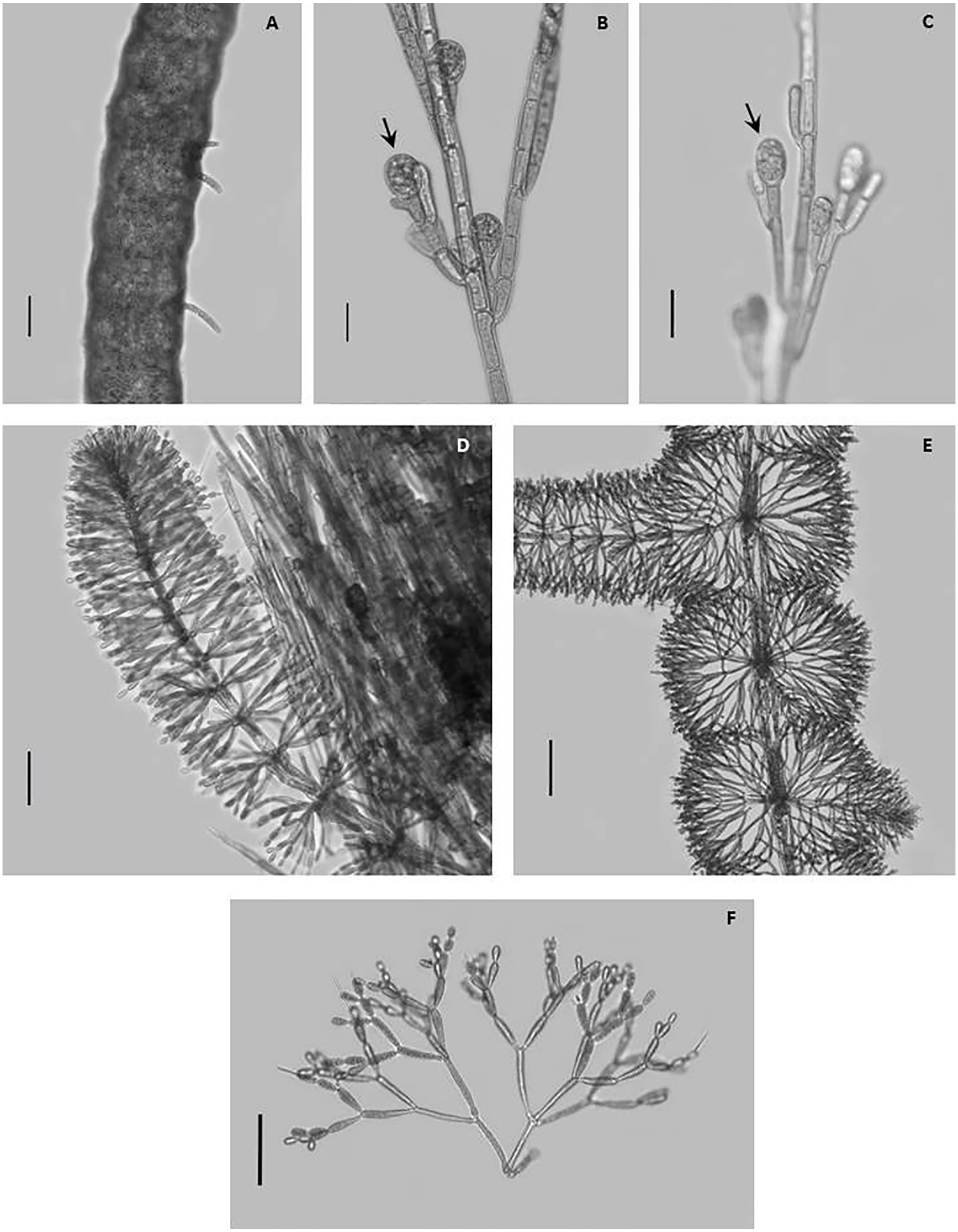


Plate 5. A; *Compsopogon caeruleus*; B. ‘Chantransia’ *macrospora* with monosporangia (arrow); C. ‘Chantransia’ *pygmaea* with monosporangia (arrow); D-F. *Batrachospermum* cf. *macrosporum*: D. *B.* cf. *macrosporum* associated with ‘Chantransia’ *macrospora*; E. General view of the whorls; F. Primary fascicle. Scale bars = 100 µm (Figures A, D, F); 20 µm (Figures B-C); 200 µm (Figure E).

Environmental conditions (n=2): Temp. 26.8-29.4°C ($\bar{x}$ = 28.1°C); Cond. 130-321 µS.cm^{-1} ($\bar{x}$ = 225 µS.cm^{-1}); pH 6.6-7.8 ($\bar{x}$ = 7.2); Vel. 0.5-0.8 m.s^{-1} ($\bar{x}$ = 0.6 m.s^{-1}); Depth 5.0-17.6 cm ($\bar{x}$ = 11.3 cm); Width 2.1-14.4 m ($\bar{x}$ = 8.2 m); Subst. bedrock; Shading 48-63% ($\bar{x}$ = 55%).

3.* *Batrachospermum* cf. *macrosporum* Montagne, Annales des Sciences Naturelles, Botanique, Troisième série 14: 293. 1850.
Plate 5. Figure D-F.
Multiseriate filaments moderately mucilaginous, irregularly and abundantly branched; straight apex, whorls well developed, lax, spherical or doliform, generally contiguous, 577.6-1642.1 µm diam.; internodes with 234.1-715.6 µm long and 37.1-71.8 µm diam., L/D 5.9-10.1; primary fascicles with 5-7 cells, 2-4 di- or trichotomously branched; proximal cells ellipsoidal or cylindrical, 55.4-97.7 µm long and 6.9-12.5 µm diam., L/D 4.4-11.8; distal cells ellipsoidal or obovoidal, 16.7-35.5 µm long and 7.9-15.5 µm diam., L/D 1.2-3.9; carposporophytes and carpogonia non observed.
Distribution in the Parnaíba River Basin (n=1): Site PI-15. BRAZIL, MARANHÃO: 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5802).
Environmental conditions (n=1): Temp. 26.9°C; Cond. 51 µS.cm^{-1}; pH 6.6; Vel. 1.2 m.s^{-1}; Depth 25.2 cm; Width 3.0 m; Subst. boulders; Shading 32%.
Taxonomic comments: the population sampled did not have carposporophytes and carpogonia, which would make identification impossible. However, based on vegetative morphology (particularly shaped and size of primary fascicles) and specimens were closely associated with 'Chantransia' *macrospora*, which is the sporophyte phase of *B. macrosporum* (Necchi Júnior & Zucchi 1997). Thus, we indicate this species as *conferatum* until a more precise identification.

4.* *Batrachospermum* sp.
Plate 6. Figure A-C.
Multiseriate filaments moderately mucilaginous, irregularly and abundantly branched; straight apex, whorls well developed, dense or lax, obconical or doliform, generally contiguous, 202.4-350.5 µm diam.; internodes 110.4-531.6 µm long and 32.0-72.0 µm diam., L/D 3.4-10.0; primary fascicles with 6-9 cells, 2-3 di- or trichotomously branched; proximal cells cylindrical, 16.5-26.8 µm long and 4.7-7.2 µm diam., L/D 3.2-5.5; distal cells elliptical or obovoidal, 8.6-11.7 µm long and 5.2-7.3 µm diam., L/D 1.2-1.8; numerous secondary fascicles along the internode length, usually reach the length of the primary fascicles; symmetrical carpogonia with 28.7-47.6 µm long and 6.1-9.0 µm diam., L/D 3.1-7.8; carposporophytes not observed.
Distribution in the Parnaíba River Basin (n=1): Site PI-15. BRAZIL, MARANHÃO: 05º0'36.97" S and 43º1'12.97" W, 111 amsl, 31.V.2017, M.R.Auricchio (UNOP 5802-1).
Environmental conditions (n=1): Temp. 26.9°C; Cond. 51 µS.cm^{-1}; pH 6.6; Vel. 1.2 m.s^{-1}; Depth 25.2 cm; Width 3 m; Subst. boulders; Shading 32%.
Taxonomic comments: no carposporophytes were observed in the population, making it impossible to identify the material at a specific level.

5.** *Kumanoa abilii* Necchi (Reis) Necchi Júnior & Vis, Phycologia 49(1): 101. 2010.
Plate 6. Figure D-G.
Basionym: *Batrachospermum abilii* M.P. dos Reis 1965.
Free multiseriate filaments with moderate mucilage, irregularly and abundantly branched; straight apex, whorls well developed, dense or lax, obconical or doliform, generally contiguous, with 199.3-451.9 µm diam. internodes with 56.7-459.5 µm long and 15.9-60.5 µm diam., L/D 2.3-10.5; primary fascicles with 5-11 cells, 2-4 di or trichotomously branched; proximal cylindrical cells with 16.1-30.1 µm long, 4.1-9.0 µm diam., L/D 1.8-6.1; distal cells elliptical, obovoidal or spherical with 4.8-13.2 µm long and 4.3-7.8 µm diam., L/D 0.9-2.2; numerous secondary fascicles along the internode length, usually reach the length of the primary fascicles; curved carpogonial branches, differentiated from fascicles, on pericentral cells; asymmetrical carpogonia, 25.7-44.1 µm long, 4.0-8.6 µm diam., L/D 3.7-7.8; carposporophytes sessile, 1 per whorl, dense, semi-spherical, 112.8-194.1 µm diam; compact mass of gonimoblast filaments, carposporangia formed at apices with 9.5-10.2 µm diam.
Distribution in the Parnaíba River Basin (n=1): Site PI-18. BRAZIL, PIAUÍ: 05º22'27.19" S and 42º38'40.05" W, 71 amsl, 01.VI.2017, M.R.Auricchio (UNOP 5817).
Environmental conditions (n=1): Temp. 29.4°C; Cond. 79 µS.cm^{-1}; pH 7.0; Vel. 0.1 m.s^{-1}; Depth 11.3 cm; Width 5.1 m; Subst. rock, boulders, gravel; Shading 62%.
Taxonomic comments: the carposporophytes measures were slightly smaller when compared to what was described by Kumano (2002) (150.0-300.0 µm) to *Batrachospermum abilii*, however, the morphological characteristics confer with what was described for the species.

Discussion

The total number of taxa found in the present study (38) can be considered high when compared to studies carried out in other Brazilian regions that used similar methodology, namely: i) 13 taxa (Pereira & Branco 2010) and 21 taxa (Almeida et al. 2011) in the northwest of the São Paulo State; ii) 23 taxa (Branco et al. 2008) and 34 taxa (Krupek et al. 2008), in the central-southern region of the Paraná State; iii) 24 taxa (Branco et al. 2009) in the mid-western region of the Paraná State, and; iv) 19 taxa (Peres et al. 2008) reported to the eastern region of Paraná State. Likewise, when considering the relative richness, found 1.81 taxa per sampling point, which is also higher when compared to what was reported in those other regions (taxa per sampling point and respective studies: 1.09 in Branco et al. 2009, 1.23 in Almeida et al. 2011, 1.35 in Peres et al. 2008, 1.78 in Krupek et al. 2008, 2.09 in Branco et al. 2008). Studies in tropical streams have shown that biomes without a dense canopy cover can sustain richer macroalgae communities (Necchi Júnior et al. 2003, 2008). Apparently, a larger radiation area in the drainage basin (which is typical in the Caatinga and Cerrado biomes, here sampled) may provide support for a bigger regional species pool that enables greater local richness (as demonstrated for green algae in Peres et al. al. 2017).

In terms of the algal groups that are commonly found in streams, there were predominant species of green algae (Chlorophyta plus Charophyta) (13 taxa, 34.2%) followed by Cyanophyta (10 taxa, 26.3%). These data corroborate the pattern observed by Sheath & Cole (1992, 35% and 24%, green algae and cyanobacteria, respectively), Vis et al.

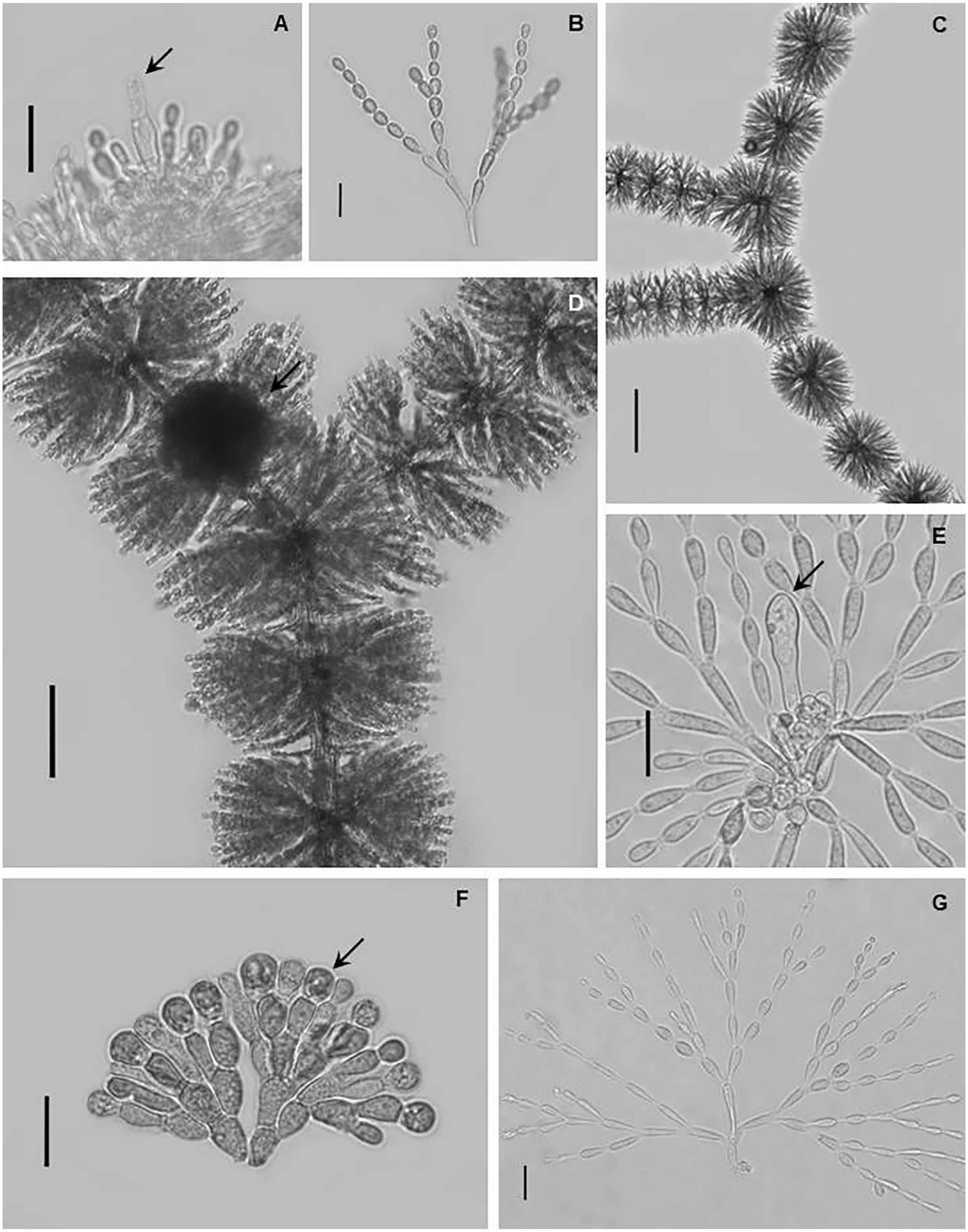


Plate 6. A-C. *Batrachospermum* sp.: A. Carpogonia (arrow); B. Primary fascicle; C. General view of the whorls. D-G. *Kumanoa abilii*: D. General view of the whorls with a carposporofite (arrow); E. Carpogonia (arrow); F. Gonimoblast filaments with carposporangia at apices (arrow); G. Primary fascicle. Scale bars = 20 µm (figs. A-B, E-G);100 µm (fig. D); 200 µm (fig. C).

(1994, 47% and 32%), Branco et al. (2008, 52.2% and 26.1%), Branco et al. (2009, 54% and 21%), Almeida et al. (2011, 43% and 38%) and Krupek & Branco (2014, 58.4% and 25%). Most surveys cited above include disturbed regions or open riparian vegetation. It appears that this predominance of green algae species in these regions occurs due to the better adaptation of the group to high light intensity regimes due to characteristics of its photosynthetic complex (Branco et al. 2017). Although Bacillariophyta was not the richest group, in this study a bigger number of diatom species was found than conventionally for stream macroalgae studies (e.g. Necchi et al. 2000, Branco et al. 2008, 2009). Even though unicellular, diatoms can form monospecific macroscopic colonies (Biggs & Kilroy 2000, as shown in the Plate 1, Figure A in this study) that can dominate the streambed - hence macroalga, sensu Sheath & Cole (1992).

The results of this work make very clear the importance of floristic surveys to the reduction of the algae biodiversity conservation shortfalls, specially the Wallacean Shortfall (Whittaker et al. 2005). Of the 38 species recorded by this taxonomic survey, 37 are new records for the Parnaíba River Basin where only *Cladophora glomerata* was already registered for the basin. Following the same pattern, 23 species are new records for the Brazilian Northeast region (60.5% of the total) and *Microcoleus lacustris* represented the first record for the Brazilian territory. Despite the fact that it remains the Brazilian watershed with the lowest number of algal species documented, this work contributed to the increase of almost five times the number of species sampled in the Parnaíba River Basin (from only 10 to 47 species, Flora do Brasil 2020 under construction 2018). These data reinforce that the differences presented in the algal diversity in the Brazilian regions are much more related to the sampling effort than to environmental differences. In addition, this study illustrates not only the group's lack of information in the region but also shows the importance of this type of study as a tool for expanding knowledge about biodiversity and its conservation.

Acknowledgements

We are grateful to PRPPG/UNILA for support (Edital 109/2017) and field work help provided by Paulo Auricchio, Pedro Henrique Soares Melo, Caio Frederico Saraiva da Silveira, Gabriel Saraiva Lima e Silva, Yasnailson Euflávio de Souza and Cláudia Renata Madella-Auricchio.

Author Contributions

Marina Ramos Auricchio: Contributed in the concept and design of the study, data collection and analysis, and manuscript preparation.

Richard Wilander Lambrecht: Contributed in the data analysis, taxonomic confirmation and manuscript preparation.

Cleto Kaveski Peres: Contributed in the data analysis, taxonomic confirmation and manuscript preparation.

Conflicts of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ALMEIDA, F.V., NECCHI JÚNIOR, O. & BRANCO, L.H.Z. 2011. Flora de comunidades de macroalgas lóticas de fragmentos florestais remanescentes da região noroeste do Estado de São Paulo, Brasil. Hoehnea 38(4):553-568.

AGÊNCIA NACIONAL DE ÁGUAS (BRASIL). 2015. Conjuntura dos recursos hídricos no Brasil: regiões hidrográficas brasileiras – Edição Especial. Brasília: ANA.

AZEVEDO, M.T.P., NOGUEIRA, N.M.C. & SANT'ANNA, C.L. 1996. Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas, 8: Cyanophyceae. Hoehnea 23:1-38.

BICUDO, C.E. & MENEZES, M. 2006. Gêneros de algas de águas continentais do Brasil: chave para identificação e descrição. RiMa, São Carlos.

BIGGS, B.J.F. & KILROY, C. 2000. Stream periphyton monitoring manual. Published by NIWA for Ministry for the Environment.

BINI, L.M., DINIZ-FILHO, J.A.F., RANGEL, T.F.L.V.B., BASTOS, R.P. & PINTO, M.P. 2006. Challenging Wallacean and Linnean shortfalls: knowledge gradients and conservation planning in a biodiversity hotspot. Divers. Distrib. 12(5):475-482.

BRANCO, C.C.Z., PERES, C.K., KRUPEK, R.A. & BERTUSSO, F.R. 2009. Stream macroalgae from the Midwestern region of Paraná State, southern Brazil. Biota Neotrop. 9(2):227-235.

BRANCO, C.C.Z., EMED, R.G., BRANCO, L.H.Z. & NECCHI JUNIOR, O. 2008. Macroalgas de riachos da Floresta Nacional de Irati, região centro-sul do Estado do Paraná, Sul do Brasil. Acta Bot. Bras. 22(2):437-451.

BRANCO, C.C.Z., RIOLFI, T.A., CRULHAS, B.P., TONETTO, A.F., BAUTISTA, A.I.N. & NECCHI JÚNIOR, O. 2017. Tropical lotic primary producers: Who has the most efficient photosynthesis in low-order stream ecosystems? Freshw. Biol. 62:1623-1636.

BRANCO, C.C.Z., RIOLFI, T.A., PERES, C.K. & NECCHI JUNIOR, O. 2011. Rhodophyta de ambientes lóticos de Unidades de Conservação da região Sul do Brasil. Biota Neotrop. 11(3): 365-375.

BRANCO, C.C.Z. & NECCHI JÚNIOR, O. 1996. Distribution of stream macroalgae in the eastern Atlantic Rainforest of São Paulo State, southeastern Brazil. Hydrobiologia 333(3):139-150.

BRANCO, C.C.Z.; NECCHI JÚNIOR, O. & BRANCO, L.H.Z. 2002. Taxonomy and ecological distribution of Chaetophoraceae (Chaetophorales, Chlorophyta) in lotic ecosystems from São Paulo State, southeastern Brazil. Algol. Stud. 106:43-75.

BROWN, J. H. & LOMOLINO, M. V. 1998. Biogeography. Sinauer Associates, USA.

COSTA, L.F., WETZEL, C.E., LANGE-BERTALOT, H., ECTOR, L. & BICUDO, D.C. 2017. Taxonomy and ecology of *Eunotia* species (Bacillariophyta) in southeastern Brazilian reservoirs. Bibl Diatomol. 64: 302.

DESIKACHARY, T.V. 1959. Cyanophyta. Indian Council of Agricultural Research, New Delhi.

FLORA DO BRASIL 2020 UNDER CONSTRUCTION. 2018. (http://floradobrasil.jbrj.gov.br). Último acesso em: 29/06/2018.

GORDON, N.D., McMAHON, T.A. & FINLAYSON, B.L. 1992. Stream Hydrology: an introduction for ecologists. Chichester, John Wiley & Sons.

GUIRY, M.D. & GUIRY, G.M. 2018. AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Disponível em: <http://www.algaebase.org> Acessado em: jun. 2018.

KOMÁREK, J. & ANAGNOSTIDIS, K. 2005. Cyanoprokaryota 2.Teil: Oscillatoriales. Elsevier GmbH, Munchen.

KRUPEK, R.A., BRANCO, C.C.Z. & PERES, C.K. 2008. Levantamento florístico das comunidades de macroalgas da bacia do rio das Pedras, região centro-sul do Estado do Paraná, Sul do Brasil. Hoehnea 35(2):189-208.

KRUPEK, R.A. & BRANCO, C.C.Z. 2014. Estrutura das comunidades de macroalgas da bacia de drenagem do rio Cascavel, Estado do Paraná, Brasil. Hoehnea 41(1):41-50.

KUMANO, S. 2002. Freshwater red algae of the world. Biopress Ltd, Bristol.

LOMOLINO, M.V. 2004. Conservation biogeography. In: LOMOLINO M.V. & HEANEY L.R. (Eds.) Frontiers of Biogeography: new directions in the geography of nature. Sinauer Associates, Sunderland, Massachusetts.

MACE, G.M. 2004. The role of taxonomy in species conservation. Philos. T. R. Soc. B 359(1444): 711-719.

MENEZES, M., BICUDO, C.E.M., MOURA, W.N., ALVES, A.M., SANTOS, A.A., PEDRINI, A.G., ARAÚJO, A., TUCCI, A., FAJAR, A., MALONE, C., KANO, C.H., SANT`ANNA, C., BRANCO, C.C.Z., ODEBRECHT, C., PERES, C.K., NEUHAUS, E.B., ESKINAZI-LEÇA, E., AQUINO, E., NAUER, F., SANTOS, G.N., AMADO FILHO, G.M., LYRA, G.M., BORGES, G.C.P., COSTA, I.O., NOGUEIRA, I.S., OLIVEIRA, I.B., PAULA, J.C., NUNES, J.M.C., LIMA, J.C., SANTOS, K.R.S., FERREIRA, L.C., GESTINARI, L.M.S., CARDOSO, L.S., FIGUEIREDO, M.A.O., SILVA, M.H., BARRETO, M.B.B.B., HENRIQUES, M.C.O., CUNHA, M.G.G.S., BANDEIRA-PEDROSA, M.E., OLIVEIRA-CARVALHO, M.F., SZÉCHY, M.T.M., AZEVEDO, M.T.P., OLIVEIRA, M.C., CABEZUDO, M.M., SANTIAGO, M.F., BERGESH, M., FUJII, M.T., BUENO, N.C., NECCHI JUNIOR, O., JESUS, P.B., BAHIA, R.G., KHADER, S., ALVES-DA-SILVA, S.M., GUIMARÃES, S.M.P.B., PEREIRA, S.M.B., CAIRES, T.A., MEURER, T., CASSANO, V., WERNER, V.R., GAMA JÚNIOR, W.A. & SILVA, W.J. 2015. Update of the Brazilian floristic list of Algae and Cyanobacteria. Rodriguésia 66(4):1047-1062.

METZELTIN, D. & LANGE-BERTALOT, H. 1998. Tropical Diatoms of South America I: About 700 predominantly rarely known or new taxa representative of the neotropical flora. In: LANGE-BERTALOT, H. Iconographia Diatomologica. Vol. 5. Diversity-Taxonomy-Geobotany. Koeltz Scientific Books. Alemanha: Königstein.

MMA: MINISTÉRIO DO MEIO AMBIENTE. 2006. Caderno da Região Hidrográfica do Parnaíba. MMA, Brasília.

NECCHI JÚNIOR, O. 1990. Revision of the genus *Batrachospermum* Roth (Rhodophyta, Batrachospermales) in Brasil. J. Cramer, Berlin.

NECCHI JÚNIOR, O. & ZUCCHI, M.R. 1997. *Audouinella macrospora* (Acrochaetiaceae, Rhodophyta) is the ‘Chantransia’ stage of *Batrachospermum* (Batrachospermaceae). Phycologia 36(3):220-224.

NECCHI JÚNIOR, O.; BRANCO, C.C.Z. & BRANCO, L.H.Z.. 2000. Distribution of stream macroalgae in São Paulo State, southeastern Brazil. Algol. Stud. Stuttgart, 97:43-57.

NECCHI JÚNIOR, O., BRANCO, C.C.Z. & SPEZAMIGLIO, D. 2008. Distribuição ecológica de comunidades de macroalgas de ambientes lóticos do Parque Nacional de Itatiaia (MG, RJ), Brasil. Braz. J. Bot. 31:135-145.

NECCHI Jr., O., BRANCO, L.H.Z. & BRANCO, C.C.Z. 2003. Ecological distribution of stream macroalgal communities from a drainage basin in the Serra da Canastra National Park, Minas Gerais, Southeastern Brazil. Braz. J. Biol. 63:1-12.

NECCHI JÚNIOR, O., SPEZAMIGLIO, D.N., BRANCO, C.C.Z. & BRANCO, L.H.Z. 2001. Estudo taxonômico e distribuição ecológica do gênero *Vaucheria* (Xanthophyta, Vaucheriaceae) em ecossistemas lóticos do Estado de São Paulo, Brasil. Hoehnea. 28(3):231-242.

NECCHI JÚNIOR, O., PASCOALOTO, D., BRANCO, C.C.Z. & BRANCO, L.H.Z. 1997. Stream macroalgal flora from the northwest region of São Paulo State, Southeastern Brazil. Algol. Stud. 84:91-112.

NECCHI JÚNIOR, O., DIP, M.R. & GOES, R.M. 1991. Macroalgae of a stream in southeastern Brazil: composition, seasonal variation and relation to physical and chemical variables. Hydrobiologia 213(3):241-250.

NOVIS, P. M. 2004. A taxonomic survey of *Microspora* (Chlorophyceae, Chlorophyta) in New Zealand. New Zeal. J. Bot. 42:153-165.

PEREIRA, J.L. & BRANCO, L.H. 2010. Macroalgas em nascentes e arredores de riachos na região noroeste do Estado de São Paulo. Hoehnea. 37(3):435-444.

PERES, C.K. & BRANCO, C.C.Z. 2014. Algas verdes macroscópicas de ambientes lóticos do Sul do Brasil: taxonomia e aspectos ecológicos. Novas Edições Acadêmicas, SIA OmniScriptum Publishing, Letônia, 2014.

PERES, C.K., TONETTO, A.F., GAREY, M.V. & BRANCO, C.C.Z. 2017. Canopy cover as the key factor for occurrence and species richness of subtropical stream green algae (Chlorophyta). Aquat. Bot. 137:24-29.

PERES, C.K., BRANCO, C.C.Z. & KRUPEK, R.A. 2008. Macroalgas de riachos da Serra da Prata, leste do Estado do Paraná, sul do Brasil. Acta Bot. Bras. 22(2):333-344.

PYKE, G.H. & EHRLICH, P.R. 2010. Biological collections and ecological/ environmental research: a review, some observations and a look to the future. Biol. Rev. Camb. Philos. 85(2):247-266.

SENNA, P.A. & FERREIRA, L.V. 1987. Nostocophyceae (Cyanophyceae) da Fazenda Água Limpa, Distrito Federal, Brasil, 2: Famílias Nostocaceae, Scytonemaceae e Stigonemataceae. Rickia 14: 7-19.

SHEATH, R.G. & COLE, K.M. 1992. Biogeography of stream macroalgae in North America. J. Phycol. 28(4):448-460.

SKINNER, S. & ENTWISLE, T.J. 2001. Non-marine algae of Australia: 2. Some conspicuous tuft-forming Cyanobacteria. Telopea 9(3):685-712.

VIS, M.L., SHEATH, R.G., HAMBROOK, J.A. & COLE, K.M. 1994. Stream macroalgae of the Hawaiian islands: a preliminary study. Pac. Sci. 48(2):175-187.

WHITTAKER, R., ARAÚJO, M.B., JEPSON, P., LADLE, R.J., WATSON, J.E.M. & WILLIS, K.J. 2005. Conservation biogeography: assessment and prospect. Divers. Distrib. 11(1):3-23.

Received: 31/10/2018
Revised: 01/02/2019
Accepted: 07/03/2019
Published online: 01/04/2019

Biota Neotropica 19(2): e20180606, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Article

Community structure and tree diversity in a subtropical forest in southern Brazil

Kauane Maiara Bordin[1]*, Letícia Daiana Ferreira[2], Adriano Rosina[2], Marciana Malacarne[2], Patricia Zanotelli[2], Samuel Fernando Adami[3] & Giovana Secretti Vendruscolo[3]

[1]Universidade Federal do Rio Grande do Sul, Laboratório de Ecologia Vegetal, Programa de Pós-Graduação em Ecologia, Porto Alegre, RS, Brasil
[2]Universidade Comunitária da Região de Chapecó, Chapecó, SC, Brasil
[3]Universidade Federal da Integração Latino-Americana, Programa de Pós-Graduação em Biodiversidade Neotropical, Foz do Iguaçu, PR, Brasil
**Corresponding author: Kauane Maiara Bordin, e-mail: kauanembordin@gmail.com*

BORDIN, K.M., FERREIRA, L.D., ROSINA, A., MALACARNE, M., ZANOTELLI, P., ADAMI, S.F., VENDRUSCOLO, G.S. **Community structure and tree diversity in a subtropical forest in southern Brazil**. Biota Neotropica. 19(2): e20180606. http://dx.doi.org/10.1590/1676-0611-BN-2018-0606

Abstract: Local and regional environmental variations lead to different species composition, creating transitional areas. An example is the Araucaria and Seasonal forest in southern Brazil. Our objectives were (1) to describe the tree community structure and composition of a subtropical forest in southern Brazil and (2) to compare the floristic relationships between two forest typologies (Araucaria and Seasonal forest) in order to characterize the study area and the distribution patterns of tree species. We conducted a survey at Chapecó National Forest (in southern Brazil) in an area of 1.2 ha, where all individuals ≥ 30 cm of circumference at breast height were sampled. Community structure was described using the traditional phytosociological parameters. The floristic relationships were obtained by comparing our results with compiled data from other scientific papers through cluster analyses using an unweighted average linkage method, based on Jaccard similarity coefficient. We sampled 809 individuals belonging to 61 species and 28 families. The richest family was Fabaceae and *Coussarea contracta* (Walp.) Müll.Arg. was the most abundant species. Taxonomic diversity was 3.06 and the evenness was 0.74. The floristic similarity revealed that species composition of our study area is more similar to Seasonal forest. Species composition is related to environmental factors such as great thermal amplitude and seasonality. This subtropical forest is well structured, highly diverse and extremely important for the local and regional biodiversity conservation.

Keywords: *Araucaria forest, Biodiversity conservation, Floristic similarity, Phytosociology, Seasonal forest, Transitional forest.*

Estrutura da comunidade e diversidade de espécies arbóreas em uma floresta subtropical no sul do Brasil

Resumo: Variações ambientais locais e regionais levam à composição de espécies diferentes e áreas de transições. Um exemplo é a floresta com araucária e a floresta estacional no sul do Brasil. Nossos objetivos foram (1) descrever a composição e estrutura da comunidade arbórea de uma floresta subtropical no sul do Brasil e (2) comparar as relações florísticas entre duas tipologias florestais (Floresta com Araucária e Floresta Estacional) a fim de caracterizar a área de estudo e o padrão de distribuição de espécies arbóreas. Nós realizamos uma pesquisa na Floresta Nacional de Chapecó (no sul do Brasil) em uma área de 1.2 hectares, onde todos os indivíduos com circunferência à altura do peito ≥ 30 cm foram amostrados. A estrutura da comunidade foi descrita utilizando os parâmetros fitossociológicos tradicionais. Obtivemos as relações florísticas através da comparação dos nossos resultados com dados compilados de outros trabalhos científicos, através de análises de agrupamento (método de ligação de distância média não ponderada, UPGMA, baseado no coeficiente de similaridade de Jaccard). Foram amostrados 809 indivíduos pertencentes a 61 espécies e 28 famílias. A família mais rica foi Fabaceae e *Coussarea contracta* (Walp.) Müll.Arg. a espécie mais abundante. A diversidade taxonômica foi de 3.06 e a equabilidade foi de 0.74. A similaridade florística revelou que a composição de espécies da nossa área de estudo é mais similar a da floresta estacional. A composição de espécies está relacionada a fatores ambientais como a amplitude térmica e sazonalidade. Esta floresta subtropical é bem estruturada, altamente diversa e muito importante para a conservação da biodiversidade local e regional.

Palavras-chave: *Floresta com araucária, Conservação da biodiversidade, Similaridade florística, Fitossociologia, Floresta estacional, Floresta de transição.*

Introduction

The community species composition results from processes that rule the assemblages. In general, the processes are related to neutral models (Hubbell 2001), niche-based models (Cornwell & Ackerly 2009) or both, in different scales (Xu et al. 2017). In general, the first are related to stochastic variations in the community (*eg*. dispersal limitations), and the second, to the influence of the environmental filters on species. These environmental filters are related to abiotic factors that prevent the establishment or persistence of a species in a location (*eg*. temperature, precipitation, and soil conditions) (Kraft et al. 2015, Oliveira-Filho et al. 2001).

The environmental characteristics can vary along the extent of an area, creating some special conditions to the development and occurrence of a set of species. Factors such as soil composition, rainfall patterns, latitudinal and altitudinal ranges lead to floristic differentiations (Liebsch et al. 2008). These variations create gradients (or ecotones), known as transitional areas with great biodiversity, especially because of the overlap of different physiognomies (Risser 1995). In general, studies in these areas are mainly in the transition of forested and non-forested areas (Junyan et al. 2014), and the forest-forest dynamics is poorly documented, especially because of the gaps in knowledge about the capacity of rearrangement of species distribution (Oliveira-Filho et al. 2014).

The Brazilian Atlantic Forest is one of the biodiversity hotspots of the world, because of the great endemism of species in this area (Myers et al. 2000). Originally, it covered more than 1,400.000 km² of Brazilian territory (Joly et al. 2014) and the geographical location ranges from the northeastern to southern Brazil (Morellato & Haddad 2000, Joly et al. 2014). The Atlantic Forest is defined by Oliveira-Filho and Fontes (2000) as *sensu stricto* (*s.s.*) and *sensu lato* (*s.l.*). The first is related to the forests along the Atlantic Ocean and the second, as a biome, to a set of physiognomies present at the extent of its distribution. In this study, we considered the concept of Atlantic Forest *s.l.*, including the forest physiognomies (Oliveira-Filho & Fontes 2000).

The floristic differences can be generated by local environmental factors, such as river valleys (important corridors of arboreal species dispersal) (Spichiger et al. 2004), edaphic factors, local topography and disturbances (Higuchi et al. 2012, Gonçalves & Souza 2014, Estevan et al. 2016). In the southern Brazil region, heterogeneous climatic conditions allow the occurrence of many different groups of species (Higuchi et al. 2012). In general, they are a mixture of tropical elements from Amazon region and temperate Austral-Antartic and Andean floras (Rambo 1951).

As a consequence of this heterogeneity, many transitional areas are found in this region. For example, the Araucaria forest is surrounded by the Atlantic forest *s.s.* (*i.e.* Dense ombrophilous forest), Seasonal forest, grasslands and other vegetation types (Klein 1978; Overbeck et al. 2007; IBGE 2012). However, little is known about the floristic patterns of the arboreal components of these forests (Higuchi et al. 2012), especially in transitional areas. In order to answer these questions, we had two objectives in this study. In the first, we aimed at describing the tree community structure and composition of a subtropical forest in southern Brazil. In the second, we aimed at comparing the floristic relationships between two forest typologies (Araucaria and Seasonal forests) in order to characterize the study area and to contribute to the knowledge on the distribution patterns of tree species.

Material and Methods

1. *The southern Brazilian Atlantic Forest: the Araucaria and Seasonal forests*

The forest typologies in Santa Catarina state were initially described in an extensive fieldwork by the botanists Raulino Reitz and Roberto Miguel Klein (Klein 1978, 1960, Reitz et al. 1978, Klein 1963). Their results were summarized in two publications – the “Flora Ilustrada Catarinense” (in many papers since 1965) and the “Projeto Madeira de Santa Catarina” (Reitz et al. 1978), who became important for the knowledge about Santa Catarina’s flora. Recently, another project was conducted in the state (“The Floristic and Forest Inventory of Santa Catarina State” (Vibrans et al. 2010)) and resulted in many papers and books about the species occurrence, plant genetic information and economic aspects of the population surrounding the forest (Vibrans et al. 2013a, b, 2010).

The results obtained from the inventory for Araucaria forest (AF) and Seasonal forest (SF) were summarized by Gasper, Uhlmann, et al. (2013) and Gasper, Sevegnani, et al. (2013) where they recorded 366 and 204 tree species, respectively. In the southern Atlantic forest *s.l.*, the presence of the conifer *Araucaria angustifolia* (Bertol.) Kuntze characterizes the AF. This species occurs at the Santa Catarina’s plateau in elevations between 500 m to 1,500 m above sea level (a.s.l.) (Backes 2009). The climatic conditions for its occurrence are low temperature (12 °C in average) and high humidity (1.830 mm in average) (Rambo 1953, Backes 2009). Other species frequently associated with this forest are *Nectandra lanceolata* Nees, *Eugenia involucrata* DC., *Ocotea diospyrifolia* (Meisn.) Mez, *Ilex paraguariensis* A.St.-Hil. and *Cupania vernalis* Cambess. (Klein 1978; Jarenkow and Budke 2009). Otherwise, the Seasonal forest, also known as “Upper Uruguay River Basin” forest, is characterized by deciduous species (who lose their leaves in the cold season). It occurs in elevations between 150 m to 800 m a.s.l (Klein 1978, Gasper, Uhlmann, et al. 2013) and the temperature in the region is lower than 15 °C in average. Other species associated with this forest are *Apuleia leiocarpa* (Vogel) J.F.Macbr., *Diatenopteryx sorbifolia* Radlk., *Peltophorum dubium* (Spreng.) Taub. and *Myrocarpus frondosus* Allemão (Gasper, Uhlmann, et al. 2013, Klein 1978).

The west of the Santa Catarina state was traditionally characterized as a transitional area, composed by Araucaria and Seasonal forests. According to IBGE (2012), this region is substantially covered by Seasonal forest; However, Klein (1960) described it as Araucaria forest, mainly. Furthermore, many authors agree that the dynamics of these forests follow a west-east pattern and the species that compose the SF advances over the AF (Backes 2009; Waechter 2009; Jarenkow and Budke 2009; Gasper et al. 2013b, 2015).

2. *Study area*

The sampling was conducted at Chapecó National Forest (glebe one), a Conservation Unit for sustainable use located in the west of Santa Catarina, southern Brazil (27º05’19.9’’S and 52º46’47.3’’W) (Figure 1) (see details in Lucas & Fortes 2008). The climate is subtropical (Nimer 1989); the annual rainfall is well distributed during the year (the average rainfall is 2000 mm) and the average temperature ranges from 22 °C to 14 °C for the hotter and coldest month, respectively (http://www.inmet.gov.br). The regional landforms are characterized by the hilly topography of the Araucarias’ Plateau, between 520 m

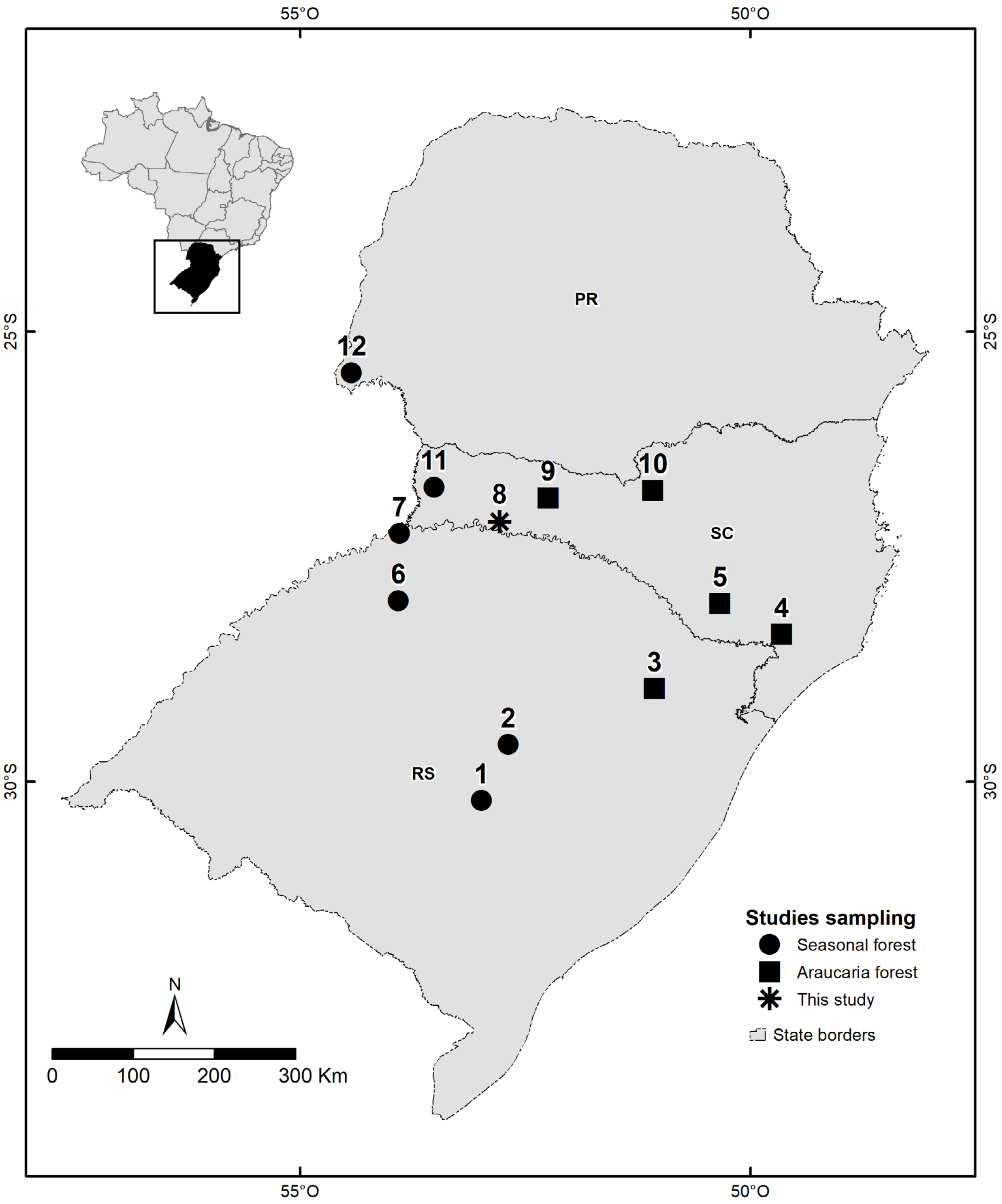


Figure 1. Location of the study area, at Chapecó National Forest, west Santa Catarina, southern Brazil (code number 8 - *). Information about Araucaria forest is presented as quadrats (numbers 9-12) and Seasonal forest as circles (numbers 1-7). Complete information about data sources data can be accessed in Table 1.

and 617 m a.s.l. and the predominant soils are Inceptisols and Oxisols (ICMBio 2013).

3. *Sampling units and data analysis*

The survey was conducted in a secondary forest, with more than 60 years without disturbance (in 2017). There, 24 permanent plots were systematically selected with 500 m² each one (50 m x 10 m), corresponding to a total area of 1.2 ha. To describe the community structure we measured trees ≥ 30 cm of circumference at breast height (c.b.h.) (which means ≥ 9.54 cm of diameter at breast height, d.b.h.). The maximum height was estimated for all individuals. Whenever possible, the individuals had species determination in field. For those which determination was not possible, we collected material for determination in laboratory. Voucher specimens were deposited in the

UNO Herbarium (UNO, at Universidade Comunitária da Região de Chapecó). The circumscription adopted for angiosperm families are in accordance with Angiosperm Phylogeny Group (The Angiosperm Phylogeny Group 2016).

The community structure was described using the phytosociological parameters (Mueller-Dombois & Ellenberg 1974). We evaluated the sample adequacy using a rarefaction curve (Magurran 2004) with the software Estimates 8.2, considering $\alpha \leq 0.05$. Taxonomic diversity was estimated using the Shannon-Weiner Index (H') and Pielou's evenness Index (J) (R version 3.3.1, *vegan* package) (R Core Team 2016).

To evaluate the floristic similarity we compiled information from scientific papers. Our selection criterion followed the conditions: a) survey at Araucaria or Seasonal forests and b) range of study restricted to southern Brazil, with the maximum distance of 350 km from the Chapecó National Forest (our study area) and more than 50 km between the surveys (data sources) to remove a possible effect of proximity, also considering, as possible, studies with similar sampling method and data collection effort (Fig. 1). We selected 12 papers, and most part of surveys employed plots for vegetation sampling; once a study used point-centered quarters (Benvenuti-Ferreira & Coelho 2009). The studies differ in regard to sampling effort and size criteria for individual inclusion (minimum c.b.h.). The information about each study is described in Table 1. We integrated our results to the matrix of compiled data and used an abundance matrix. The final matrix was composed by 145 arboreal species (genus and families identifications were excluded). We performed cluster analyses using an unweighted average linkage method (UPGMA) based on a Jaccard similarity coefficient (Legendre & Legendre 2012) (R version 3.3.1, *vegan* package).

Results

We sampled 809 individuals belonging to 61 species and 28 families. Families with the largest number of species were: Fabaceae (11), Lauraceae (5), Rutaceae (5), Salicaceae (4) and Sapindaceae (4) (Table 2). The mean density was 674 individuals/ha. *Coussarea contracta* (Walp.) Müll.Arg., *Ocotea diospyrifolia* (Meisn.) Mez, *Cupania vernalis* Cambess., *Araucaria angustifolia*, *Casearia sylvestris* Sw., *Diatenopteryx sorbifolia* Radlk. and *Myrocarpus frondosus* Allemão were the species with biggest relative density, representing 62.7% of the individuals sampled. These species even had the biggest relative dominance in basal area (more than 54%), relative frequency (37.9%), coverage value (58.2%) and importance value (51.5%) (Table 2). According to the Shannon index, the taxonomic diversity was 3.06 and the evenness (J) was 0.74 (Table 1). However, the rarefaction curve did not reach an asymptotic richness level (Figure 2).

The diameter structure followed the traditional inverted-J curve, with most of the individuals at the first diameter class (almost 50%) (Figure 2). Individuals with largest diameter are represented by *Luehea divaricata* Mart. & Zucc. (107 cm d.b.h) and *A. angustifolia* (81.5 cm d.b.h.). Most of the individuals were from six to 15 meters height and, in general, the abundance decreased as the height grew up (Figure 2). The emergent species (over 20 m height) were *A. angustifolia*, *D. sorbifolia*, *M. frondosus*, *O. diospyrifolia*, *Helietta apiculata* Benth., *Lamanonia ternata* Vell., *Matayba elaeagnoides* Radlk., *Parapiptadenia rigida* (Benth.) Brenan, *Peltophorum dubium* (Spreng.) Taub. and *Prunus myrtifolia* (L.) Urb.

Table 1. Data compiled from studies at Araucaria and Seasonal forests in order to evaluate the species similarity. Source: papers where the data were obtained; SL: sampling location; CO: site code; A: total sampled area (in hectares); c.b.h: minimum tree circumference criteria (cm); N: number of species sampled; RF: Richest families at community; Abundant species: Most abundant species at community; Total abundance: number of individuals sampled at community; RD: *Araucaria angustifolia* relative dominance at community; H': Shannon-Wiener diversity index; J: Pielou's evenness index.

Source	SL	CO	A	c.b.h.	N	RF	Abundant species (N)	Total abundance	RD (%)	H'	J
This study	SC	8	1,2	30	61	Fabaceae (11); Lauraceae (5); Rutaceae (5); Salicaceae (4); Sapindaceae (4)	*Coussarea contracta* (177); *Ocotea diospyrifolia* (110); *Cupania vernalis* (84); *Araucaria angustifolia* (45); *Casearia sylvestris* (32).	809	5,5	3,06	0,74
							Seasonal forest				
Lindenmaier & Budke (2006)	RS	1	1	15	49	Myrtaceae (7); Meliaceae (4); Salicaceae (5); Fabaceae (5).	*Eugenia ramboi* (397); *Cordia americana* (111); *Sorocea bomplandii* (94); *Casearia sylvestris* (68); *Cabralea canjerana* (56).	1097	-	2,68	0,66
Jarenkow & Waechter (2001)	RS	2	1	15,7	55	Fabaceae (7); Myrtaceae (6); Euphorbiaceae (5); Lauraceae (5); Meliaceae (5)	*Gymnanthes concolor* (632); *Euterpe edulis* (434); *Sorocea bomplandii* (255).	1855	-	2,24	0,56
Benvenuti-Ferreira & Coelho (2009)	RS	6	0,25	15,7	63	Fabaceae (11); Myrtaceae (4); Rutaceae (4); Sapindaceae (4); Lauraceae (4); Euphorbiaceae (4)	*Cordia americana* (45); *Diatenopteryx sorbifolia* (39); *Prunus myrtifolia* (27); *Matayba elaeagnoides* (26); *Machaerium stipitatum* (22).	508	-	3,68	0,89

Continuation Table 1.

Source	SL	CO	A	c.b.h.	N	RF	Abundant species (N)	Total abundance	RD (%)	H’	J
Balbinot et al. (2016)	RS	7	1	31,4	83	Fabaceae (14); Myrtaceae (4); Euphorbiaceae (4); Rutaceae (4); Meliaceae (4)	*Syagrus romanzoffiana* (130); *Sebastiania commersoniana* (53); *Nectandra megapotamica* (49); *Lonchocarpus muehlbergianus* (44); *Eugenia subterminalis* (30).	842	-	3,72	-
Schneider & Rocha (2014)	SC	11	0,4	25	54	Fabaceae (9); Myrtaceae (5); Meliaceae (5); Sapincaceae (4); Lauraceae (4);	*Sorocea bomplandii* (52); *Nectandra megapotamica* (50); *Syagrus romanzoffiana* (27); *Trichilia claussenii* (27).	512	-	3,47	0,87
Gris et al. (2014)	PR	12	0,4	15	70	Fabaceae (10); Meliaceae (7); Myrtaceae (5); Lauraceae (4); Rutaceae (4)	*Euterpe edulis* (159); *Sorocea bomplandii* (99); *Guarea kunthiana* (57).	518	-	3,37	0,79
Araucaria forest											
Rondon-Neto et al. (2002)	RS	3	0,8	15,7	37	Myrtaceae (9); Lauraceae (3); Sapindaceae (3); Asteraceae (2); Rutaceae (2)	*Araucaria angustifolia* (217); *Sebastiania commersoniana* (43); *Lithraea brasiliensis* (41); *Zanthoxylum rhoifolium* (39); *Myrcia* sp. (38)	673	32,2	2,77	-
Ferreira et al. (2016)	SC	4	1	15,7	55	Myrtaceae (10); Asteraceae (5); Anacardiaceae (4); Lauraceae (4)	*Lithraea brasiliensis* (176); *Podocarpus lambertii* (125); *Araucaria angustifolia* (97); *Blepharocalix salicifolius* (97); *Acca sellowiana* (96).	1457	6,6	3,21	0,79
Silva et al. (2012)	SC	5	1	15,7	87	Myrtaceae (18); Asteraceae (6); Lauraceae (5); Salicaceae (5)	*Araucaria angustifolia* (198); *Myrcia splendens* (159); *Podocarpus lambertii* (139); *Jacaranda puberula* (103); *Myrcia bombycina* (78).	1783	11,1	3,60	0,82
Santos et al. (2012)	SC	9	0,66	30	36	Lauraceae (7); Myrtaceae (4); Bignoniaceae (3); Fabaceae (3); Sapindaceae (3)	*Prunus myrtifolia* (80); *Cupania vernalis* (37); *Nectandra megapotamica* (30); *Matayba elaeagnoides* (25); *Ocotea spixiana* (21).	439	3,5	2,79	-
Lingner et al. (2007)	SC	10	2,5	60	41	Myrtaceae (5); Asteraceae (3); Sapindaceae (3); Euphorbiaceae (2); Cunoniaceae (2)	*Araucaria angustifolia* (162); *Ocotea porosa* (75); *Cupania vernalis* (62); *Capsicodendron dinisii* (33); *Matayba elaeagnoides* (23).	502	32,2	2,54	-

Floristic similarity revealed that our study area (code number 8 - *) was more similar to the species composition of Seasonal forest (cophenetic correlation coefficient: 0.9092) (Figure 3). The cluster indicated two groups, and the first division was between species composition from both two forest typologies. Seasonal forest group was divided according to the hydrography: forests from the upper Uruguay and Paraná and Jacuí river basins. On the other hand, the higher altitude was important for the Araucaria forest in the southern Brazilian mountains. Furthermore, surveys geographically closer were more similar in species composition; Fabaceae has a greater richness in Seasonal forests from Uruguay and Paraná river basins, Myrtaceae is highly represented in Araucaria forests from northeastern Rio Grande do Sul, and Lauraceae is well distributed in both forest typologies (Table 1, Fig.1). In terms of species composition, *M. elaeagnoides*, *P. myrtifolia*, *Allophylus edulis* (A.St.-Hil., Cambess. & A. Juss.) Radlk, *Campomanesia xanthocarpa* O.Berg, *Cedrela fissilis* Vell. and *Nectandra megapotamica* (Spreng.) were registered in most part of the studies and also in our sampling. Otherwise, *A. angustifolia* was sampled only in Araucaria forests, broadly associated to higher altitudes in Santa Catarina and Rio Grande do Sul states.

Discussion

1. *Tree community structure and composition in a subtropical forest in southern Brazil*

The tree taxonomic diversity and evenness indexes found in this study had intermediate values when compared to other surveys in southern Brazil (Table 1). These indexes indicated the balance of few dominant species and many species with lower abundance. This result reflects the ecological stability and maturity of the community (Wright 2007, Warring et al. 2016).

Table 2. Structural parameters of the tree community surveyed at Chapecó National Forest, west Santa Catarina, southern Brazil. Species: identification of the species sampled in this study; N: number of individuals; RD: relative density (%); RF: relative frequency (%); RDo: relative dominance (%); IV: importance value (%); CV: coverage value (%).

Family	Species	N	RD	RF	RDo	IV	CV
Rubiaceae	*Coussarea contracta* (Walp.) Müll.Arg.	177	21,88	6,6	5,56	11,3	13,72
Lauraceae	*Ocotea diospyrifolia* (Meisn.) Mez	110	13,6	7,2	20,52	13,8	17,06
Sapindaceae	*Cupania vernalis* Cambess.	84	10,38	6,3	6,46	7,7	8,42
Araucariaceae	*Araucaria angustifolia* (Bertol.) Kuntze	45	5,56	6,0	14,79	8,8	10,18
Salicaceae	*Casearia sylvestris* Sw.	32	3,96	4,2	1,38	3,2	2,67
Sapindaceae	*Diatenopteryx sorbifolia* Radlk.	30	3,71	4,2	2,80	3,6	3,25
Fabaceae	*Myrocarpus frondosus* Allemão	27	3,34	3,6	2,54	3,2	2,94
Myrtaceae	*Campomanesia xanthocarpa* (Mart.) O.Berg	20	2,47	3,9	1,31	2,6	1,89
Rutaceae	*Helietta apiculata* Benth.	19	2,35	3,0	1,70	2,3	2,02
Sapindaceae	*Matayba elaeagnoides* Radlk.	19	2,35	3,9	4,21	3,5	3,28
Primulaceae	*Myrsine umbellata* Mart.	18	2,22	3,6	0,83	2,2	1,53
Boraginaceae	*Cordia americana* (L.) Gottschling & J.S.Mill.	17	2,1	1,5	3,50	2,4	2,80
Salicaceae	*Banara tomentosa* Clos	16	1,98	2,7	0,52	1,7	1,25
Meliaceae	*Cedrela fissilis* Vell.	16	1,98	4,2	3,92	3,4	2,95
Sapotaceae	*Chrysophyllum marginatum* (Hook. & Arn.) Radlk.	16	1,98	3,0	1,24	2,1	1,61
Malvaceae	*Luehea divaricata* Mart. & Zucc.	12	1,48	1,8	6,71	3,3	4,10
Salicaceae	*Casearia decandra* Jacq.	11	1,36	2,4	0,32	1,4	0,84
Fabaceae	*Ateleia glazioveana* Baill.	10	1,24	1,8	2,47	1,8	1,85
Lauraceae	*Nectandra megapotamica* (Spreng.) Mez	9	1,11	1,5	0,79	1,1	0,95
Rutaceae	*Pilocarpus pennatifolius* Lem.	9	1,11	0,6	0,24	0,7	0,68
Fabaceae	*Peltophorum dubium* (Spreng.) Taub.	7	0,87	1,8	2,73	1,8	1,80
Loganiaceae	*Strychnos brasiliensis* Mart.	7	0,87	1,2	0,34	0,8	0,60
Meliaceae	*Cabralea canjerana* (Vell.) Mart.	6	0,74	1,8	0,29	0,9	0,51
Sapotaceae	*Chrysophyllum gonocarpum* (Mart. & Eichler ex Miq.) Engl.	6	0,74	0,9	0,25	0,6	0,50
Annonaceae	*Annona rugulosa* (Schltdl.) H.Rainer	5	0,62	1,5	0,17	0,8	0,40
Salicaceae	*Casearia obliqua* Spreng.	5	0,62	1,5	0,25	0,8	0,43
Aquifoliaceae	*Ilex paraguariensis* A.St.-Hil.	5	0,62	1,2	0,57	0,8	0,59
Fabaceae	*Parapiptadenia rigida* (Benth.) Brenan	5	0,62	1,5	3,34	1,8	1,98
Moraceae	*Sorocea bonplandii* (Baill.) W.C.Burger et al.	5	0,62	0,9	0,12	0,6	0,37
Lauraceae	*Nectandra lanceolata* Nees	4	0,49	0,9	0,91	0,8	0,70
Euphorbiaceae	*Gymnanthes klotzschiana* Müll.Arg.	4	0,49	0,9	0,34	0,6	0,42
Sapindaceae	*Allophylus edulis* (A.St.-Hil. et al.) Hieron. ex Niederl.	3	0,37	0,6	0,26	0,4	0,31
Rutaceae	*Balfourodendron riedelianum* (Engl.) Engl.	3	0,37	0,3	0,11	0,3	0,24
Boraginaceae	*Cordia trichotoma* (Vell.) Arráb. ex Steud.	3	0,37	0,9	0,49	0,6	0,43
Myrtaceae	*Eugenia involucrata* DC.	3	0,37	0,9	0,08	0,5	0,23
Fabaceae	*Muellera campestris* (Mart. ex Benth.) M.J. Silva & A.M.G. Azevedo	3	0,37	0,9	0,74	0,7	0,55
Polygonaceae	*Ruprechtia laxiflora* Meisn.	3	0,37	0,9	0,26	0,5	0,32
Rutaceae	*Zanthoxylum petiolare* A.St.-Hil. & Tul.	3	0,37	0,9	0,22	0,5	0,29
Apocynaceae	*Aspidosperma australe* Müll.Arg.	2	0,25	0,6	0,42	0,4	0,34
Fabaceae	*Calliandra foliolosa* Benth.	2	0,25	0,6	0,04	0,3	0,14
Fabaceae	*Erythrina falcata* Benth.	2	0,25	0,6	0,51	0,5	0,38
Fabaceae	*Holocalyx balansae* Micheli	2	0,25	0,6	0,73	0,5	0,49
Aquifoliaceae	*Ilex brevicuspis* Reissek	2	0,25	0,6	0,40	0,4	0,32
Bignoniaceae	*Jacaranda micrantha* Cham.	2	0,25	0,6	0,39	0,4	0,32
Cunoniaceae	*Lamanonia ternata* Vell.	2	0,25	0,3	1,77	0,8	1,01

Continuation Table 2.

Family	Species	N	RD	RF	RDo	IV	CV
Lauraceae	*Ocotea puberula* (Rich.) Nees	2	0,25	0,6	0,56	0,5	0,40
Euphorbiaceae	*Sebastiania brasiliensis* Spreng.	2	0,25	0,3	0,11	0,2	0,18
Euphorbiaceae	*Actinostemon concolor* (Spreng.) Müll.Arg.	1	0,12	0,3	0,02	0,2	0,07
Annonaceae	*Annona neosalicifolia* H.Rainer	1	0,12	0,3	0,02	0,2	0,07
Fabaceae	*Apuleia leiocarpa* (Vogel) J.F.Macbr.	1	0,12	0,3	0,22	0,2	0,17
Erythroxylaceae	*Erythroxylum deciduum* A.St.-Hil.	1	0,12	0,3	0,24	0,2	0,18
Myrtaceae	*Eugenia pyriformis* Cambess.	1	0,12	0,3	0,12	0,2	0,12
Fabaceae	*Inga virescens* Benth.	1	0,12	0,3	0,03	0,2	0,07
Fabaceae	*Machaerium stipitatum* Vogel	1	0,12	0,3	0,05	0,2	0,08
Lauraceae	*Ocotea pulchella* (Nees & Mart.) Mez	1	0,12	0,3	0,03	0,2	0,08
Simaroubaceae	*Picrasma crenata* (Vell.) Engl.	1	0,12	0,3	0,03	0,2	0,07
Rosaceae	*Prunus myrtifolia* (L.) Urb.	1	0,12	0,3	0,84	0,4	0,48
Rubiaceae	*Randia ferox* (Cham. & Schltdl.) DC.	1	0,12	0,3	0,04	0,2	0,08
Styraceae	*Styrax leprosus* Hook. & Arn.	1	0,12	0,3	0,07	0,2	0,09
Apocynaceae	*Tabernaemontana catharinensis* A.DC.	1	0,12	0,3	0,08	0,2	0,10
Rutaceae	*Zanthoxylum kleinii* (R.S.Cowan) P.G.Waterman	1	0,12	0,3	0,02	0,2	0,07

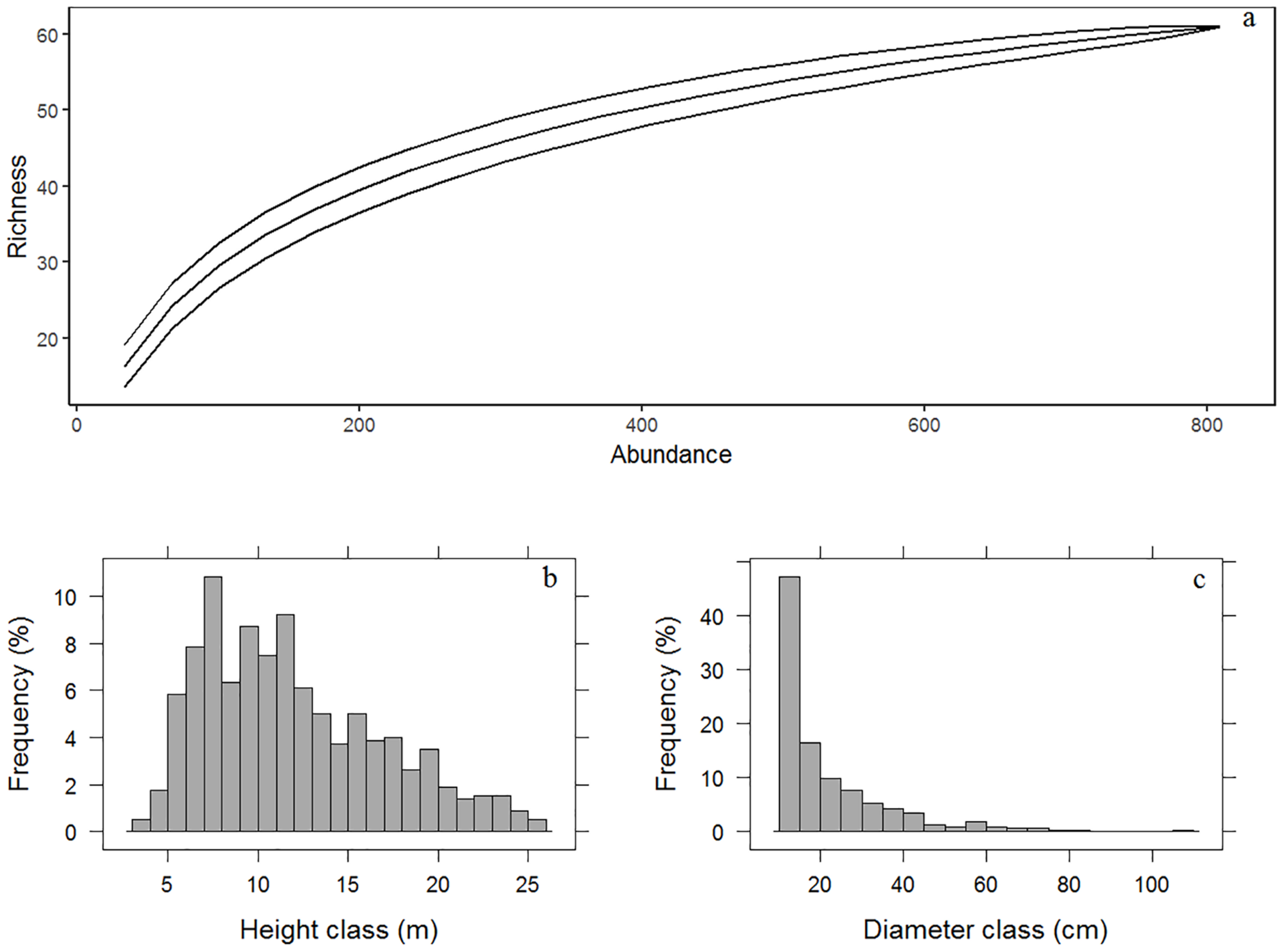


Figure 2. Tree community structure of Chapecó National Forest, west Santa Catarina, southern Brazil. a) Sample adequacy represented by a rarefaction curve standardizing a number of species and individuals. Upper and lower line estimates are also showed. b) Height distribution from three to 26 meters height. c) Diameter classes from 9.54 cm to 107 cm, with intervals of five centimeters each class. The first class presents all individuals with 9.54 cm to 15 cm d.b.h.

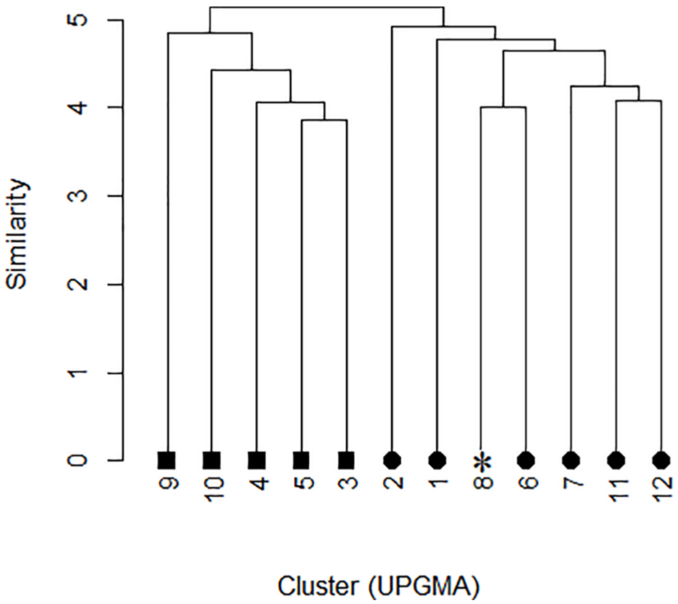


Figure 3. Cluster analysis, using the unweighted pair group method with arithmetic mean (UPGMA) and Jaccard similarity coefficient demonstrating the floristic similarities of 12 studies sampled at Araucaria and Seasonal forests, in southern Brazil. Cophenetic Correlation Coefficient = 0.9092. See table 1 and fig 1 for site codes.

The species with higher importance values are common and important in other surveys in the Araucaria and Seasonal forests of Santa Catarina (Gasper, Uhlmann, et al. 2013, Gasper, Sevegnani, et al. 2013, Gasper et al. 2015, Vibrans et al. 2011, Schneider & Rocha 2014, Vibrans et al. 2013a, 2012). *Ocotea diospyrifolia*, *Cupania vernalis*, *Casearia sylvestris*, *Diatenopteryx sorbifolia* and *Myrocarpus frondosus* are common at Seasonal forest (Fontana & Sevegnani 2012) and *A. angustifolia*, *Coussarea contracta* e *Cupania vernalis,* are typical from components of early and intermediate successional stages in the Araucaria forest of Santa Catarina (Klauberg et al. 2010, Vibrans et al. 2011). According to Gasper et al. (2015), *Cupania vernalis*, *Diatenopteryx sorbifolia* and *Myrocarpus frondosus* characterize the transitional forests from the west of Santa Catarina.

We did not observe the asymptotic richness level in the rarefaction curve. It was found in other studies and was justified by the environmental heterogeneity or human influence in the landscape (Ruschel et al. 2007, Benvenuti-Ferreira & Coelho 2009). Also, it is important to remember that forest communities are dynamic entities, where species are associated with some spatial and temporal variations (Schilling & Batista 2008). In our study, this result is probably related to this dynamic and either the small number (24) and big size of each sampling unit (500 m²), whereas the usually recommended for these studies are sampling units that maximize the sampling area (*e.g.* more sampling units with a smaller size).

The inverted-J curve of diameter classes found in this study is common for different forest typologies and it indicates the forest regeneration and permanence of the species in forest structure (Balbinot et al. 2016). Most individuals sampled are small trees of the forest understory and it is also indicative of forest regeneration capacity (Cunha & Silva Jr. 2012). On the other hand, individuals in the biggest size classes usually represent species with greater longevity (Chao et al. 2008) and their mortality contributes to de gap dynamics in forests (Yamamoto 2000).

Fabaceae frequently exhibits great species richness at Seasonal forests in southern Brazil (Jurinitz & Jarenkow 2003, Benvenuti-Ferreira & Coelho 2009). It is related to the immigration corridor of the Uruguay and Paraná river basins, which connects the species of Fabaceae from the north to southern Brazil. However, further from the corridor towards the south, the number of species of this family decreases (Budke et al. 2005, Benvenuti-Ferreira & Coelho 2009, Jurinitz & Jarenkow 2003). On the other hand, Lauraceae species are usually described as highly abundant in the Araucaria forest, acting as indicator species associated to this forest typology (Gasper, Sevegnani, et al. 2013). Leyser et al. (2009) demonstrated high species richness of Fabaceae and Lauraceae in the transitional area between Araucaria and Seasonal forest, as found in this study, which may also indicate an association between these families in ecotonal areas. In general, Myrtaceae species are widely distributed in Atlantic forest *s.l.*, but with lower abundance in the SF of Santa Catarina, leading to a rare occurrence specially driven by the climatic filters in the region (Fontana et al. 2014). These associations reflect complex species interactions which may also contribute powerfully to forest structuring and patterns of species distributions, leading to more studies and efforts in ecotonal areas.

2. *Floristic relationships between two forest typologies*

Despite the presence of *A. angustifolia*, the transitional forest of this study has great floristic similarity to the Seasonal forest. According to the cluster analysis, the vegetation type was the main grouping factor, followed by geographical proximity, as observed by other authors (Oliveira-Filho et al. 2001, Gonçalves & Souza 2014, Estevan et al. 2016). The species composition of our study area must be also related to the altitude of the region, which is close to Uruguay river valley (around 600 m a.s.l.). In general, altitude is considered an important variable for the species distribution in the Atlantic forest *s.l.* (Eisenlohr et al. 2015), especially for Araucaria forest species, which occurrence is conditioned by higher altitudes, leading to higher local densities (Backes 2009, Higuchi et al. 2012, Viani et al. 2011). In our case, the lower altitude could be acting as an environmental filter (Higuchi et al. 2012), associated with the great amplitude and thermal seasonality and higher maximum temperature in the hottest month leading to the limitation of the species range of the Araucaria forest and with a pronounced presence of the Seasonal forest species (Gasper, Uhlmann, et al. 2013, Gasper et al. 2015).

Among the potential drivers associated to species occurrence and distribution, the biogeographic factor is another important driver related to actual species occurrence and communities diversity in southern Brazil (Rezende et al. 2016). It may be thus related to the species composition of Seasonal forests, which is also promoted by dispersal from Paraná river basin and the Misiones forests (Gasper et al. 2012) and leads to higher species diversity and great floristic similarity to rainforests (Eisenlohr & Oliveira-Filho 2015; Rezende et al. 2016). In the other hand, the Araucaria forest has a low number of unique species (Eisenlohr & Oliveira-Filho 2015) and presents physiognomic, floristic and structural differences throughout its distribution (Jarenkow and Budke 2009; Higuchi et al. 2012), leading to a particular forest structure and composition, different from other forest types (Eisenlohr & Oliveira-Filho 2015). This forest occurs mainly in regions with higher altitude and has a gradual overlap of the species towards southwestern Brazil, making contact with the Seasonal forest species on Uruguay and Paraná river basins (Jarenkow and Budke 2009).

Regard to the *A. angustifolia* dynamics along the time in this forest, there is a clear pattern of reduction of the population (Bordin 2018), which reinforces the Seasonal forest species turnover on the Araucaria forest species. Paleoecological analysis in the Araucaria forest areas at Rio Grande do Sul state revealed that population of *A. angustifolia* was much larger than today, where we can find typical semi-deciduous species occurring in these areas (Behling et al. 2016). It indicates that same pattern of turnover from Araucaria to Seasonal forest species composition is a trend found in other Atlantic Forest *s.l.* areas.

The subtropical forest presented here is well structured and highly diverse. Also, is an extremely important forest fragment for the local and regional biodiversity conservation, especially due to the presence of Seasonal forest species (Bergamin et al. 2017).

Concerning to forest characterization, despite the presence of *Araucaria angustifolia*, the species composition is closely related to the Seasonal forests, reinforcing the strong west-east pattern of species composition. Briefly, Seasonal forest is the floristic classification that should be considered in further analysis. This turnover of species along the regions strengthens the conservation necessity for protecting this pool of species and remaining forested areas, especially in this conservation unit.

Acknowledgements

We are grateful to ICMBio (License nº 56024/2017) for the logistical support during the sampling; to the fieldworkers Cristiano Ilha, Marciela Batistela and Francieli Delazeri and to Unochapecó for the logistical support and equipment. We also thank the two anonymous reviewers for the valuable suggestions. KMB has financial support from Coordenação de Aperfeiçoamento Pessoal de Nível Superior.

Author Contributions

Kauane Maiara Bordin: contributed in the concept and design of the study, data collection, data analysis, interpretation and to manuscript preparation, providing critical revision, and adding intellectual content.

Letícia Daiana Ferreira, Adriano Rosina, Marciana Malacarne, Patricia Zanotelli: contributed to data collection and intellectual content.

Samuel Fernando Adami: data analysis, interpretation and to manuscript preparation and intellectual content.

Giovana Secretti Vendruscolo: contributed in the concept and design of the study, data collection, data analysis, interpretation and to manuscript preparation, providing critical revision, and adding intellectual content.

Conflict of interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

BACKES, A. 2009. Distribuição geográfica atual da Floresta com Araucária: condicionamentos climáticos. In Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável (C. R. Fonseca, A. F. Souza, A. Leal-Zanchet, T. Dutra, A. Backes, & G. Ganade, eds) Holos Editora, p.328.

BALBINOT, R., LAMBRECHT, F.R., BREUNIG, F.M., TRAUTENMULLER, J.W., GALVÃO, L.S., DENARDI, L. & VENDRUSCOLO, R. 2016. Análise fitossociológica de um fragmento de Floresta Estacional Decidual: Parque Estadual do Turvo, RS. Pesqui. Florest. Bras. - Brazilian J. For. Res. 36(86):103–113.

BEHLING, H., VERISSIMO, N., BAUERMANN, S., BORDIGNON, S. & EVALDT, A. 2016. Late holocene vegetation history and early evidence of Araucaria angustifolia in Caçapava do sul in the lowland region of Rio Grande do Sul State, southern Brazil. Brazilian Arch. Biol. Technol. 59(December):1–10.

BENVENUTI-FERREIRA, G. & COELHO, G.C. 2009. Floristics and structure of the tree component in a Seasonal Forest remnant, Chiapetta, Rio Grande do Sul State, Brazil. Brazilian J. Biosci. 7(4):344–353.

BERGAMIN, R.S., BASTAZINI, V.A.G., VÉLEZ-MARTIN, E., DEBASTIANI, V., ZANINI, K.J., LOYOLA, R. & MÜLLER, S.C. 2017. Linking beta diversity patterns to protected areas: lessons from the Brazilian Atlantic Rainforest. Biodivers. Conserv. 26(7):1557–1568.

BORDIN, K.M. (2018) Dinâmica da biomassa em uma floresta subtropical: efeito de componentes bióticos e abióticos. Dissertação. Universidade Federal do Rio Grande do Sul.

BUDKE, J.C., ATHAYDE, E.A., GIEHL, E.L.H., ZÁCHIA, R.A. & EISINGER, S.M. 2005. Composição florística e estratégias de dispersão de espécies lenhosas em uma floresta ribeirinha, arroio Passo das Tropas, Santa Maria, RS, Brasil. Iheringia - Ser. Bot. 60(1):17–24.

CHAO, K.J., PHILLIPS, O.L., GLOOR, E., MONTEAGUDO, A., TORRES-LEZAMA, A. & MARTÍNEZ, R.V. 2008. Growth and wood density predict tree mortality in Amazon forests. J. Ecol. 96(2):281–292.

CORNWELL, W.K. & ACKERLY, D.D. 2009. Community Assembly and Shifts in Plant Trait Distributions across an Environmental Gradient in Coastal California Published by: Ecological Society of America Community across an environmental in plant trait distributions assembly and shifts gradient and. Ecol. Monogr. 79(1):109–126.

CUNHA, M.D.C.L. & SILVA JR., M.C. 2012. Estrutura diamétrica e hipsométrica na Floresta Estacional Semidecidual Montana do Pico do Jabre - PB. Rev. Bras. Ciencias Agrar. 7(2):292–300.

EISENLOHR, P. V. & OLIVEIRA-FILHO, A.T. de. 2015. Revisiting Patterns of Tree Species Composition and their Driving Forces in the Atlantic Forests of Southeastern Brazil. Biotropica 47(6):689–701.

EISENLOHR, P. V., OLIVEIRA-FILHO, A.T. de & PRADO, J. 2015. The Brazilian Atlantic Forest: new findings, challenges and prospects in a shrinking hotspot. Biodivers. Conserv. 24(9):2129–2133.

ESTEVAN, D.A., VIEIRA, A.O.S. & GORENSTEIN, M.R. 2016. Estrutura E Relações Florísticas De Um Fragmento De Floresta Estacional Semidecidual, Londrina, Paraná, Brasil. Ciência Florest. 26(3):713.

FERREIRA, T. de S., MARCON, A.K., SALAMI, B., RECH, C.C.C., MENDES, A.R., CARVALHO, A.F., MISSIO, F. de F., PSCHEIDT, F., GUIDINI, A.L., DORNELLES, R. da S., SILVA, A.C. & HIGUCHI, P. 2016. Composição florístico-estrutural ao longo de um gradiente de borda em fragmento de floresta ombrófila mista alto-montana em Santa Catarina. Cienc. Florest. 26(1):123–134.

FONTANA, C., GASPER, A.L. & SEVEGNANI, L. 2014. Espécies raras e comuns de Myrtaceae da Floresta Estacional Decidual de Santa Catarina, Brasil. Rodriguesia 65(3):767–776.

FONTANA, C. & SEVEGNANI, L. 2012. Quais são as espécies arbóreas comuns da Floresta Estacional Decidual em Santa Catarina? Rev. Estud. Ambient. 14(1esp):74–88.

GASPER, A.L. de, SEVEGNANI, L. & VIBRANS, A. 2013. Inventário florístico florestal de Santa Catarina: espécies da Floresta Ombrófila Mista. Rodriguésia 64(2):201–210.

GASPER, A.L. De, UHLMANN, A., SEVEGNANI, L., LINGNER, D.V., RIGON-JÚNIOR, M.J., VERDI, M., STIVAL-SANTOS, A., DREVECK, S., SOBRAL, M., VIBRANS, A.C. & DECIDUAL, E. 2013. Inventário Florístico Florestal de Santa Catarina: espécies da Floresta Estacional Decidual. Rodriguésia 64(3):427–443.

GASPER, A.L. de, UHLMANN, A., VIBRANS, A.C. & SEVEGNANI, L. 2015. Variação da estrutura da floresta estacional decidual no estado de Santa Catarina e sua relação com a altitude e clima. Ciência Florest. 77–89.

GASPER, A.L., SEVEGNANI, L., MEYER, L., SOBRAL, M., VERDI, M., STIVAL-SANTOS, A., DREVECK, S., KORTE, A. & UHLMANN, A. 2012. Flora vascular da Floresta Estacional Decidual em Santa Catarina. In Inventário Florístico Florestal de Santa Catarina: espécies da Floresta Estacional Decidual Edifurb, p.111–122.

GONÇALVES, E.T. & SOUZA, A.F. 2014. Floristic variation in ecotonal areas: Patterns, determinants and biogeographic origins of subtropical forests in South America. Austral Ecol. 39(1):122–134.

GRIS, D., TEMPONI, L. & DAMASCENO JUNIOR, G. 2014. Structure and floristic diversity of remnant semideciduous forest under varying levels of disturbance. Acta Bot. Brasilica 28(4):569–576.

HIGUCHI, P., SILVA, A.C. da, FERREIRA, T.S., SOUZA, S.T. de, GOMES, J.P., SILVA, K.M. da & SANTOS, K.F. do. 2012. Floristic composition and phytogeography of the tree component of Araucaria Forest fragments in southern Brazil. Brazilian J. Bot. 35(2):145–157.

HUBBELL, S.. 2001. The Unified Neutral Theory of Biodiversity and Biogeography. Monograps in population biology.

IBGE - INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA. 2012. Manual Técnico da Vegetação Brasileira. 2a edição ed. Instituto Brasileiro de Geografia e Estatística - IBGE, Rio de Janeiro.

ICMBio. 2013. Plano de Manejo da Floresta Nacional de Chapecó. ICMBio, Instituto Chico Mendes de Conservação da Biodiversidade, Florianópolis.

JARENKOW, J. A., BUDKE, J. 2009. Padrões florísticos e análise estrutural de remanescentes de Florestas com Araucária no Brasil. In Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável (C. R. Fonseca, A. F. Souza, A. Leal-Zanchet, T. Dutra, A. Backes, & G. Ganade, eds) Holos Editora, p.328.

JARENKOW, J.A. & WAECHTER, J.L. 2001. Composição, estrutura e relações florísticas do componente arbóreo de uma floresta estacional no Rio Grande do Sul, Brasil. Rev. Bras. Botânica 24(3):263–272.

JOLY, C.A., METZGER, J.P., TABARELLI, M., JOLY, C.A., JOLY, C.A., METZGER, J.P. & TABARELLI, M. 2014. Experiences from the Brazilian Atlantic Forest: ecological findings and conservation initiatives. New Phytol. 204(3):459–473.

JUNYAN, Z., KEWU, C., RUNGUO, Z. & YI, D. 2014. Changes in floristic composition, community structure and species diversity across a tropical coniferous- broadleaved forest ecotone. Trop. Conserv. Sci. 7(1):126–144.

JURINITZ, C.F. & JARENKOW, J.A. 2003. Estrutura do componente arbóreo de uma floresta estacional na Serra do Sudeste, Rio Grande do Sul, Brasil. Rev. Bras. Botânica 26(4):475–487.

KLAUBERG, C., PALUDO, G.F., BORTOLUZZI, R.L.C. & MANTOVANI, A. 2010. Florística e estrutura de um fragmento de Floresta Ombrófila Mista no Planalto Catarinense. Biotemas 23(1):35–47.

KLEIN, R.M. 1960. O aspecto dinâmico do pinheiro brasileiro. Sellowia 1217–44.

KLEIN, R.M. 1963. Observações e considerações sobre o Planalto nordeste Catarinense. Sellowia 1539–56.

KLEIN, R.M. 1978. Mapa fitogeográfico do estado de Santa Catarina. In Flora Ilustrada Catarinense. (R. Reitz, ed.) Herbário Barbosa Rodrigues, p.24.

KRAFT, N.J.B., ADLER, P.B., GODOY, O., JAMES, E.C., FULLER, S. & LEVINE, J.M. 2015. Community assembly, coexistence and the environmental filtering metaphor. Funct. Ecol. 29(5):592–599.

LEGENDRE, P. & LEGENDRE, L. 2012. Numerical Ecology. Elsevier.

LEYSER, G., VINISKI, M., DONIDA, A.L., ZANIN, E.M. & BUDKE, J.C. 2009. Espectro de dispersão em um fragmento de transição entre Floresta Ombrófila Mista e Floresta Estacional na região do Alto Uruguai, Rio Grande do Sul, Brasil. Pesqui. Série Botânica 60355–366.

LIEBSCH, D., MARQUES, M.C.M. & GOLDENBERG, R. 2008. How long does the Atlantic Rain Forest take to recover after a disturbance? Changes in species composition and ecological features during secondary succession. Biol. Conserv. 141(6):1717–1725.

LINDENMAIER, D. de S. & BUDKE, J.C. 2006. Florística, Diversidade E Distribuição Espacial Das Espécies Arbóreas Em Uma Floresta Estacional Na Bacia Do Rio Jacuí, Sul Do Brasil. São Leopoldo 57193–216.

LINGNER, D.V.; OLIVEIRA, Y.M.M.D.; ROSOT, N.C. & DLUGOSZ, F.L. 2007. Caracterização da estrutura e da dinâmica de um remanescente de floresta de araucária no Planalto Catarinense. Pesquisa Florestal Brasileira 55: 55–66.

LUCAS, E.M. & FORTES, V.B. 2008. Frog diversity in the Floresta Nacional de Chapecó, Atlantic Forest of southern Brazil. Biota Neotrop. 8(3):0–0.

MAGURRAN, A.E. 2004. Measuring biological diversity. Oxford, Blackwell Science. 256p.

MORELLATO, L.P.C. & HADDAD, C.F.B. 2000. Introduction: The Brazilian Atlantic Forest. Biotropica 32(4b):786–792.

MUELLER-DOMBOIS, D. & ELLEMBERG, H. 1974. Aims and methods of vegetation ecology. New York: John Wiley and Sons. 547 p

MYERS, N., MITTERMEIER, R.A., MITTERMEIER, C.G., DA FONSECA, G.A.B. & KENT, J. 2000. Biodiversity hotspots for conservation priorities. Nature 403(6772):853–858.

NIMER, E. (1989) Climatologia do Brasil. 2nd ed. IBGE, Departamento de Recursos Naturais e Estudos Ambientais, Rio de Janeiro.

OLIVEIRA-FILHO, A., CURI, N., VILELA, E.. A. & CARVALHO, D.. A. 2001. Variation in Tree Community Composition and Structure With Changes in Soil Properties Within a Fragment of Semideciduous Forest in South-Eastern Brazil. Geographical 58(1):139–158.

OLIVEIRA-FILHO, A.T., BUDKE, J.C., JARENKOW, J.A., EISENLOHR, P. V., NEVES, D.R.M. 2014. Delving into the variations in tree species composition and richness across South American subtropical Atlantic and Pampean forests. J. Plant Ecol. 8(3):242–260.

OLIVEIRA-FILHO, A.T. & FONTES, M.A.L. 2000. Patterns of Floristic Differentiation among Atlantic Forests in Southeastern Brazil and the Influence of Climate. Biotropica 32(2):793–810.

OVERBECK, G.E., MÜLLER, S.C., FIDELIS, A., PFADENHAUER, J., PILLAR, V.D., BLANCO, C.C., BOLDRINI, I.I., BOTH, R. & FORNECK, E.D. 2007. Brazil's neglected biome: The South Brazilian Campos. Perspect. Plant Ecol. Evol. Syst. 9(2):101–116.

R CORE TEAM. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https://www.r-project.org/.

RAMBO, B. 1951. O Elemento Andino no Pinhal Riograndense. An. Botânicos do Herbário Barbosa Rodrigues 2(2):7–39.

RAMBO, B. 1953. História da Flora do Planalto Riograndense. An. Botânicos do Herbário Barbosa Rodrigues 5185–232.

REITZ, R., KLEIN, R.M. & REIS, A. 1978. Projeto Madeira de Santa Catarina. Sellowia 2811–320.

REZENDE. V.L., BUENO, M.L., OLIVEIRA-FILHO, A,T. (2016) Patterns of tree composition in the southern cone of South America and its relevance to the biogeographic regionalization. Plant Ecology 217:97–110

RISSER, P.G. 1995. The status of the science examining ecotones. Bioscience 45(5):318–325.

RONDON-NETO, R.M., WATZLAWICK, L.F., CALDEIRA, M.V.W. & SCHOENINGER, E.R. 2002. Análise florística e estrutural de um fragmento de Floresta Ombrófila Mista Montana, situado em Criúva, RS - Brasil. Ciência Florest. 12(1):29–37.

RUSCHEL, A.R., NODARI, R.O. & MOERSCHBACHER, B.M. 2007. Woody plant species richness in the Turvo State park, a large remnant of deciduous Atlantic forest, Brazil. Biodivers. Conserv. 16(6):1699–1714.

SANTOS, C.S. dos, CHIOSSI, R.Y., GASPARIN, AVILA, A.L. de & GASPARIN, E. 2012. Levantamento florístico e fitossociológico de um fragmento florestal no município de Faxinal dos Guedes, SC. Unoesc e Ciência - ACET 3(1):7–22.

SCHILLING, A.C. & BATISTA, J.L.F. 2008. Curva de acumulação de espécies e suficiência amostral em florestas tropicais. Rev. Bras. Botânica 31(1):179–187.

SCHNEIDER, G. & ROCHA, F.S. 2014. Levantamento florístico e fitossociológico do componente arbóreo de um fragmento de Floresta Estacional Decidual em São Miguel do Oeste, Santa Catarina. Biotemas 27(2):43–55.

SILVA, A.C. da, HIGUCHI, P., AGUIAR, M.D. de, NEGRINI, M., NETO, J.F. & HESS, A.F. 2012. Relações florísticas e fitossociologia de uma floresta ombrófila mista montana secundária em lages, Santa Catarina. Ciência Florestalncia Florest. 22(1):193–206.

SPICHIGER, R., CALENGE, C. & BISE, B. 2004. Geographical zonation in the Neotropics of tree species characteristic of the Paraguay-Paraná Basin. J. Biogeogr. 31(9):1489–1501.

THE ANGIOSPERM PHYLOGENY GROUP. 2016. An update of the Angiosperm Phylogeny Group Classification for the orders and families of floweringplants: APG IV. Bot. J. Linn. Soc. 1811–20.

VIANI, R.A.G., COSTA, J.C., ROZZA, A. de F., BUFO, L.V.B., FERREIRA, M.A.P. & OLIVEIRA, A.C.P. de. 2011. Caracterização florística e estrutural de remanescentes florestais de Quedas do Iguaçu, Sudoeste do Paraná. Biota Neotrop. 11(1):115–128.

VIBRANS, A.C., SEVEGNANI, L., GASPER, A.L. de & LINGNER, D. V. 2012. Inventário Florístico Florestal de Santa Catarina, vol II: Floresta Estacional Decidual. Edifurb.

VIBRANS, A.C., SEVEGNANI, L., GASPER, A.L. de & LINGNER, D.V. 2013a. Inventário Florístico Florestal de Santa Catarina, vol IV: Floresta Ombrófila Densa. Edifurb.

VIBRANS, A.C., SEVEGNANI, L., GASPER, A.L. & LINGNER, D.V. 2013b. Inventário Florístico Florestal de Santa Catarina, vol. III: Floresta Ombrófila Mista. Edifurb.

VIBRANS, A.C., SEVEGNANI, L., LINGNER, D.V., GASPER, A.L. de & SABBAGH, S. 2010. Inventário florístico florestal de Santa Catarina (IFFSC): aspectos metodológicos e operacionais. Pesqui. Florest. Bras. 30(64):291–302.

VIBRANS, A.C., SEVEGNANI, L., UHLMANN, A., SCHORN, L.A., SOBRAL, M.G., GASPER, A.L., LINGNER, D. V., BROGNI, E., KLEMZ, G., GODOY, M.B. & VERDI, M. 2011. Structure of mixed ombrophyllous forests with Araucaria angustifolia (Araucariaceae) under external stress in Southern Brazil. Rev. Biol. Trop. 59(3):1371–1387.

WAECHTER, J.L. 2009. Epífitos vasculares da Floresta com Araucária do sul do Brasil. In Floresta com Araucária: Ecologia, Conservação e Desenvolvimento Sustentável (C. R. Fonseca, A. F. Souza, A. Leal-Zanchet, T. Dutra, A. Backes, & G. Ganade, eds) Holos Editora, p.328.

WARRING, B., CARDOSO, F.C.G., MARQUES, M.C.M. & VARASSIN, I.G. 2016. Functional diversity of reproductive traits increases across succession in the Atlantic forest. Rodriguesia 67(2):321–333.

WRIGHT, S.J. 2007. Plant Diversity in Tropical Forests. In Functional plant ecology (F. I. Pugnaire & F. Valladares, eds) Taylor & Francis Group, p.748.

XU, J., CHEN, Y., ZHANG, L., CHAI, Y., WANG, M., GUO, Y., LI, T. & YUE, M. 2017. Using phylogeny and functional traits for assessing community assembly along environmental gradients: A deterministic process driven by elevation. Ecol. Evol. 7(14):5056–5069.

YAMAMOTO, S.I. 2000. Forest gap dynamics and tree regeneration. J. For. Res. 5(4):223–229.

Received: 17/07/2018
Revised: 21/01/2019
Accepted: 25/02/2019
Published online: 18/03/2019

Biota Neotropica 19(2): e20180568, 2019
www.scielo.br/bn

biotaneotropica
ISSN 1676-0611 (online edition)
Inventory

Periphytic diatoms from an oligotrophic lentic system, Piraquara I reservoir, Paraná state, Brazil

Angela Maria da Silva-Lehmkuhl[1,2], Priscila Izabel Tremarin[3], Ilka Schincariol Vercellino[4] &*

Thelma A. Veiga Ludwig[5]

[1]Universidade Federal do Amazonas, Estrada Parintins Macurany, 1805, 69152-240, Parintins, AM, Brasil
[2]Universidade Estadual Paulista Julio de Mesquita Filho, 13506-900, Rio Claro, SP, Brasil
[3]Acqua Diagnósticos Ambientais Ltda., Curitiba, PR, Brasil
[4]Centro Universitario São Camilo, São Paulo, SP, Brasil
[5]Universidade Federal do Paraná, Av. Cel. Francisco H. dos Santos, Jardim das Américas, 81531980, Curitiba, PR, Brasil
**Corresponding author: Angela Maria da Silva-Lehmkuhl, e-mail: angela_ecologia@yahoo.com.br*

SILVA-LEHMKUHL, A.M., TREMARIN, P.I., VERCELLINO, I.S., LUDWIG, T.A.V. **Periphytic diatoms from an oligotrophic lentic system, Piraquara I reservoir, Paraná state, Brazil**. Biota Neotropica. 19(2): e20180568. http://dx.doi.org/10.1590/1676-0611-BN-2018-0568

Abstract: Knowledge of biodiversity in oligotrophic aquatic ecosystems is fundamental to plan conservation strategies for protected areas. This study assessed the diatom diversity from an urban reservoir with oligotrophic conditions. The Piraquara I reservoir is located in an Environmental Protection Area and is responsible for the public supply of Curitiba city and the metropolitan region. Samples were collected seasonally between October 2007 and August 2008. Periphytic samples were obtained by removing the biofilm attached to *Polygonum hydropiperoides* stems and to glass slides. The taxonomic study resulted in the identification of 87 diatom taxa. The most representative genera regarding the species richness were *Pinnularia* (15 species) and *Eunotia* (14 species). Five species were registered for the first time in Brazil and seven in the State of Paraná. Taxonomic and ecological comments of the species registered are provided.
Keywords: *artificial substrate, diatom ecology, macrophytes, reservoirs.*

Diatomáceas perifíticas de um sistema lêntico oligotrófico, reservatório do Piraquara I, estado do Paraná, Brasil

Resumo: O conhecimento da biodiversidade em ecossistemas aquáticos oligotróficos é fundamental para planejar estratégias de conservação de áreas protegidas. Este estudo teve como objetivo conhecer a diversidade de diatomáceas em um reservatório urbano com condições oligotróficas. O reservatório do Piraquara I está inserido em uma Área de Proteção Ambiental em região de manancial e é responsável pelo abastecimento público de Curitiba e região metropolitana. Foram realizadas coletas estacionais entre os meses de outubro de 2007 e agosto de 2008. As amostras perifíticas foram obtidas pela remoção do biofilme aderido a caules de *Polygonum hydropiperoides* e de lâminas de vidro. O estudo taxonômico resultou na identificação de 87 táxons. Os gêneros mais representativos, considerando a riqueza de espécies, foram *Pinnularia* (15 espécies) e *Eunotia* (14 espécies). Cinco espécies de diatomáceas foram registradas pela primeira vez no Brasil e sete no estado do Paraná. Comentários taxonômicos e ecológicos também são apresentados para as espécies encontradas.
Palavras-chave: *substrato artificial, ecologia de diatomáceas, macrófitas, reservatório.*

Introduction

Diatoms are considered one of the most representative groups of microalgae due to the number of described species. Approximately 12,000 species of diatoms have been described hitherto (Guiry 2012) although the estimated number of species is thought to be at least 30,000 (Mann e Vanormeligen 2013). Diatoms are excellent bioindicators of water quality, being sensible to environmental pollution and a number of anthropogenic pressures, such as the increase in nutrient concentrations, acidification, land use, and the presence of toxic chemicals dissolved in water (Leskinen & Hällfors 1990, Lowe & Pan 1996, Lobo et al. 2002, Rimet et al. 2015).

Diatoms release mucilage through raphe, rimoportulae and apical pore fields, facilitating the frustule adhesion to different types of substrates. This strategy contributes to diatoms representativeness in the periphytic community (Round et al. 1990, Lowe 1996). The species composition of periphytic assemblages can be influenced by the substrate micro-topography (Murdock and Dodds 2007, Souza & Ferragut 2012). Inventories using substrates with different surface types usually hold a great algal diversity (Ács et al. 2000). Therefore, diatom inventories should be carried out in a great number of different environments and using diverse substrates to maximize the recovery of new species, extend the distribution of known species and better define their ecological preferences, which increase the reliability of environmental diagnostics.

Man-made lentic environments with oligotrophic conditions are rare in urban areas and are examples of environmental health. Unfortunately, human activities in the surrounding watershed have been accelerating the processes of eutrophication, affecting the water quality and the biodiversity, leading to the loss of important ecological functions (Tundisi 2003, Torrisi et al. 2010). Due to the undesirable impact of these activities on the water physical and chemical conditions, efforts have been generally focused on studying eutrophic environments rather than on preserving oligotrophic water bodies. For this reason, there is usually a gap in the knowledge of the algal diversity of protected areas, which creates exceptional opportunities to study the remaining oligotrophic environments (Kociolek & Stoermer 2009).

Recent studies in oligotrophic Brazilian reservoirs contributed to the description of new diatom species in the genera *Kurtkrammeria* and *Encyonema* (Marquardt et al. 2016, Marquardt et al. 2017) and extended the geographic distribution of the already known species (Canani et al. 2011). Paleolimnological studies with diatoms are important in providing a further understanding of biodiversity, detecting the response of the assemblages over time. Generally, a decrease in the diatom diversity during the eutrophication process can be observed when comparing environments that were oligotrophic but are currently eutrophised (Faustino et al. 2016, Wengrat et al. 2017).

Previous diatom inventories were carried out in urban reservoirs of the Iguaçu river basin along a trophic gradient ranging from mesotrophic (Passaúna, Bertolli et al. 2010 and Piraquara II, Marra et al. 2016), eutrophic (Iraí, Silva et al. 2010) to hypereutrophic (Itaqui, Faria et al. 2010). The Piraquara I reservoir, which was surveyed in the present study, is connected to Piraquara II reservoir. The macrophyte *Polygonum hydropiperoides* Michaux and glass slides were used as substrates to assess the periphytic diatoms in these reservoirs.

The present study aimed at assessing the composition of periphytic diatom assemblages from the oligotrophic reservoir Piraquara I. We provided measures, illustrations, taxonomical comments and ecological data based on scientific literature, in order to contribute to the knowledge of Brazilian diatoms from oligotrophic environments.

Material and Methods

The Piraquara I Reservoir (25°30’24.16” S, 49°1’29.4” W) is a man-made public water supply of the Iguaçu River basin located in an Environmental Protection Area which is in a transition zone between the dense and mixed ombrophilous forest. The Cayuguava river was dammed to construct the reservoir in 1979 (Figure 1) (Guimarães 2008). The reservoir has a 3.3 km^2 of flooded area, 7 meters in depth, a flow rate of 600 l/s, and a water residence time of 438 days (Júnior et al. 2005).

The physical and chemical data were provided by the Paraná Environmental Institute (IAP) (unpublished data) and by the water company of Paraná State (SANEPAR) (unpublished data). The Trophic State Index (TSI) was calculated according to Lamparelli (2004) (Zorzal-Almeida et al. 2017).

Samplings were carried out seasonally (i.e. in spring, summer, autumn, and winter) from October 2007 to August 2008, at two sampling stations. Periphytic samples were obtained from natural and artificial substrates. As natural substrates, we used stems of the macrophyte *Polygonum hydropiperoides* Michaux that were collected near the reservoir margin. The periphyton was obtained by scraping the stems with a razor blade wrapped in foil. Glass slides (7.5 cm x 2.5 cm) were used as artificial and inert substrates fixed to woody frames that were left submerged in the water column for 30 days. The artificial substrates were placed at about 80 meters far from the margin of the reservoir to prevent being eventually trapped in the macrophytes by the wind action.

Substrate fragments were fixed in Transeau’s solution (6:3:1) (Bicudo & Menezes 2017). The samples were treated by oxidation according to the method of Simonsen (1974) modified by Moreira-Filho & Valente-Moreira (1981). The material was air-dried onto glass slides and mounted in Naphrax® resin. Observations, measurements, and diatom photomicrographs were performed at 1000× magnification in an Olympus BX40 microscope equipped with a DP71 Olympus camera. Diatom identification was performed up to the lowest level of taxonomic hierarchy based on recent and classic literature (e.g. Lange-Bertalot 1993, 1999, 2001, Lange-Bertalot & Metzeltin 1996, Metzeltin & Lange-Bertalot 1998, 2002, 2005, 2007, Bahls 2015). For each taxon, we included information on morphometry, remarks on the autoecology (when available in literature) as well as on the occurrence in the samples (see also table 2). Taxonomic comments were provided only for poorly known species in Brazil. We considered that a species is recorded for the first time in Brazil and in the state of Paraná if it was not previously recorded in a published article. The studied materials were deposited at the herbarium of the State University of Paraná (UPCB) under the numbers 63371 to 63374 (glass slides) and 63375 to 63378 (macrophyte *P. hydropiperoides*).

Results and Discussion

The Piraquara I reservoir was classified as oligotrophic by the TSI index, characterized by low concentrations of phosphorus and nitrogen, high transparency of the water column (up to 3.2 meters), and a slightly acid to nearly neutral pH. Summer showed the highest

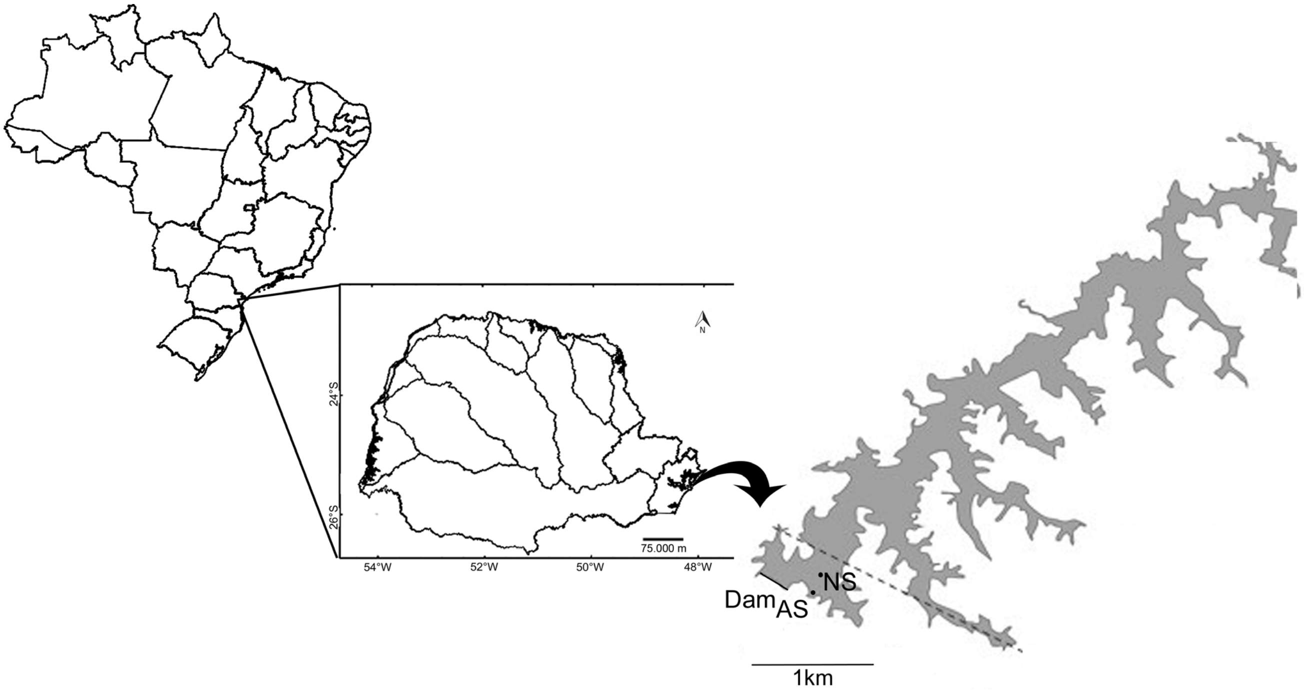


Figure 1. Sampling site in Piraquara I reservoir, Paraná state, Brazil. Artificial Substrate (AS) and Natural Substrate (NS). (shapefile map from SUDERHSA 2007).

values of temperature and accumulated rainfall occurred in the summer sample (27°C and 326.9 mm, respectively). The physical and chemical water parameters are shown in Table 1.

We identified 88 infrageneric taxa that belong to 35 genera. *Pinnularia* Ehrenberg (15 spp.) and *Eunotia* Ehrenberg (14 spp.) were the most representative diatoms in both substrates. The macrophyte *P. hydropiperoides* showed the highest richness (77) and the highest number of exclusive species (35) that represented 45.4% of the total substrate richness, whereas the artificial substrate presented 52 species and 10 exclusive taxa, which corresponded to 19.2% of the total substrate richness.

The species first recorded in Brazil were *Achnanthidium neomicrocephalum* Lange-Bertalot & Staab, *Craticula riparia* var. *mollenhaurei* Lange-Bertalot, *Frustulia australocrassinervia* Casa, Mataloni & Van de Vijver, *Gomphonema spiculoides* Gandhi and *Stauroneis neohyalina* Lange-Bertalot & Krammer. Seven diatoms are recorded for the first time in Paraná State: *Craticula acidoclinata* Lange-Bertalot & Metzeltin, *Fragilaria billingsii* Wengrat, Wetzel & Morales, *Eunotia longicamelus* Costa, Bicudo & Wetzel, *Eunotia pseudoimplicata* Lange-Bertalot, Costa & Wetzel, *Humidophila biscutella* (Moser, Lange-Bertalot & Mezeltin) Lowe, Kociolek, Johansen, Van de Vijver,

Table 1. Physical and chemical characteristics of the Piraquara I reservoir registered seasonally between October 2007 and August 2008.

Abiotic Variables	**spring (October/2007)**	**summer (February/2008)**	**autumn (May/2008)**	**winter (August/2008)**
Temperature (°C)	20.8	27	18.6	16
Accummulated rainfall (mm)	96.6	326.9	200.5	104.7
Transparency (m)	3.2	2.6	2.9	2.3
Conductivity (μS.cm^{-1})	24.0	24.5	22.5	24.5
Turbidity (NTU)	1.9	1.9	1.5	2.0
pH	6.85	6.75	6.7	6.0
NO_3 (μg.L^{-1})	95	40	45	35
NH_4^+ (μg.L^{-1})	120	175	180	100
TN (μg.L^{-1})	1500.0	2400.0	800	600
TP (μg.L^{-1})	12	15	17	17
OD (mg.L^{-1})	6.05	5.35	7.77	7.7
Chl-a (μg.L^{-1})	3.06	3.09	1.55	1.55
TSI	50.9	51.6	50.3	50.3

Lange-Bertalot & Kopalová, *Nitzschia semirobusta* Lange-Bertalot and *Pinnularia viridiformis* var. *minor* Krammer.

Pinnularia and *Eunotia* (15 and 14 species, respectively) presented a higher species richness in Piraquara I reservoir. The presence of these genera was favored by the slight acidity, low conductivity and water oligotrophy conditions of the studied reservoir (Round et al. 1990, Metzeltin & Lange-Bertalot 1998, Spaulding & Edlund 2009, Costa et al. 2017). *Eunotia* was also the most representative genus in a eutrophic reservoir (Silva et al. 2010) located near to the Piraquara I. However, only three *Eunotia* species (*E. naegelii, E. minor* and *E. subarcuatoides*), which have a wide tolerance to trophic gradients (Van Dam et al. 1994, Silva et al. 2010, Costa et al. 2017) and they are able to occur under oligotrophic and eutrophic conditions, were common to both reservoirs.

The genera *Navicula*, *Gomphonema,* and *Nitzschia,* which are commonly registered in nutrient-rich environments (Goldsborough & Robinson 1996), showed a high species number in both substrates at the mesotrophic and hypereutrophic reservoirs located nearby to Piraquara I (Bertolii et al. 2010, Faria et al. 2010, Marra et al. 2016).

The richest assemblage was obtained from *Polygonum hydropiperoides* (natural substrate), 77 species, and can be explained by the glabrous or pubescent petiole of this macrophyte (Melo 2008), which offers a greater architectural complexity than the smooth and uniform surface of glass slides. The roughness difference between substrates usually does not contribute to an increase in algal biomass, but it is an important factor in selecting the species composition of the periphyton (Burkholder 1996, Souza & Ferragut 2012).

The diatom species richness found in the oligotrophic Piraquara I reservoir was lower than in the mesotrophic reservoirs Piraquara II and Passaúna (135 and 106 taxa, respectively), the eutrophic reservoir Iraí (96 taxa) and the hypereutrophic reservoir Itaqui (124 taxa) (Bertolli et al. 2010, Silva et al. 2010, Faria 2010, Marra et al. 2016). The Piraquara II is a mesotrophic reservoir located downstream from Piraquara I. Both reservoirs shared the presence of 31 diatom species, of which 11 were recorded from oligotrophic to mesotrophic environments and 20 species had a wide trophic tolerance. The diatom assemblage had more species that are tolerant in Piraquara II than in Piraquara I. Although eutrophication might lead to a loss in diatom diversity (Wengrat et al. 2017), the species richness in shallow lakes might be also influenced by the presence, abundance and diversity of macrophytes (Sayer et al. 1999, Bicudo et al. 2007). Piraquara II and Itaqui had more diatom richness than the other reservoirs, which can be related to the fact that in the former reservoir were sampled three different species of macrophytes (Marra et al. 2016), and that the latter reservoir was almost totally covered by the floating macrophyte *Pistia stratiotes* L. (Faria et al. 2010, Faria et al. 2013).

Summer showed the highest accumulated rainfall (326.9 mm) during the sampling period and the lowest species number in both substrates. High rainfall lead to the detachment of the periphyton from the substrates (Casartelli et al. 2016), which certainly contributed to the less number of species registered in Piraquara I. We registered nine diatom species (*Spicaticribra kingstonii, Eunotia intermedia, Eunotia parasiolii, Eunotia pseudoimplicata, Encyonopsis subminuta, Cymbopleura naviculiformis, Planothidium incuriatum, Capartogramma crucicula* and *Nitzschia nana*) that occurred in spring on both substrates, but were not recorded in summer. These species release mucilage and remain attached to the substrates and are therefore commonly observed from the periphytic samples (Round et al. 1990, Santos et al. 2011, Montoya-Moreno et al. 2011). In summer, we registered temporary losses of species that were retrieved again during the sampling period. As shown in Table 2, the planktonic and benthic diatoms registered were *Aulacoseira ambigua, Cyclotella meneghiniana, Fragilaria recapitellata, Fragilaria* sp., *Ulnaria delicatissima, Eunotia intricans, Eunotia paratridentula, Encyonopsis schubartii, Achnanthidium minutissimum, Achnanthidium* sp., *Sellaphora tridentula, Pinnularia brauniana, Pinnularia divergens* var. *media*, *Pinullaria obscura, Pinnularia pseudosudetica, Navicula leptostriata, Nitzschia acidoclinata* and *Nitzschia semirobusta*.

Diatoms taxonomy and ecology

Family STEPHANODISCACEAE
Spicaticribra kingstonii Johansen, Kociolek & Lowe
Figure: 2A
Valve diameter: 14.8-15.2 µm; areolae: 13 in 10 µm; fultoportulae: 3-5 per valve; rimoportulae: 1-3 per valve.
In Brazil, this species was described by Ludwig et al. (2008) as *Thalassiosira rudis* Tremarin, Ludwig, Becker & Torgan and later synonimized with *S. kingstonii* by Rivera & Cruces (2013).
Ecology: previously registered in Brazilian tropical and subtropical reservoirs with oligotrophic to eutrophic conditions (Bartozek et al. 2018, Silva et al. 2010, Bertolli et al. 2010), and neutral pH with moderate conductivity (Ludwig et al. 2008). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm-^{1}) and oligotrophic conditions, only in spring (temperature < 21°C),
Occurrence in samples: artificial substrates (UPCB 63371).

Cyclotella meneghiniana Kützing
Figure: 2B
Valve diameter: 10-12 µm; striae: 16 in 10 µm; rimoportulae: 1-2 per valve.
Ecology: it is recorded in oligotrophic to eutrophic environments, with a higher abundance in eutrophic waters (Bartozek et al. 2018, Silva et al. 2010) and in acidic waters (van Dam et al. 1994). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{1}) and oligotrophic conditions, in spring and winter (temperature < 21°C).
Occurrence in samples: artificial (UPCB 63374) and natural (UPCB 63375; UPCB 63378) substrates.

Discostella stelligera (Cleve & Grunow) Houk & Klee
Figure: 2C
Valve diameter: 12-21 µm; striae: 9.6-18.3 in 10 µm.
Ecology: common in oligo-mesotrophic environments (Yang & Dickman 1993, Potapova & Charles 2007), and in alkaline waters with high conductivity (Bartozek et al. 2018). It was common in our samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm-^{1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Table 2. Diatom species and seasonal occurrence on artificial and natural substrates in Piraquara I reservoir.

Species	**Artificial substrates**				**natural substrates**			
	spring (October/2007)	**summer (February/2008)**	**autumn (May/2008)**	**winter (August/2008)**	**spring (October/2007)**	**summer (February/2008)**	**autumn (May/2008)**	**winter (August/2008)**
Spicaticribra kingstonii	X	-	-	-	-	-	-	-
Cyclotella meneghiniana	-	-	-	X	X	-	-	X
Discostella stelligera	X	X	X	X	X	X	X	X
Aulacoseira ambigua	X	-	X	X	X	X	X	X
A. tenella	-	-	X	X	X	X	X	X
Fragilaria billingsii	X	X	X	X	X	X	X	X
F. capucina	-	X	X	X	-	X	X	X
F. recapitellata	X	X	X	X	X	-	-	X
F. tenera	X	X	X	X	-	X	X	-
Fragilaria sp.	X	-	X	X	-	-	-	X
Staurosira construens	-	-	-	-	-	-	-	X
Staurosirella pinnata	-	-	-	X	-	-	-	-
Ulnaria delicatissima	X	X	-	X	X	-	-	X
U. ulna	-	-	-	-	-	X	-	-
Eunotia desmogonioides	X	X	X	-	X	X	X	X
E. intermedia	X	-	-	-	-	-	-	-
Eunotia intricans	-	-	-	-	X	-	X	-
E. longicamelus	-	-	-	-	-	-	-	X
E. minor	-	-	-	-	-	-	-	X
E. naegelii	-	-	-	-	-	-	-	X
E. parasiolii	-	-	-	-	X	-	-	-
E. paratridentula	X	-	X	-	X	X	-	X
E. pseudoimplicata	X	-	-	-	X	-	-	-
E. pseudosudetica	X	X	X	-	X	X	X	X
E. pyramidata f. *capitata*	-	-	-	-	-	-	X	-
E. rabenhorstii var. *monodon*	-	-	-	-	-	X	X	-
E. subarcuatoides	-	-	-	-	X	X	X	X
E. yanomami	-	-	-	-	-	-	X	-
Placoneis elginensis	-	-	X	-	-	-	-	-
Encyonema neogracile	X	X	X	X	X	X	X	X
E. silesiacum	X	-	-	-	X	X	-	X
Encyonopsis cf. *reichardtii*	-	X	X	-	-	X	X	X
E. schubartii	X	X	X	-	X	-	X	-
E. subminuta	X	-	-	-	-	-	-	-
Gomphonema gracile	X	X	X	X	X	X	X	X
G. lagenula	-	-	-	-	-	-	X	X
G. pantropicum	-	-	-	X	-	-	-	-
G. spiculoides	X	X	X	X	X	X	X	X
Cymbopleura naviculiformis	-	-	-	-	X	-	-	-
A. minutissium	X	-	-	X	X	-	X	X
A. neomicrocephalum	-	-	-	X	-	-	-	-
Achnanthidium sp.	X	-	X	-	X	X	X	X
Planothidium incuriatum	-	-	-	-	X	-	-	-
Humidophila biscutella	-	-	X	X	-	-	-	X
Luticola simplex	-	-	-	-	-	-	-	X
Luticola permuticoides	-	-	-	-	-	X	-	-

Continuation Table 2.

Species	**Artificial substrates**				**natural substrates**			
	spring (October/2007)	**summer (February/2008)**	**autumn (May/2008)**	**winter (August/2008)**	**spring (October/2007)**	**summer (February/2008)**	**autumn (May/2008)**	**winter (August/2008)**
Frustulia australocrassinervia	X	X	X	X	X	X	X	X
F. undosa	-	-	X	X	-	X	X	-
Brachysira neoexilis	X	X	X	X	X	X	X	X
Neidium affine	-	-	-	-	-	-	X	-
Sellaphora rectangularis	-	-	X	X	-	-	X	-
S. tridentula	-	-	-	-	X	-	X	-
Pinnularia borealis	-	-	-	X	X	-	-	-
P. brauniana	X	-	X	X	X	-	X	X
P. divergens var. *divergens*	-	-	-	-	-	-	X	-
P. divergens var. *media*	-	-	-	-	X	-	X	X
P. grunowii	-	-	X	-	X	X	-	-
P. obscura	-	-	-	-	X	-	X	-
P. pseudogibba	X	-	-	X	X	X	X	-
P. rumrichae	-	-	X	-	X	X	-	X
P. schoenfelderi	-	-	-	-	-	-	X	-
P. subcapitata var. *elongata*	-	-	-	X	-	-	-	-
P. subcapitata var. *subrostrata*	-	-	X	-	X	X	X	-
P. subgibba var. *undulata*	-	-	X	-	X	X	X	-
P. submicrostauron	-	-	X	-	-	X	X	-
P. viridiformis	-	-	-	-	X	X	-	X
P. viridiformis var. *minor*	-	-	-	-	-	-	-	X
Chamaepinnularia brasilianopsis	-	-	-	-	-	-	X	-
C. mediocris	-	X	-	-	-	-	X	X
Kobayasiella sp.	-	-	-	-	-	-	X	-
Navicula cryptocephala	X	X	X	-	X	X	-	X
N. cryptotenella	X	X	X	X	X	X	X	X
N. leptostriata	-	-	-	X	X	-	X	-
N. veneta	-	-	-	-	-	-	X	-
Capartogramma crucicula	X	-	-	-	-	-	-	-
Stauroneis neohyalina	-	-	X	-	-	X	-	X
S. gracilis	-	-	-	-	-	-	X	-
S. phoenicenteron	-	-	X	X	-	-	X	-
Craticula acidoclinata	-	-	-	-	-	-	X	-
C. riparia var. *mollenhaurei*	-	-	-	-	-	-	X	-
C. submolesta	-	-	-	-	-	-	X	-
Nitzschia acidoclinata	-	-	X	X	X	-	-	X
N. nana	-	-	-	-	X	-	-	-
N. semirobusta	X	-	-	X	X	X	X	X
Hantzschia amphyoxis	-	-	-	-	-	X	-	-
Rhopalodia gibberula var. *vanheurckii*	-	-	-	-	-	-	-	X
Surirella grunowii	X	-	-	-	-	-	X	-
Iconella curvula	-	-	-	-	-	X	-	-
Total of species	30	18	34	31	42	35	49	41

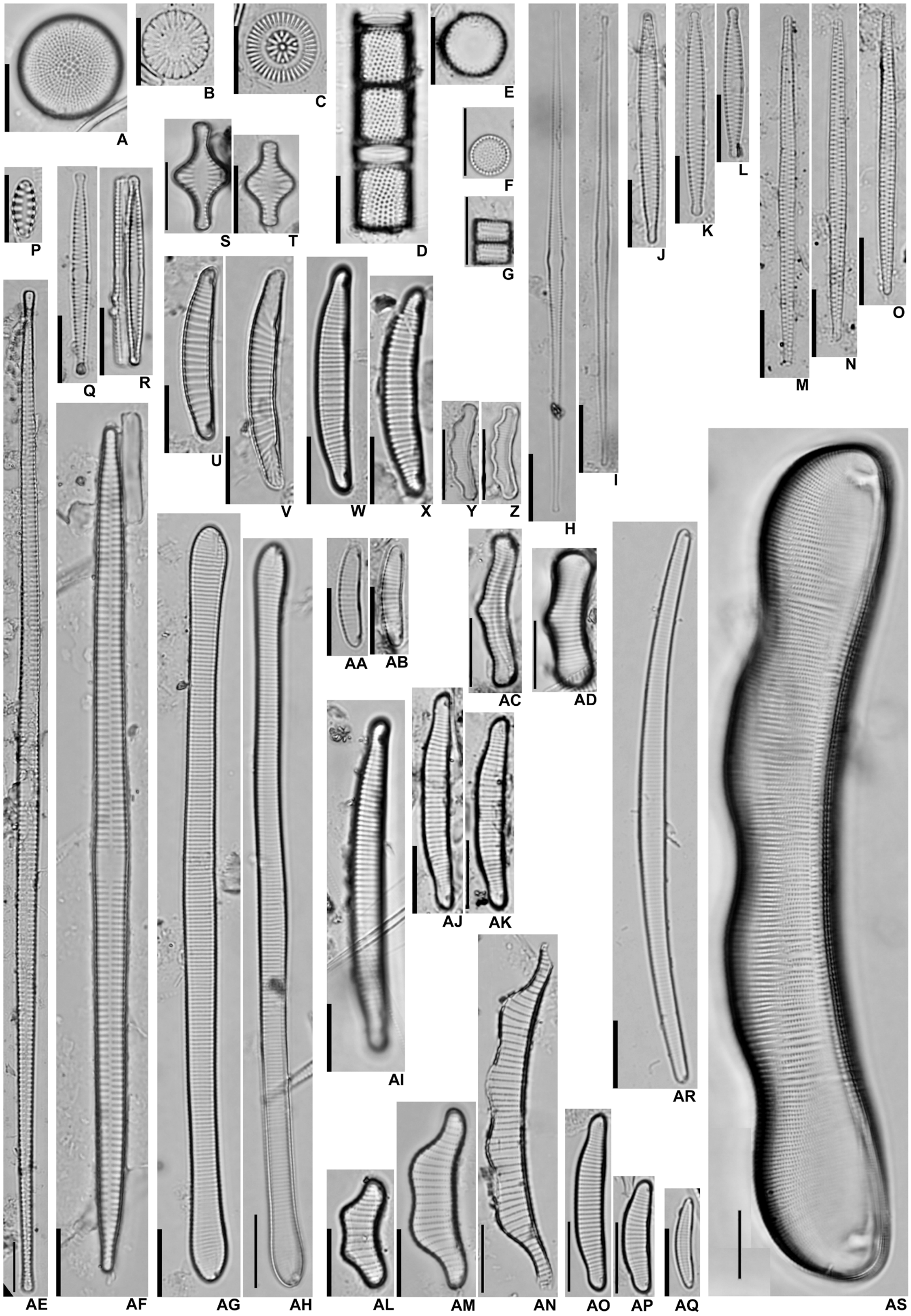


Figure 2. A. *Spicaticribra kingstonii*; B. *Cyclotella meneghiniana*; C. *Discostella stelligera*; D–E. *Aulacoseira ambigua*; F–G. *Aulacoseira tenella*; H–I. *Fragilaria billingsii*; J. *Fragilaria capucina*; K–L. *Fragilaria recapitellata*; M–O. *Fragilaria tenera*; P. *Staurosirella pinnata*; Q–R. *Fragilaria* sp., S–T. *Staurosira construens*; U–V. *Eunotia parasiolii*; W–X. *Eunotia pseudosudetica*; Y–Z. *Eunotia paratridentula*; AA–AB. *Eunotia intermedia*; AC. *Eunotia pyramidata* f. *capitata*; AD. *Eunotia rabenhorstii* var. *monodon*; AE. *Ulnaria delicatissima*; AF. *Ulnaria ulna*; AG–AH. *Eunotia desmogonioides*; AI. *Eunotia intricans*; AJ–AK. *Eunotia pseudoimplicata*; AL–AN. *Eunotia longicamelus*; AO–AP. *Eunotia minor*; AQ. *Eunotia subarcuatoides*; AR. *Eunotia naegelii*; AS. *Eunotia yanomami*.

Family AULACOSEIRACEAE

Aulacoseira ambigua (Grunow) Simonsen

Figures: 2D–2E

Valve diameter: 4-9.4 µm; striae: 8-17 in 10 µm; areolae: 8-17 in 10 µm.

Ecology: this species has a wide ecological tolerance, registered in oligotrophic (Stenger-Kovács et al. 2007), mesotrophic (Bicudo et al. 2016) and meso-eutrophic (Gómez & Licursi 2001, Ivanov & Kirilova 2004, Dela-Cruz et al. 2006) environments. In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm-^{1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Aulacoseira tenella (Nygaard) Simonsen

Figures: 2F–2G

Valve diameter: 6.3-8 µm; striae: 15 in 10 µm.

Ecology: common in oligotrophic to mesotrophic environments (Bicudo et al. 2016, Siver & Kling 1997). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm-^{1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Family FRAGILARIOPHYCEAE

Fragilaria billingsii Wengrat, Wetzel & Morales

Figures: 2H–2I

Length: 66-73.6 µm; width (central region): 2-2.3 µm; striae: 22 in 10 µm.

Fragilaria billingsii is characterized by long and narrow valves with a bilaterally inflated central area. The striae are distinct and the specimes found in the present study have a higher striae density than those registered by Wengrat et al. (2016) (striae 17-20 in 10 µm).

Ecology: previously registered only for meso-eutrophic environments, with slightly acid pH and low conductivity (Wengrat et al. 2016) In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and oligotrophic conditions.

This is the first record for Paraná state.

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Fragilaria capucina Desmazières

Figure: 2J

Length: 27-34.2 µm; width: 2.8-2.9 µm; striae: 15-16 in 10 µm.

The taxonomy of *Fragilaria capucina* is very confusing. Based on the type materials, there are three different morphotypes of *F. capucina* documented in the literature. One of the morphotypes has lanceolate valves; the second one has linear to lanceolate valves that are shorter than in the other two morphotypes, and the third one has a linear valve with a rhombic central area and slightly rostrate valve ends (Tuji & William 2006, Krammer & Lange-Bertalot 1991a).

Ecology: probably cosmopolitan, found in oligo-mesotrophic (Delgado et al. 2015) to mesotrophic (vam Dam et al. 1994) and eutrophic environments (Hofmann 1994). Considered as a planktonic, benthic and terrestrial species (Delgado et al. 2015). In our samples, it occurred attached to artificial substrates and macrophytes, in slightly acid to circumneutral pH (6-6.75), low conductivity (24-24.5 µS cm-^{1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Fragilaria recapitellata Lange-Bertalot & Metzeltin

Figures: 2K–2L

Length: 22.2-30.4 µm; width: 2-4 µm; striae: 16-18 in 10 µm.

This species has lanceolate valves with capitate ends and a unilateral central area (Metzeltin et al. 2009).

Ecology: this species is fairly common in alpha-mesosaprobic and eutrophic conditions (Cantonati et al. 2017, van Dam et al. 1994). In contrast to previous studies, it was common in our samples, indicating a tolerance to oligotrophic waters, poor in nutrients, with slightly acid to circumneutral pH (6-6.85), and low conductivity (24-24.5 µS cm-^{1}). Although it is cosmopolitan and often present in periphyton samples from lakes, rivers, and streams of temperate regions (Delgado et al. 2015), it is rarely found in tropical regions (Silva et al. 2010, Tremarin et al. 2009).

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63378) substrates.

Fragilaria tenera (W. Smith) Lange-Bertalot

Figures: 2M–2O

Length: 38.8-51.8 µm; width: 2.3-2.8 µm; striae: 15 in 10 µm.

The specimens analyzed in the present study are shorter, with a lower striae density, and the absence of a central area. Hofmann et al. (2013) referred specimens of 30-100 µm long, 2-3 µm wide and with a striae density of 17-20 in 10 µm, and the often absence of a central area.

Ecology: this species was registered for oligo-mesotrophic environments (van Dam et al. 1994). In our samples it occurred attached to the substrates in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63376; UPCB 63377) substrates.

Fragilaria sp.

Figures: 2Q–2R

Length: 24.5-31.7 µm; width: 2.3-3.1 µm; striae: 16-17 in 10 µm.

Fragilaria sp. resembles *Fragilaria austriaca* (Grunow) Lange-Bertalot in the outline but has wider valves (3-4 µm) and a lower striae density (12-15 µm). *Fragilaria pararumpens* Lange-Bertalot, Hofmann & Werum has similar dimensions to *Fragilaria* sp., but a more lanceolate valve with subcapitate ends (Cantonati et al. 2017).

Ecology: In our samples, it occurred only in temperatures < 21°C, slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371; UPCB 63373; UPCB 63374) and natural (UPCB 63378) substrates.

Family STAURORIRACEAE

Staurosira construens Ehrenberg

Figures: 2S–2T

Length: 15.7 µm; width: 8.6 µm; striae: 18 in 10 µm.

Ecology: this species is registered for meso-eutrophic environments (van Dam et al. 1994) and is considered as tolerant to different water conditions (Hofmann 1994). In our samples, it occurred in slighltly acid pH (6), low conductivity (24.5 µS cm-^{1}) and oligotrophic conditions, only in winter (temperature 16°C).

Occurrence in samples: natural substrates (UPCB 63378).

Staurosirella pinnata (Ehrenberg) Williams & Round.

Figure: 2P

Length: 8-14.4 µm; width: 3.3-3.8 µm; striae: 8-11 in 10 µm.

Ecology: this species is considered as tolerant (Hofmann 1994) to indifferent (van Dam et al. 1994) to the trophic state. In our samples, it occurred in slighltly acid pH (6), low conductivity (24.5 µS cm-^{1}) and oligotrophic condition, only in winter (temperature 16°C).

Occurrence in samples: artificial substrates (UPCB 63374).

Family ULNARIACEAE

Ulnaria delicatissima (Smith) Aboal & Silva

Figure: 2AE

Length: 62.4-109.5 µm; width: 2.4-4.7 µm; striae: 13-16 in 10 µm.

Ecology: common in oligo-mesotrophic environments (van Dam et al. 1994, Hofmann 1994). In our samples, it occurred in slighltly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm-^{1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63374) and natural (UPCB 63375; UPCB 63378) substrates.

Ulnaria ulna (Nitzsch) Compère.

Figure: 2AF

Length: 90-189 µm; width: 4-4.8 µm; striae: 9-14 in 10 µm.

Ecology: considered as indifferent to tolerant to the trophic state of the environment (van Dam et al. 1994, Hofmann 1994). In our samples, it occurred in circumneutral pH (6.75), low conductivity (24.5 µS cm-^{1}) and oligotrophic conditions, only in summer (temperature of 27°C and accumulated rainfall 326.9 mm).

Occurrence in samples: natural substrates (UPCB 63376).

Family EUNOTIACEAE

Eunotia desmogonioides Metzeltin & Lange-Bertalot

Figures: 2AG–2AH

Length: 99-159.3 µm; width: 3.5-5 µm; striae: 17-20 in 10 µm.

This species has a valve outline similar to *Eunotia flexuosa* (Brébisson) Kützing, although with narrower valves and a higher striae density. *Eunotia rabenhorstiana* var. *elongata* (Patrick) Metzeltin & Lange-Bertalot is similar to *E. flexuosa* but has wider valves with more inflated ends (Costa et al. 2017).

Ecology: previously registered for Brazilian oligo- to mesotrophic reservoirs, with low conductivity and slightly acidic to neutral pH (Costa et al. 2017). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371, UPCB 63372, UPCB 63373) and natural (UPCB 63375, UPCB 63376, UPCB 63377, UPCB 63378) substrates.

Eunotia intermedia (Krasske ex Hustedt) Nörpel & Lange-Bertalot

Figures: 2AA–2AB

Length: 13.3-16.8 µm; width: 3.1-3.3 µm; striae: 14-15 in 10 µm.

Ecology: previously registered for slightly acidic waters with low conductivity (Lange-Bertalot et al. 2011). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm-^{1}) and oligotrophic conditions, only in spring (temperature < 21°C).

Occurrence in samples: artificial substrate (UPCB 63371).

Eunotia intricans Lange-Bertalot & Metzeltin

Figure: 2AI

Length: 35-51.7 µm; width: 4.5-5.1 µm; striae: 11-13 in 10 µm.

Eunotia intricans is similar to *Eunotia parasiolii* Metzeltin & Lange-Bertalot in the valve outline, although it has a lower striae density (6.5-11/10 µm, according to Metzeltin & Lange-Bertalot 1998). In Piraquara reservoir, the specimens were longer than the type specimen (17-48 µm, according to Lange-Bertalot & Metzeltin 2009), but have a similar length to other specimens registered in Brazilian reservoirs (Costa et al. 2017).

Ecology: previously registered for oligo-mesotrophic environments, with a preference for acid waters with low conductivity (Costa et al. 2017). In our samples, it occurred in circumneutral pH (6.7-6.85), low conductivity (22.5-24 µS cm^{-1}) and oligotrophic conditions, in spring and autumn (temperature < 21°C).

Occurrence in samples: natural substrates (UPCB 63375, 63377).

Eunotia longicamelus Costa, Bicudo & Wetzel

Figure: 2AL–2AN

Length: 16.2-52.6 µm; width: 5.4-5.26 µm; striae: 11 in 10 µm; valvar undulations: 2-4.

Similar to *Eunotia camelus* Ehrenberg, but differs in the type and number of undulations on the dorsal margin (two undulations subdivided into two more) and the presence of wider valves (length: 22-30 µm; width: 6-8.8 µm; striae density 8-12 in 10 µm and two undulations) (Costa et al. 2017, Reichardt 1995).

Ecology: previously registered for mesotrophic environments, with a preference for slightly acidic waters (pH 6.6) with medium values of conductivity (71 µS cm^{-1}) (Costa et al. 2017). In our samples, it occurred only in winter (temperature 16°C) and expanded the occurrence to oligotrophic environments with slightly acid pH (6), and low conductivity (24.5 µS cm^{-1}).

This is the first record for Paraná state.

Occurrence in samples: natural substrates (UPCB 63378).

Eunotia minor (Kützing) Grunow.

Figures: 2AO–2AP

Length: 17-24.7 µm; width: 3.8 µm; striae: 17-18 in 10 µm.

Ecology: considered as tolerant to different trophic states, ranging from oligo- to eutrophic (van Dam et al. 1994, Hofmann 1994). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C).

Occurrence in samples: natural substrates (UPCB 63378).

Eunotia naegelii Migula.
Figure: 2AR
Length: 40-48.4 µm; width: 1.8-3 µm; striae: 21-24 in 10 µm.
Ecology: considered as acidophile; registered for ultra-oligotrophic to oligotrophic environments (Costa et al. 2017, van Dam et al. 1994). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C).
Occurrence in samples: natural substrates (UPCB 63378).

Eunotia parasiolii Metzeltin & Lange-Bertalot.
Figures: 2U–2V
Length: 18.5-32.6µm; width: 3-4.7 µm; striae: 9-12 in 10 µm.
Ecology: previously registered for acidic waters (pH 4.4-5.3) with a low conductivity (9.4-12.6 µS cm^{-1}) (Ferrari et al. 2007) to slightly acidic (pH 6.6-6.8) and oligotrophic environments (Costa et al. 2017). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature < 21°C).
Occurrence in samples: natural substrates (UPCB 63375).

Eunotia paratridentula Lange-Bertalot & Kulikovskyi.
Figures: 2Y–2Z
Length: 13-17.6 µm; width: 2.8-4 µm; striae: 14-16 in 10 µm; undulations: 3-4.
Previously reported for Paraná State as *Eunotia muscicola* Krasske var. *tridentula* Nörpel & Lange-Bertalot (Tremarin et al. 2009). It differs from *E. muscicola* Krasske by the lower striae density; and from *E. paramuscicola* Krstić, Levkov & Pavlov by the broadly protracted apices (Krstić et al. 2013).
Ecology: considered as acidophile (van Dam et al. 1994); registered for mesotrophic environments (Marra et al. 2016). Our study expanded its occurrence to environments with pH ranging from slightly acid to circumneutral (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63378) substrates.

Eunotia pseudoimplicata Lange-Bertalot, Costa & Wetzel
Figures: 2AJ–2AK
Length: 28.7-43 µm; width: 4.2-5.3 µm; striae: 14-16 in 10 µm.
Ecology: oligo-mesotrophic reservoirs, with slightly acid to neutral waters (Costa et al. 2017). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature < 21°C).
This is the first record for Paraná state.
Occurrence in samples: artificial (UPCB 63371) and natural (UPCB 63375) substrates.

Eunotia pseudosudetica Metzeltin, Lange-Bertalot & García-Rodríguez
Figures: 2W–2X
Length: 30.4-33.26 µm; width: 4.9-5.4 µm; striae: 12 in 10 µm.
Ecology: this species occurred in slightly acidic to circumneutral pH (6-6.85), low conductivity (22.5-24 µS cm^{-1}) and in oligotrophic conditions, in agreement with previously studies (Costa et al. 2017), but can also be registered for nutrient-rich environments (Bertolli et al. 2010, Faria et al. 2010, Marra et al. 2016). Usually found in the periphyton, but can be also registered from phytoplankton (Bartozek et al. 2013) and surface sediments (Costa et al. 2017).
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Eunotia pyramidata f. *capitata* Krasske
Figure: 2AC
Length: 25.5 µm; width: 4.6 µm; striae: 15 in 10 µm; undulations: 3.
It differs from *Eunotia rabenhorstii* var. *triodon* Cleve & Grunow by the presence of a more pronounced mark in the central undulation (Costa et al. 2017).
Ecology: previously registered for moody pools with sphagnum moss (Krasske 1948), and from samples of surface sediments collected in environments with acid pH and poor in nutrients (Costa et al. 2017). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, only in autumn (temperature 18.6°C).
Occurrence in samples: natural substrates (UPCB 63377).

Eunotia rabenhorstii var. *monodon* Cleve & Grunow
Figure: 2AD
Length: 20 µm; width: 6.2 µm; striae: 17 in 10 µm.
Ecology: previously registered for a mesotrophic reservoir in Paraná state (Bertolli et al. 2010, Marra et al. 2016) and for ultraoligotrophic to eutrophic reservoirs with slightly acidic to alkaline waters (pH 6.2-7.3) in São Paulo state (Costa et al. 2017). In our samples, it occurred in circumneutral pH (6.7-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions, in summer and autumn (temperature from 18.6-27°C).
Occurrence in samples: natural substrates (UPCB 63376; UPCB 63377).

Eunotia subarcuatoides Alles, Nörpel & Lange-Bertalot
Figure: 2AQ
Length: 13.5-17.3 µm; width: 2.4-2.9 µm; striae: 23-24 in 10 µm.
Similar to the small forms of *Eunotia bilunaris* (Ehrenberg) Schaarschmidt, but with a lower density of striae (13-17 in 10 µm) that are less conspicuous (Costa et al. 2017). The specimens found herein presented a less width than the type material (3-4.5 µm, according to Alles et al. 1991), but presented a similar width to the specimens (2.5-3.3 µm) registered by Costa et al. (2017) in Brazilian reservoirs.
Ecology: considered as acidobiontic (van Dam et al. 1994). In Brazil, it was previously registered for ultraoligo to eutrophic conditions (Costa et al. 2017, Silva et al. 2010), with acid to alkaline pH (Costa et al. 2017). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378).

Eunotia yanomami Metzeltin & Lange-Bertalot
Figure: 2AS
Length: 120 µm; width: 13.5 µm; striae: 15 in 10 µm; undulations: 4.
Similar to *Eunotia zygodon* Ehrenberg in the valve outline, but *E. zygodon* has a smaller size (less than 55 µm in length, according to Metzeltin & Lange-Bertalot 1998).

Ecology: previously registered for oligotrophic to mesotrophic environments (Marra et al. 2016, Costa et al. 2017), with slightly acid waters (pH 5.4-6.4) and low to medium conductivity (12-70 µS cm^{-1}) (Bicca & Torgan 2009). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, only in autumn (temperature 18.6°C).
Occurrence in samples: natural substrates (UPCB 63377).

Family GOMPHONEMATACEAE
Placoneis elginensis (Gregory) Cox
Figure: 3A
Length: 34.7 µm; width: 10 µm; striae: 13 in 10 µm.
Ecology: previously registered for highly alkaline waters, and for mesotrophic and eutrophic environments (van Dam et al. 1994, Hofmann 1994, Poulíčková et al. 2008). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, only in autumn (temperature 18.6°C).
Occurrence in samples: artificial substrates (UPCB 63373).

Encyonema neogracile Krammer.
Figures: 3B–3C
Length: 35.5-56.6 µm; width: 6.5-7.5 µm; dorsal striae: 14 in 10 µm; ventral striae: 15-16 in 10 µm.
Ecology: this species was frequent in our samples and occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions, but was also previously registered for oligo-mesotrophic and mesotrophic environments (van Dam et al. 1994, Marra et al. 2016).
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Encyonema silesiacum (Bleisch) Mann.
Figure: 3D
Length: 17.4-21.5 µm; width: 5-7.5 µm; dorsal striae: 11-17 in 10 µm; ventral striae: 12-16 in 10 µm.
Ecology: widely distributed, considered as a circumneutral species (van Dam et al. 1994), occurring in waters ranging from oligo to eutrophic (van Dam et al. 1994, Hofmann 1994, Bertolli et al. 2010, Silva et al. 2010, Marra et al. 2016). This study expands its occurrence to slightly acid pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 6371) and natural (UPCB 63375; UPCB 63376; UPCB 63378) substrates.

Encyonopsis cf. *reichardtii* Krammer
Figures: 3Q–3S
Length: 36.5-40 µm; width: 5.8-6 µm; maximum ratio L/W= 6.8; dorsal striae: 18-20 in 10 µm; ventral striae: 17-21 in 10 µm.
The specimens from Piraquara I reservoir presented the valve outline, striation pattern, and isolated punctum near the central striae similar to *Encyonopsis reichardtii* Krammer. As the type specimens have a slightly more rhombo-lanceolate contour, wider valves (7-7.7 µm) and a maximum ratio L/W of 6.1 (Krammer 1997b: 131) we preferred to identify the taxon as *conferatum.*
Ecology: this species occurred in slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63372; UPCB 63373) and natural (UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Encyonopsis schubartii (Hustedt) Krammer
Figure: 3E
Length: 29.5-33.6 µm; width: 6-6.4 µm; dorsal striae: 9-11 in 10 µm; ventral striae: 9-12 in 10 µm.
Ecology: previously registered for oligotrophic (Krammer 1997b), and mesotrophic environments, with acid pH (Wengrat et al. 2015). In our samples, it occurred in circumneutral pH (6.7-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373) and natural (UPCB 63375; UPCB 63377) substrates.

Encyonopsis subminuta Krammer & Reichardt.
Figure: 3F
Length: 15.3-21.3 µm; width: 3.7-4.8 µm; striae: 26 in 10 µm.
Ecology: common in oligo- and mesotrophic environments, and rare in electrolyte-poor environments (Bertolli et al. 2010, Cantonati et al. 2017). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature 20.8°C).
Occurrence in samples: artificial substrate (UPCB 63371).

Gomphonema gracile Ehrenberg
Figure: 3J–3K
Length: 28-43 µm; width: 5.8-7.2 µm; striae: 14-16 in 10 µm.
The *Gomphonema gracile* species-complex includes representatives with valves of diverse forms, sizes and measures. *Gomphonema naviculoides* Smith is very similar to *G. gracile* in the valve outline and striae density, but they differ in the width range, being of 4.2-9 µm in *G. gracile* and of 7.8-9.5 µm in *G. naviculoides* (Reichardt 2015a).
Ecology: typical of environments with low conductivity and nutrients (Krammer & Lange-Bertalot 1986). It occurred in all our samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Gomphonema lagenula Kützing
Figures: 3L–3M
Length: 17.2-21.7 µm; width: 4.5-4.8 µm; striae: 18-19 in 10 µm
Ecology: considered as tolerant (Hofmann 1994), this species is widely distributed in environments ranging from oligotrophic to hypereutrophic (Bertolli, et al. 2010, Silva et al. 2010, Faria et al. 2010, Marra et al. 2016, Bartozek et al. 2018). In our samples, it occurred in slightly acid to circumneutral pH (6-6.7), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions, in autumn and winter (temperature < 19°C).
Occurrence in samples: natural substrates (UPCB 63377; UPCB 63378).

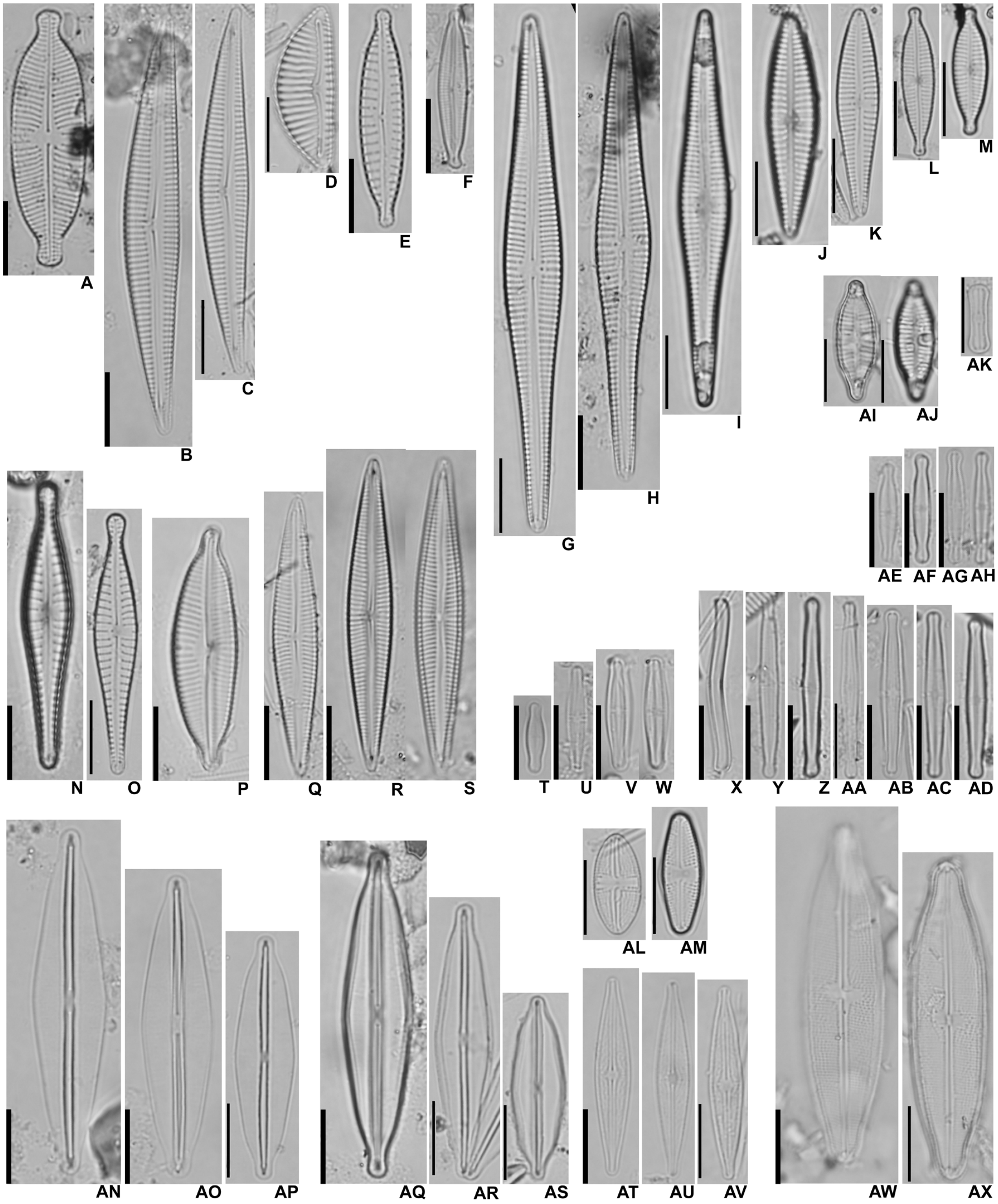


Figure 3. A. *Placoneis elginensis*; B–C. *Encyonema neogracile*; D. *Encyonema silesiacum*; E. *Encyonopsis schubartii*; F. *Encyonopsis subminuta*; G–I. *Gomphonema spiculoides*; J–K. *Gomphonema gracile*; L–M. *Gomphonema lagenula*; N–O. *Gomphonema pantropicum*; P. *Cymbopleura naviculiformis*; Q–S. *Encyonopsis* cf. *reichardtii*; T–W. *Achnanthidium minutissimum*; X–AD. *Achnanthidium neomicrocephalum*; AE–AH. *Achnanthidium* sp.; AI–AJ. *Planothidium incuriatum*; AK. *Humidophila biscutella*; AL. *Luticola permuticoides*; AM. *Luticola simplex*; AN–AP. *Frustulia australocrassinervia*; 3AQ–3AS. *Frustulia undosa*; AT–AV. *Brachysira neoexilis*; AW–AX. *Neidium affine*.

Gomphonema pantropicum Reichardt
Figure: 3N–3O
Length: 35.2-38 µm; width: 6-6.3 µm; striae: 10-11 in 10 µm.
Valves lanceolate, with constricted round apices. Spaced and convergent striae towards the middle part of the valves and parallel to the apices.
In Brazilian environments, *Gomphonema pantropicum* is often misidentified as *Gomphonema subtile* Ehrenberg (Osorio et al. 2017, Marra et al. 2016, Marquardt & Bicudo 2014). *Gomphonema subtile* presents the valves with broadly capitate ends, a long and narrow "neck" and flatly-rounded apices (Reichardt 2015b).
Ecology: distributed in South America and in tropical Africa, India, and Southeast Asia (Reichardt 2015b); the ecology of this species is little known. In Brazil, it was previously registered from epiphytic samples in the North and South region (Medeiros et al. 2018, Azevedo et al. 2018). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C).
Occurrence in samples: artificial substrates (UPCB 63374).

Gomphonema spiculoides Gandhi
Figures: 3G–3I
Length: 54.2-69 µm; width: 7.3-8 µm; striae: 14 in 10 µm.
This species presents valve ends clearly apiculate, a central area weakly delimited, a narrow axial area, undulate and lateral raphe, and striae slightly radiate.
The specimens recorded in the present study have striae slightly radiate and a less width than the specimens found by Karthick & Kociolek (2012), resembling the Gandhi's original draw and description. *Gomphonema spiculoides* can be mistaken with large valves of *Gomphonema gracile* Ehrenberg.
Ecology: this species has a low tolerance to alkaline conditions and it was previously registered for oligotrophic and slightly acid waters (Riato et al. 2017). It occurred in all our samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
This is the first record for Brazil.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Family CYMBELLACEAE
Cymbopleura naviculiformis (Auerswald) Krammer
Figure: 3P
Length: 32.1 µm; width: 9 µm; dorsal striae: 13 in 10 µm; ventral striae: 15 in 10 µm.
Ecology: previously registered for oligotrophic to eutrophic waters (Bertolli et al. 2010, Silva et al. 2010, Marra et al. 2016, Cantonati et al. 2017). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature 20.8°C).
Occurrence in samples: natural substrates (UPCB 63375).

Family ACHNANTHIDIACEAE
Achnanthidium minutissimum (Kützing) Czarnecki
Figures: 3T–3W
Length: 9.6-15.4 µm; width: 2.6-2.8 µm; indistinct striae.
Ecology: very wide range of ecological preferences (Cantonati et al. 2017). In our samples, it occurred in temperature < 21°C, slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63374) and natural (UPCB 63375; UPCB 63377; UPCB 63378) substrates.

Achnanthidium neomicrocephalum Lange-Bertalot & Staab
Figures: 3X–3AD
Length: 21.6-25.3 µm; width: 2.5-2.8 µm; indistinct striae.
Valves linear-lanceolate with subcapitate apices. Axial area narrow. Central area variable in shape.
Achnanthidium neomicrocephalum has narrower valves than *Achnanthidium gracillimum* (Meister) Lange-Bertalot (3.4-4.5 µm) and *A. caledonicum* (Lange-Bertalot) Lange-Bertalot (2.7-3.8 µm). *Achnanthidium caledonicum* presents broader valve ends than *A. neomicrocephalum*.
Ecology: previously registered for oligotrophic environments, mainly springs and lakes (Cantonati et al. 2017, Cantonati & Lange-Bertalot 2010). In our samples, it occurred in slightly acid pH (6) and low conductivity (24.5 µS cm^{-1}), and oligotrophic condition, only in winter (temperature 16°C).
This is the first record for Brazil
Occurrence in samples: artificial substrates (UPCB 63374).

Achnanthidium sp.
Figures: 3AE–3AH
Length: 13.3-15.4 µm; width: 2.4-2.5 µm; indistinct striae.
Valves linear-lanceolate, with parallel margins and capitate apices. Narrow and linear axial area. Central area bordered by one striae on both sides. Resembles *Achnanthidium tropicocatenatum* Marquardt, Wetzel & Ector although the central portion is not inflated (Marquard et al. 2017).
Ecology: this species was frequent in ours samples, and occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Planothidium incuriatum Wetzel, Van de Vijver & Ector
Figures: 3AI–3AJ
Length: 20 µm; width: 7 µm; striae: 14 in 10 µm.
Planothidium incuriatum is widely reported in Brazil as *P. biporomum* (Hohn & Hellerman) Lange-Bertalot, characterized by the rostrate apices that differ from the capitate to subcapitate apices of *P. biporomum*. *Planothidium incuriatum* is also misidentified as *P. rostratum* (Østrup) Round & Bukhtiyarova but it can be distinguished by its smaller dimensions (Wetzel et al. 2013).
Ecology: widely distributed in a broad range of environmental conditions (Wetzel et al. 2013). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature 20.8°C).
Occurrence in samples: natural substrates (UPCB 63375).

Family DIADESMIDACEAE

Humidophila biscutella (Moser, Lange-Bertalot & Mezeltin) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová

Figure: 3AK

Length: 9.5 µm; width: 2.3 µm; indistinct striae.

Valves linear with concave margins and broadly rounded apices; the central area is elliptical and not forming fascia. It is possible that *Humidophila biscutella* has been previously reported as *Humidophila contenta* (Grunow) Lowe, Kociolek, Johansen, Van de Vijver, Lange-Bertalot & Kopalová. It differs from the latter species by the absence of wide apices and of the central area with fascia (Moser et al. 1998). *Humidophila deceptionensis* Kopalová, Zidarova & Van de Vijver is similar to *H. biscutella*, but has wider valves (2.7-3.2 µm) (Kopalová et al. 2015). In Brazil, it was previously registered for reservoirs in São Paulo state (Marquardt et al. 2018).

Ecology: the ecology of this species id little known. In our samples, it occurred only in temperatures < 19°C, slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

This is the first record for Paraná state.

Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63378) substrates.

Luticola permuticoides Metzeltin & Lange-Bertalot

Figure: 3AL

Length: 13.5-19.1 µm; width: 6.4-7.8 µm; striae: 19 in 10 µm.

Ecology: found in epiphytic samples collected in rivers with slightly acidic waters (Straube et al. 2017). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C).

Occurrence in samples: natural substrates (UPCB 63378).

Luticola simplex Metzeltin, Lange-Bertalot & García-Rodríguez

Figure: 3AM

Length: 12.8-16 µm; width: 4.8-5 µm; striae: 24 in 10 µm.

Ecology: previously recorded from sediment samples of subfossil assemblages in oligotrophic conditions and from modern assemblages in eutrophic conditions (Faustino et al. 2016). In our samples, it occurred in circumneutral pH (6.75), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in summer (temperature of 27°C and accumulated rainfall of 326.9 mm).

Occurrence in samples: natural substrates (UPCB 63376).

Family AMPHIPLEURACEAE

Frustulia australocrassinervia Casa, Mataloni & Van de Vijver

Figures: 3AN–3AP

Length: 32-43.2 µm; width: 8-9.6 µm; delicate striae, ratio 3.8-4.6.

This taxon has lanceolate valves, with slightly triundulate margins, protracted and rostrate apices. It differs from *Frustulia crassinervia* (Brébisson) Lange-Bertalot & Krammer by the presence of wider valves (11.5-12.5 µm) (Casa et al. 2018).

Ecology: previously registered for acidic waters with low conductivity (Casa et al. 2018). It occurred in all samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

This is the first record for Brazil.

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Frustulia undosa Metzeltin & Lange-Bertalot

Figures: 3AQ–3AS

Length: 24.5-44.1 µm; width: 7.5-10 µm; delicate striae.

It differs from *Frustulia crassinervia* (Brébisson) Lange-Bertalot & Krammer by the presence of a narrower and longer tri-undulated valve (Metzeltin & Lange-Bertalot 1998).

Ecology: previously registered for a mesotrophic reservoir (Marra et al. 2016). In our samples, it occurred in slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63376; UPCB 63377) substrates.

Family BRACHYSIRACEAE

Brachysira neoexilis Lange-Bertalot

Figures: 3AT–3AV

Length: 16-32 µm; width: 4-5.6 µm; striae: difficult to distinguish

Wolfe & Kling (2001) have suggested that *Brachysira neoexilis* is a synonym of *Brachysira microcephala* (Grunow) Compère. Some authors consider that both species differ significantly (Cantonati et al. 2017) since the latter species presents clearly capitate apices and usually wider valves than *B. neoexilis*.

Ecology: considered as a species distributed in oligotrophic and acidic conditions (Lange-Bertalot & Moser 1994, Cantonati et al. 2017), and previously registered for mesotrophic and eutrophic reservoirs (Bertolli et al. 2010, Silva et al. 2010, Marra et al. 2016). It occurred in all our samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.

Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Family NEIDIACEAE

Neidium affine (Ehrenberg) Pfitzer

Figures: 3AW–3AX

Length: 42.9-46.1 µm; width: 10-10.8 µm; striae 25-26 in 10 µm.

Ecology: previously registered for oligotrophic and mesotrophic environments (Hofmann 1994, Marra et al. 2016). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, only in autumn (temperature of 18.6°C).

Occurrence in samples: natural substrates (UPCB 63377).

Family SELLAPHORACEAE

Sellaphora rectangularis (Gregory) Lange-Bertalot & Metzeltin

Figure: 4C

Length: 31.3-48.8 µm; width: 7.1-11.7 µm; striae: 21-27 in 10 µm.

Ecology: previously registered for oligotrophic to eutrophic environments (van Dam et al. 1994, Lange-Bertalot & Metzeltin 1996). In our samples, it occurred in autumn and winter samples (temperature < 19°C). Thys study extends its occurrence to slightly acid to circumneutral pH (6-6.7), and low conductivity (22.5-24.5 µS cm^{-1}) conditions.

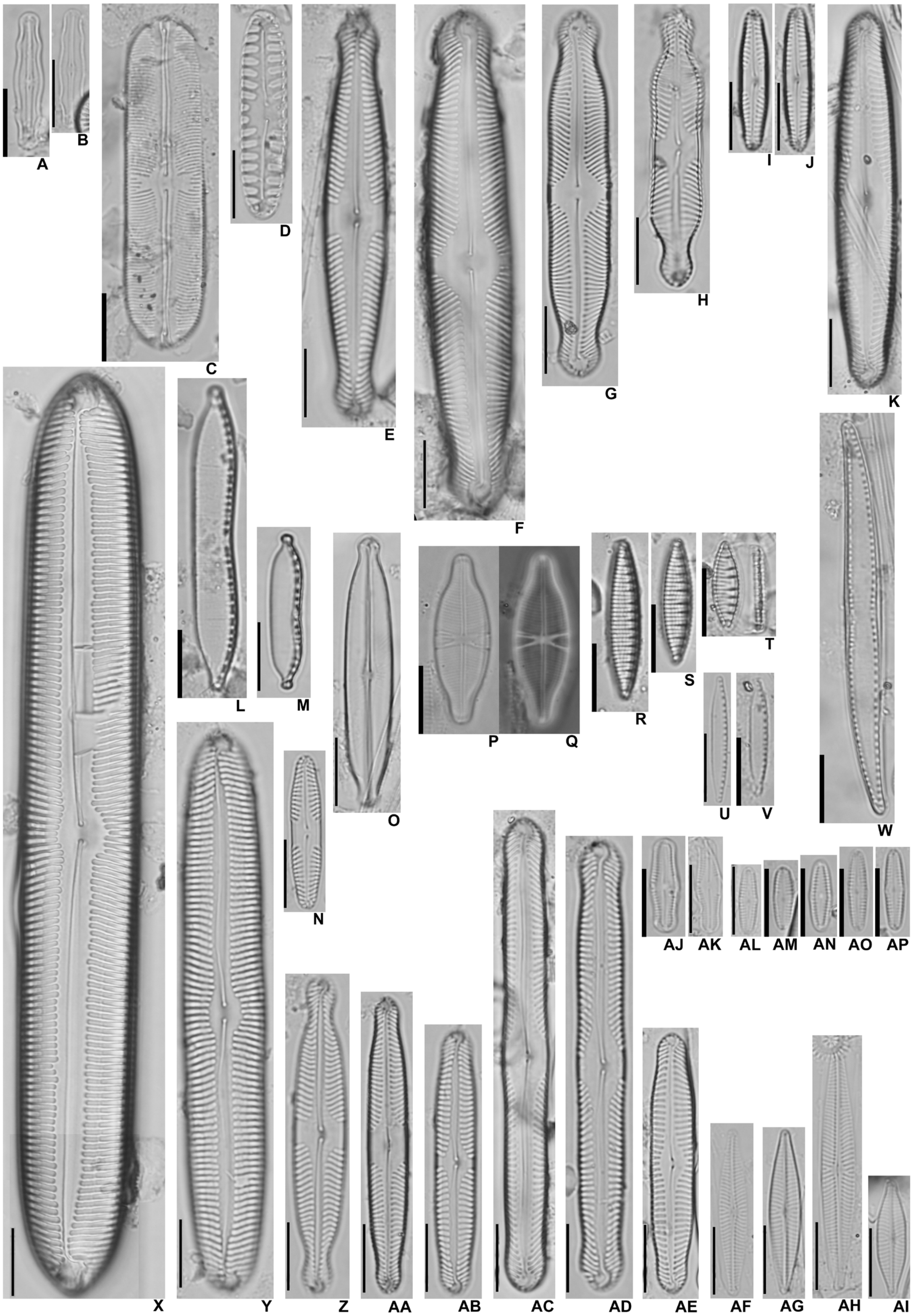


Figure 4. A–B. *Sellaphora tridentula*; C. *Sellaphora rectangularis*; D. *Pinnularia borealis*; E. *Pinnularia brauniana*; F. *Pinnularia divergens* var. *divergens*; G. *Pinnularia divergens* var. *media*; H. *Pinnularia grunowii*; I–J. *Pinnularia obscura*; K. *Pinnularia pseudogibba*; L–M. *Hantzschia amphioxys*; N. *Pinnularia schoenfelderi*; O. *Kobayasiella* sp.; P–Q. *Capartogramma crucicula*; R–T. *Nitzschia semirobusta*; U–V. *Nitzschia acidoclinata*; W. *Nitzschia nana*; X. *Pinnularia viridiformis* var. *viridiformis*; Y. *Pinnularia viridiformis* var. *minor*; Z. *Pinnularia rumrichiae*; AA. *Pinnularia subcapitata* var. *elongata*; AB. *Pinnularia subcapitata* var. *subrostrata*; AC–AD. *Pinnularia subgibba* var. *undulata*; AE. *Pinnularia submicrostauron*; AF. *Navicula cryptocephala*; AG. *Navicula cryptotenella*; AH. *Navicula leptostriata*; AI. *Navicula veneta*; AJ–AK. *Chammaepinularia brasilianopsis*; AL–AP. *Chammaepinnularia mediocris*.

Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63377) substrates.

Sellaphora tridentula (Krasske) C.E.Wetzel
Figure: 4A–4B
Length: 17.5-20.8 µm; width: 4-4.6 µm; striae: difficult to distinguish.
Ecology: considered as an acidophile species (van Dam et al. 1994). In our samples, it occurred in spring and autumn (temperature < 21°C). This study extends the occurrence of this species to circumneutral pH (6.7-6.85), low conductivity (22.5-24 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63375; UPCB 63377).

Family PINNULARIACEAE
Pinnularia borealis Ehrenberg var. *borealis*.
Figure: 4D
Length: 31-31.2 µm; width: 7-7.2 µm; striae: 7 in 10 µm.
Ecology: considered as indifferent to environmental conditions (Hofmann 1994) *P. borealis* is common in oligo and mesotrophic environments (van Dam et al. 1994, Bertolii et al. 2010), while rarely registered for eutrophic conditions (Silva et al. 2010). In our samples, it occurred in temperature s < 21°C, slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and in oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63374) and natural (UPCB 63375) substrates.

Pinnularia brauniana (Grunow) Studnicka.
Figure: 4E
Length: 52.2-60 µm; width: 8.5-9.6 µm; striae: 11-12 in 10 µm.
Ecology: previously registered for oligotrophic, mesotrophic and eutrophic conditions (Van Dam et al. 1994, Silva et al. 2010, Marra et al. 2016). According to Krammer (1992), *P. brauniana* is not considered commonly found in waters with low conductivity and pH < 6. This study extends its occurrence to temperatures < 21°C, acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63377; UPCB 63378) substrates.

Pinnularia divergens Smith var. *divergens*
Figure: 4F
Length: 74.5 µm; width: 12.5 µm; striae: 12 in 10 µm.
Ecology: in agreement with previously studies (van Dam et al. 1994, Cantonati et al. 2017), we found this species in pH < 7, low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions only in autumn (temperature 18.6°C).
Occurrence in samples: natural substrates (UPCB 63377).

Pinnularia divergens var. *media* Krammer
Figure: 4G
Length: 39-54.5 µm; width: 7.7-9.5 µm; striae: 14-15 in 10 µm.
Ecology: according to previous studies, this species prefers oligotrophic environments (van Dam et al. 1994, Cantonati et al. 2017) but can also occur in eutrophic waters (Silva et al. 2010). In our samples, it occurred in in temperatures < 21°C, slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and in oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63375; UPCB 63377; UPCB 63378).

Pinnularia grunowii Krammer
Figure: 4H
Length: 31-41 µm; width: 6-7.5 µm; striae: 14 in 10 µm.
Ecology: previously registered for eutrophic environments, with low to moderate electrolytes and pH > 8 (Krammer 2000) and also for mesotrophic waters (Bertolli et al. 2010). In our samples, it occurred in circumneutral pH (6.7-6.85) and low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63375; UPCB 63376) substrates.

Pinnularia obscura Krasske
Figures: 4I–4J
Length: 20.5-21.5 µm; width: 4-4.5 µm; striae: 16 in 10 µm.
Ecology: considered as a species tolerant to environments with pH < 4 (Ciniglia et al. 2007). In our samples, it occurred in circumneutral pH (6.7-6.85), low conductivity (22.5-24 µS cm^{-1}), and oligotrophic conditions, in spring and autumn (temperature < 21°C).
Occurrence in samples: natural substrates (UPCB 63375; UPCB 63377).

Pinnularia pseudogibba Krammer
Figure: 4K
Length: 54 µm; width: 9 µm; striae: 11 in 10 µm.
Pinnularia pseudogibba differs from *Pinnularia subgibba* Krammer var. *subgibba* by the presence of lanceolate valves with subrostrate apices (Krammer 2000).
Ecology: previously registered for oligotrophic environments (Krammer 2000). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377) substrates.

Pinnularia rumrichae Krammer.
Figure: 4Z
Length: 45-46.5 µm; width: 7.5-8 µm; striae: 13 in 10 µm.
Ecology: in our samples, it occurred in slightly acidic to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and in oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63378) substrates.

Pinnularia schoenfelderi Krammer.
Figure: 4N
Length: 23 µm; width: 4.8 µm; striae: 16 in 10 µm.
Ecology: previously registered for oligotrophic environments with low to moderate conductivity (Krammer 2000), and also for mesotrophic waters (Bertollit et al. 2010). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, only in autumn (temperature 18.6°C).
Occurrence in samples: natural substrates (UPCB 63377).

Pinnularia subcapitata var. *elongata* Krammer.
Figure: 4AA
Length: 43.5 µm; width: 6 µm; striae: 16 in 10 µm.
Ecology: commonly associated with sphagnum moss (Krammer 1992). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C).
Occurrence in samples: artificial substrates (UPCB 63374).

Pinnularia subcapitata var. *subrostrata* Krammer
Figure: 4AB
Length: 38.5-46.5 µm; width: 6-6.5 µm; striae: 13 in 10 µm.
Ecology: widespread species; common in waters with medium electrolyte concentrations (Krammer 1992). In our samples, it occurred in circumneutral pH (6.7-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63377) substrates.

Pinnularia subgibba var. *undulata* Krammer
Figures: 4AC–4AD
Length: 65.1-68.5 µm; width: 7-7.8 µm; striae: 11-12 in 10 µm.
Ecology: previously registered for waters with neutral pH and high conductivity (Noga et al. 2014). In Brazil, it was recorded from planktonic samples in Guaraguaçu River (Tremarin et al. 2010) and from epiphytic samples in a mesotrophic reservoir of the South region (Marra et al. 2016). This study expanded its occurrence to circumneutral pH (6.7-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63377) substrates.

Pinnularia submicrostauron Schroeter
Figure: 4AE
Length: 38.4-43.7 µm; width: 5-6.6 µm; striae: 12-13 in 10 µm.
Ecology: previously registered as epiphytic in non-polluted environments, with acid pH (3.95-5.89) and medium to high conductivity (46-94 µS cm^{-1}) (Krammer 1992, Veselá 2009, Szulc et al. 2014). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63376; UPCB 63377) substrates.

Pinnularia viridiformis var. *viridiformis* Krammer
Figure: 4X
Length: 131 µm; width: 17.3 µm; striae: 10 in 10 µm.
Ecology: this species is frequently sampled especially in oligotrophic and mesotrophic environments, with low to moderate conductivity (Krammer 2000, Bertolli et al. 2010), being more abundant in acid pH (Van de Vijver & Beyen 1997, Veselá 2014). In our study, it was only recorded from samples of periphyton associated with aquatic macrophytes (75%), in slightly acid to circumneutral pH (6-6.85), low conductivity (24-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63375; UPCB 63376; UPCB 63378).

Pinnularia viridiformis var. *minor* Krammer.
Figure: 4Y
Length: 52-82.5 µm; width: 11-12.5 µm; striae: 10-12 in 10 µm.
Pinnularia viridiformis var. *minor* differs from *Pinnularia viridis* (Nitzsch) Ehrenberg by the valve outline and by the presence of a semi complex raphe (Krammer 2000).
Ecology: considered as cosmopolitan (Krammer 2000); it is rare in waters with neutral to alkaline pH and in moderate to high conductivity (Cremer et al. 2004, Noga et al. 2014). It was previously registered for streams with acid pH and low conductivity, in Central Brazil (França et al. 2017). In our study, it was recorded from samples of periphyton associated with aquatic macrophytes, in temperatures of 16°C, acid pH (6), low conductivity (24.5 µS cm^{-1}), and oligotrophic conditions. This is the first record for Paraná state.
Occurrence in samples: natural substrates (UPCB 63378).

Order NAVICULALES
Family *incertae sedis*
Chamaepinnularia brasilianopsis Metzeltin & Lange-Bertalot
Figures: 4AJ–4AK
Length: 13.5-13.9 µm; width: 3.5-4.1 µm; striae: 24 in 10 µm.
Ecology: previously described for the Neotropical region (Metzeltin & Lange-Bertalot 1998), in mesotrophic waters (Marra et al. 2016). with circumneutral to slightly alkaline pH, and conductivity between 24.3 and 145 µS cm^{-1} (Sala et al. 2015). In our study, it was only registered in autumn, from samples of macrophytes, in temperatures of 18.6°C, circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63377).

Chamaepinnularia mediocris (Krasske) Lange-Bertalot & Krammer
Figures: 4AL–4AP
Length: 10-14.7 µm; width: 2.5-3 µm; striae: 21-24in 10 µm.
Ecology: considered as characteristic of oligotrophic environments (van Dam et al. 1994, Hofmann 1994), but also cited for mesotrophic and eutrophic waters (Silva et al. 2010, Marra et al. 2016). It is a good indicator of environments rich in minerals, with moderate to high conductivity, and acid pH (Fránková et al. 2009), and also associated with the process of deglaciation (Fell et al. 2018). In our study, it occurred in summer (temperature of 27°C and high acummulated rainfall), in waters with slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63372) and natural (UPCB 63377; UPCB 63378) substrates.

Kobayasiella sp.
Figure: 4O
Length: 39.8 µm; width: 7.4 µm; striae: 15 in 10 µm.
Kobayasiella sp. presents linear-elliptic valves with subcapitate ends. The axial area is linear and straight. The central area is elliptic and delimited by irregular striae that are radiate towards the valve center. The valve outline is similar to *Kobayasiella acidophila* Vanhoutte, *Kobayasiella subtilissima* (Cleve) Lange-Bertalot and *Kobayasiella tapajoensis* Metzeltin & Lange-Bertalot, but these three latter taxa

present smaller size (24.7-31.2 µm, 23-27 µm, and 23-34 µm, respectively) and a higher striae density (34-42, 36-40 and 34-38 in 10 µm, respectively). *Kobayasiella* sp. is also similar to *Kobayasiella floridana* Metzeltin & Lange-Bertalot and *Kobayasiella krasskei* (Metzeltin & Lange-Bertalot) Lange-Bertalot, but these species have larger valves (54-83 µm and 44-50 µm, respectively) and higher striae density (28-32 and 27-28 in 10 µm, respectively) (Metzeltin & Lange-Bertalot 2007, 1998, Vanhoutte et al. 2004).
Occurrence in samples: natural substrates (UPCB 63377).

Family NAVICULACEAE
Navicula cryptocephala Kützing
Figure: 4AF
Length: 23.2-39.2 µm; width: 4.8-6 µm; striae: 12-18 in 10 µm.
Ecology: cosmopolitan; registered for oligotrophic to eutrophic environments, but more abundant in mesotrophic waters (Bertolli et al. 2010, Bartozek et al. 2018). In our study, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373) and natural (UPCB 63375; UPCB 63376; UPCB 63378) substrates.

Navicula cryptotenella Lange-Bertalot.
Figure: 4AG
Length: 20-35 µm; width: 4.5-5.6 µm; striae: 12-18 in 10 µm.
Ecology: widely distributed in oligotrophic to eutrophic conditions (Bertolii et al. 2010, Silva et al. 2010, Marra et al. 2016, Bartozek et al. 2018, Santos & Ferragut 2018), but most commonly associated with low TP concentrations (Wang et al. 2019). It occurred in all of our samples, in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63371; UPCB 63372; UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Navicula leptostriata Jørgensen
Figure: 4AH
Length: 33-36.3 µm; width: 4.5-4.8 µm; striae: 17-18 in 10 µm.
Ecology: previously registered for environments with moderate to high nutrient concentrations (van Dam et al. 1994, Silva et al. 2010, Ma et al. 2014). In our study, it occurred in temperatures < 21°C, slightly acid and circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}), and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63374) and natural (UPCB 63375; UPCB 63377) substrates.

Navicula veneta Kützing
Figure: 4AI
Length: 17 µm; width: 4 µm; striae: 18 in 10 µm.
Ecology: commonly registered for environments rich in nutrients (van Dam et al. 1994, Hofmann 1994, Silva et al. 2010, Marra et al. 2016, Casco et al. 2018). In our samples, it only occurred in autumn (temperature of 18.6°C). This study expanded its occurrence to circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63377).

Capartogramma crucicula (Grunow ex Cleve) Ross
Figures: 4P–4Q
Length: 27.5 µm; width: 8.5 µm; striae: 21 in 10 µm.
Ecology: the ecological optimum of this species is difficult to define (Beyene et al. 2014). It was previously registered for the oligotrophic paleophase (Faustino et al. 2016) and for modern mesotrophic conditions (Marra et al. 2016). In our study, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, only in spring (temperature of 20.8°C).
Occurrence in samples: artificial substrates (UPCB 63771).

Family STAURONEIDACEAE
Stauroneis neohyalina Lange-Bertalot & Krammer
Figures: 5A–5B
Length: 43-52 µm; width: 8-8.7 µm; striae: difficult to distinguish under the LM.
Stauroneis neohyalina differs from *Stauroneis gracilior* (Rabenhorst) Reichardt by the presence of subcapitate apices, narrower valves and a higher striae density. The striae are difficult to distinguish under the light microscope and the areolae are inconspicuous (Reichardt 1995, Lange-Bertalot & Metzeltin 1996). In this species, the proximal ends of the raphe are straight and not inflated and teardrop-shaped as in *S. gracilior* (Cantonati et al. 2017).
Widely recorded in different worldwide regions (Guiry & Guiry 2018). This is the first record for Brazil.
Ecology: previously registered for oligotrophic and acidic environments (van Dam et al. 1994, Sienkiewicz 2005, Van de Vijver et al. 2005, Krstić et al. 2012). In our samples, it occurred in slightly acid to circumneutral pH (6-6.75), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions, in summer (temperature of 27°C).
Occurrence in samples: artificial (UPCB 63373) and natural (UPCB 63376; UPCB 63378) substrates.

Stauroneis gracilis Ehrenberg
Figure: 5C
Length: 83.5 µm; width: 13.5 µm; striae: 23 in 10 µm.
Ecology: considered as characteristic of oligotrophic environments (Dixit & Smol 1995); it is rare in mesotrophic and eutrophic conditions (Silva et al. 2010, Marra et al. 2016) and has its optimum in conditions of low conductivity (Pestryakova et al. 2018). In our study, it only occurred from samples of aquatic macrophytes, in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, in autumn (temperature of 18.6°C).
Occurrence in samples: natural substrates (UPCB 63377).

Stauroneis phoenicenteron (Nitzsch) Ehrenberg
Figure: 5D
Length: 96.5-116.5 µm; width: 18.2-22.5 µm; striae: 18-19 in 10 µm.
Ecology: considered as characteristic of meso-eutrophic environments (van Dam et al. 1994, Silva et al. 2010, Bertolii et al. 2010), it has its optimum in conditions of intermediate conductivity (Pestryakova et al. 2018) and acid to circumneutral pH (Ma et al. 2014). In this samples, it occurred in slightly to circumneutral pH (6-6.7), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions, in autumn (temperature < 21°C).
Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63377) substrates.

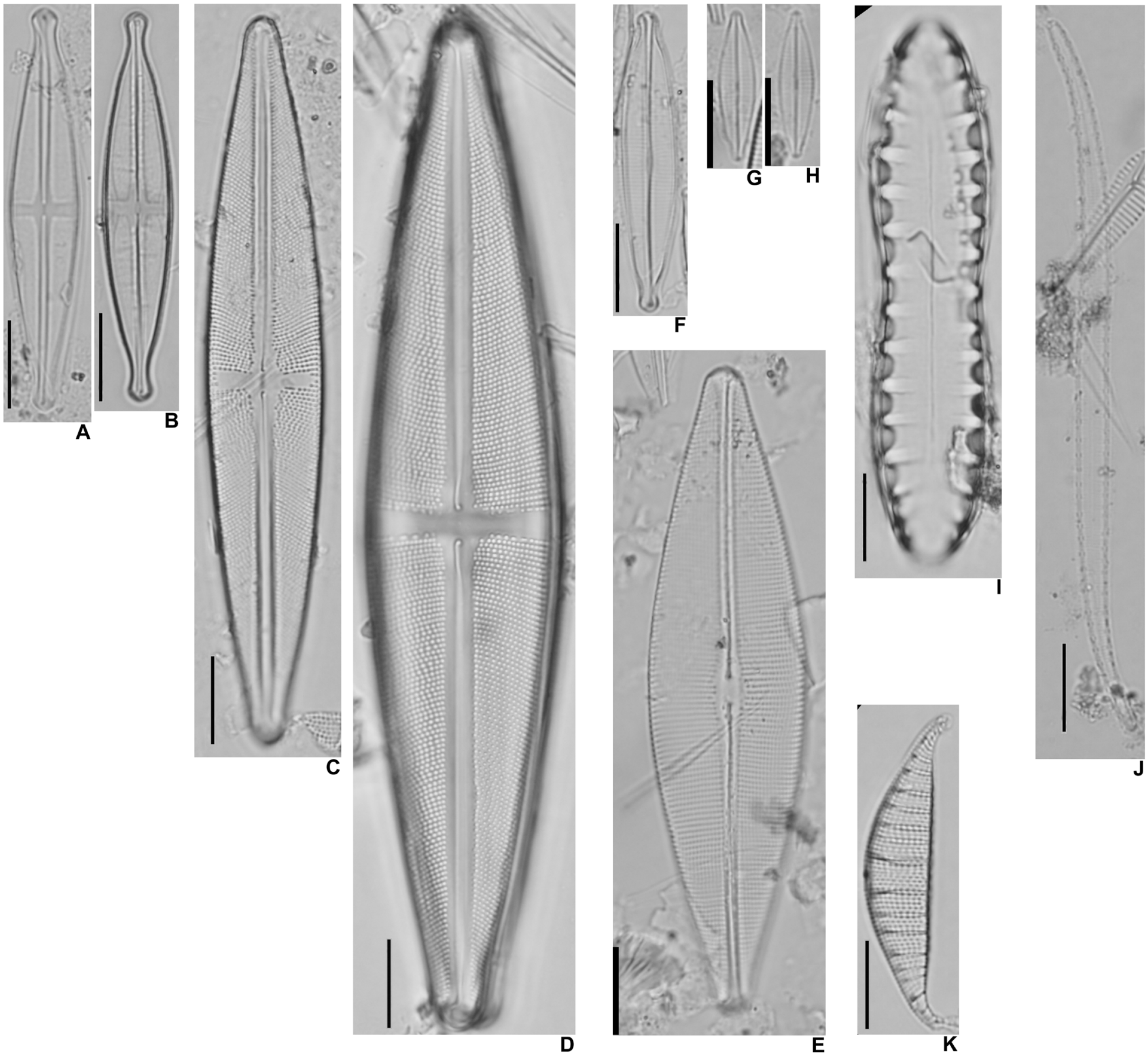


Figure 5. A–B. *Stauroneis neohyalina*; C. *Stauroneis gracilis*; D. *Stauroneis phoenicenteron*; E. *Craticula acidoclinata*; F. *Craticula riparia* var. *mollenhaurei*; G–H. *Craticula submolesta*; I. *Surirella grunowii*; J. *Iconella curvula*; K. *Rhopalodia gibberula* var. *vanheurckii*.

Craticula acidoclinata Lange-Bertalot & Metzeltin
Figure: 5E
Length: 65-1-74.2 µm; width: 17.9-18.5 µm; striae: 15 in 10 µm; areolae: 24 in 10 µm.

Craticula acidoclinata differs from *Craticula ambigua* (Ehrenberg) Mann by the presence of subrostrate apices and broadly rounded ends in the valves (distinctly protracted and rostrate in *C. ambigua*). *Craticula cuspidata* (Kützing) Mann has wider valves with narrower apices (Lange-Bertalot & Metzeltin 1996).

Ecology: registered for acid environments, poor in nutrients (Cantonati et al. 2017). It was previously found in Rio Tapajós, Brazil (Metzeltin & Lange-Bertalot 1998). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, in autumn (temperature < 21°C).

This is the first record for Paraná state.

Occurrence in samples: natural substrates (UPCB 63377).

Craticula riparia var. *mollenhaurei* Lange-Bertalot.
Figure: 5F
Length: 33.5 µm; width: 7 µm; striae: 24 in 10 µm.

Craticula riparia var. *mollenhaurei* resembles *Craticula riparia* (Hustedt) Lange-Bertalot var. *riparia* Lange-Bertalot in the valve outline but differs from the latter by the presence of longer and wider valves (35-50 µm and 8-10.5 µm) and a lower striae density (15-18 in 10 µm). *Craticula riparia* var. *riparia* is common in eutrophic environments with high conductivity (Lange-Bertalot 2001, van Dam et al. 1994).

Ecology: previously registered for slightly acid to neutral waters, with low conductivity (Lange-Bertalot 2001, Lange-Bertalot 1993). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, in.autumn (temperature < 21°C).

This is the first record for Brazil.
Occurrence in samples: natural substrates (UPCB 63377).

Craticula submolesta (Hustedt) Lange-Bertalot
Figures: 5G–5H
Length: 16.8-17.1 µm; width: 4-4.3 µm; striae: 22-24 in 10 µm.
Ecology: considered as characteristic of oligotrophic environments (Lange-Bertalot & Metzeltin 1996), it is rarely found in mesotrophic conditions (Marra et al. 2016). In our samples, it occurred in circumneutral pH (6.7), low conductivity (22.5 µS cm^{-1}) and oligotrophic conditions, in autumn (temperature < 21°C).
Occurrence in samples: natural substrates (UPCB 63377).

Family BACILLARIACEAE
Nitzschia acidoclinata Lange-Bertalot
Figure: 4U–4V
Length: 21-38.7 µm; width: 2.5-3 µm; indistinct striae; fibulae: 13-14 in 10 µm.
Ecology: previously registered for oligo-mesotrophic environments (van Dam et al. 1994, Hofmann 1994). In our samples, it occurred in temperatures < 21°C, slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: artificial (UPCB 63373; UPCB 63374) and natural (UPCB 63375; UPCB 63378) substrates.

Nitzschia nana Grunow
Figure: 4W
Length: 58.3, width: 5.2 µm; indistinct striae; fibulae: 10 in 10 µm.
Ecology: it avoids saprobic environments (Cantonati et al. 2017). In our samples, it occurred in circumneutral pH (6.85), low conductivity (24 µS cm^{-1}) and oligotrophic conditions, in spring (temperature < 21°C).
Occurrence in samples: natural substrates (UPCB 63375)

Nitzschia semirobusta Lange-Bertalot.
Figures: 4R–4T
Length: 16-20 µm; width: 4.5 -5 µm; striae: 16-18 in 10 µm; fibulae: 7 in 10 µm.
Nitzschia semirobusta differs from *Nitzschia amphibia* Grunow by the presence of longer fibulae and from *Nitzschia amphibioides* Hustedt by having a greater striae density. *Denticula kuetzingii* Grunow is distinguished from *Nitzschia semirobusta* by the presence of fibulae that are extended completely and with similar thickness from margin to margin in the first species (Lange-Bertalot 1993), while in the latter species the fibulae decrease in thickness towards the margin.
Ecology: Due to the taxonomic problems and misidentification, the ecological preference of *N. semirobusta* is unclear. Therefore, information on its occurrence and ecological preferences is of high relevance for delimiting the ecological requirements and tolerances of *N. semirobusta*. It was frequently registered for oligo- to mesotrophic reservoirs in São Paulo state (Bartozek et al. 2018). In our samples, it occurred in slightly acid to circumneutral pH (6-6.85), low conductivity (22.5-24.5 µS cm^{-1}) and oligotrophic conditions.
This is the first record for Paraná state.
Occurrence in samples: artificial (UPCB 63371; UPCB 63374) and natural (UPCB 63375; UPCB 63376; UPCB 63377; UPCB 63378) substrates.

Hantzschia amphioxys (Ehrenberg) Grunow
Figure: 4L–4M
Length: 23.5-45 µm; width: 5-5.6 µm; indistinct striae; fibulae: 8-9 in 10 µm.
Ecology: common in soil and aerial habitats (Jahn et al. 2014), and considered as tipically indifferent to trophic conditions (van Dam et al. 1994, Hofmann 1994). It is rarely found in waters poor in nutrients, being more common in mesotrophic environments (Bertolli et al. 2010, Bartozek et al. 2018, Cuña-Rodríguez et al. 2018) and eutrophic conditions (Silva et al. 2010). Previous studies associated this species with cold waters (Darling et al. 2017), however, in our samples it only occurred in summer (temperature 27°C), in circumneutral pH (6.75), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions.
Occurrence in samples: natural substrates (UPCB 63376).

Family RHOPALODIACEAE
Rhopalodia gibberula var. *vanheurckii* O. Müller
Figure: 5K
Length: 35.1-36.3 µm; width: 8-8.5 µm; striae: 14-19 in 10 µm; transapical ribs: 4-5 in 10 µm.
Ecology: considered as an alkalinophilous species (van Dam et al. 1994). In our samples, it occurred in slightly acid pH (6), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in winter (temperature 16°C),
Occurrence in samples: natural substrates (UPCB 63378).

Family SURIRELLACEAE
Surirella grunowii Kulikovskiy, Lange-Bertalot & Witkovski.
Figure: 5I
Length: 62.4-76.1 µm; width: 11.5-11.6 µm; striae: difficult to distinguish; alar canals: 3-4 in 10 µm.
Surirella linearis var. *constricta* Grunow was synonymized with *S. grunowii* by Kulikovskiy et al. (2010).
Ecology: previously registered for oligo-mesotrophic environments (van Dam et al. 1994). In our samples, it occurred in circumneutral pH (6.7-6.85), low conductivity (22.5-24 µS cm^{-1}) and oligotrophic conditions, in spring and autumn seasons (temperature < 21°C).
Occurrence in samples: artificial (UPCB 63371) and natural (UPCB 63377) substrates.

Iconella curvula (Smith) Ruck & Nakov
Figure: 5J
Length: 92 µm; width: 4 µm; indistinct striae; alar canals: 7 in 10 µm.
Ecology: cited as *Stenopterobia curvula* (Smith) Krammer, it was registered for oligotrophic (van Dam et al. 1994) and mesotrophic environments (Marra et al. 2016). In our samples, it occurred in circumneutral pH (6.75), low conductivity (24.5 µS cm^{-1}) and oligotrophic conditions, only in summer (temperature 27°C).
Occurrence in samples: natural substrates (UPCB 63376).

Conclusions

The inventory performed at the Piraquara I reservoir brings an important contribution to the diatom diversity from oligotrophic environments. We registered the first occurrence of five diatom species in Brazil and seven in the state of Paraná, contributing to the geographic distribution and the ecology of the identified species. Finally, fourteen periphytic species extended their ecological limits of pH, conductivity and trophic preferences.

Acknowledgments

To "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) for the master´s scholarship (AMSL), scientific productivity scholarship (TAVL) and financial support for the project (CTHidro-555397/2006-8). To IAP and SANEPAR for providing chemical data and field sampling support and to Laboratório de Ficologia UFPR for facilitating the use of the optical microscope.

Author Contributions

Angela Maria da Silva-Lehmkuhl: has substantial contribution in the concept and design of the study; data collection; data analysis and interpretation; manuscript preparation and to critical revision, adding intelectual content.

Priscila Izabel Tremarin: Has substantial contribution to data analysis and interpretation, and to critical revision, adding intelectual content.

Ilka Schincariol Vercellino: Has substantial contribution to data analysis and interpretation, and to critical revision, adding intelectual content.

Thelma A. Veiga Ludwig: has substantial contribution in the concept and design of the study; data collection; data analysis and interpretation; manuscript preparation and to critical revision, adding intelectual content.

Conflicts of Interest

The authors declare that they have no conflict of interest related to the publication of this manuscript.

References

ÁCS, E., KISS, K.T., SZABÓ, K. & MAKK, J. 2000. Short-term colonization sequence of Periphyton on glass slides in a large river (River Danube, near Budapest). Algological Studies 100: 135-156.

ALLES, E., NÖRPEL-SCHEMPP, M. & LANGE-BERTALOT, H. 1991. Zur Systematik und Ökologie charakteristischer Eunotia-Arten (Bacillariophyceae) in elektrolytarmen Bachoberläufen. Nova Hedwigia 53 (1-2): 171-213.

AZEVEDO, J.S., TALGATTI, D.M., TORGAN, L.C., PEREIRA, A.C. & MELO, S. 2018. O genero *Gomphonema* (Bacillariophyta) na comunidade perifitica do rio e reservatório de Curuá-Una (Santarém, Pará, Brasil). Rodriguésia 69(2): 765-776. http://dx.doi.org/10.1590/2175-7860201869234

BAHLS, L.L. 2015. *Kurtkrammeria*, a new genus of freshwater diatoms (Bacillariophyta, Cymbellaceae) separated from *Encyonopsis*. Nova Hedwigia 101: 165-190. http://dx.doi.org/10.1127/nova_hedwigia/2015/0263

BARTOZEK, E.C.R., ZORZAL-ALMEIDA, S. & BICUDO, D.C. 2018. Surface sediment and phytoplankton diatoms across a trophic gradient in tropical reservoirs: new records for Brazil and São Paulo State. Hoehnea 45(1): 69-92. http://dx.doi.org/10.1590/2236-8906-51/2017

BARTOZEK, E.C.R., BUENO, N.C., LUDWIG, T.A.V., TREMARIN, T.A.V., NARDELLI, M.S. & ROCHA, A.C.R. 2013. Diatoms (Bacillariophyceae) of Iguaçu National Park, Foz do Iguaçu, Brazil. Acta Botanica Brasilica 27(1): 108-123. http://dx.doi.org/10.1590/S0102-33062013000100012

BERTOLLI, L.M., TREMARIN, P.I. & LUDWIG, T.A.V. 2010. Diatomáceas perifíticas em *Polygonum hydropiperoides* Michaux, reservatório do Passaúna, Região Metropolitana de Curitiba, Paraná, Brasil. Acta Bot. Bras. 24(4):1065-1081. http://dx.doi.org/10.1590/S0102-33062010000400022

BEYENE, A., AWOKE, A. & TRIEST, L. 2014. Validation of a quantitative method for estimating the indicator power of, diatoms for ecoregional river water quality assessment. Ecological Indicators 37: 58-66. http://dx.doi.org/10.1016/j.ecolind.2013.09.025

BICCA, A.B. & TORGAN, L.C. 2009. Novos registros de *Eunotia* Ehrenberg (Eunotiaceae-Bacillariophyta) para o Estado do Rio Grande do Sul e Brasil. Acta Bot. Bras. 23(2): 427-435. http://dx.doi.org/10.1590/S0102-33062009000200014

BICUDO, C.E.M. & MENEZES, M. 2017. Gêneros de algas de águas continentais do Brasil (Chave de identificação e descrições). 3 ed. Rima, São Carlos.

BICUDO, D.C., TREMARIN, P.I., ALMEIDA, P.D., ZORZAL-ALMEIDA, S., WENGRAT, S., FAUSTINO, S.B., COSTA, L.F., BARTOZEK, E.C.R., ROCHA, A.C.R., BICUDO, C.E.M. & MORALES, E.A. 2016. Ecology and distribution of *Aulacoseira* species (Bacillariophyta) in tropical reservoirs from Brazil. Diat. Res. 31(3): 199-215. http://dx.doi.org/10.1080/0269249X.2016.1227376

BICUDO, D.C., FONSECA, B.M., BINI, L.M., CROSSETI, L.O., BICUDO, C.E.M. & ARAÚJO-JESUS, T. 2007. Undesirable side-effects of water hyacinth control in a shallow tropical reservoir. Freshwater Biology 52: 1120-1133. http://dx.doi.org/10.1111/j.1365-2427.2007.01738.x

BURKHOLDER, J.M. 1996. Interactions of benthic algae with their substrata. In Algal ecology: freshwater benthic ecosystems (R.J. Stevenson, M.L. Bothwell & R.L. Lowe, eds). Academic Press, San Diego, p. 253-297.

CANANI, L.G.C., MENEZES, M. & TORGAN, L.C. 2011. Diatomáceas epilíticas de águas oligotróficas e ácidas do Sudeste do Brasil. Acta Bot. Bras. 25(1): 130-140. http://dx.doi.org/10.1590/S0102-33062011000100016

CANTONATI, M. & LANGE-BERTALOT, H. 2010. Diatom biodiversity of springs in the Berchtesgaden National Park (North-Eastern Alps, Germany), with the ecological and morphological characterization of two species new to science. Diat. Res. 25(2): 251-280. http://dx.doi.org/10.1080/0269249X.2010.9705849

CANTONATI, M., KELLY, M.G. & LANGE-BERTALOT, H. 2017. Freshwater benthic diatoms of Central Europe: over 800 common species used in ecological assessment. English edition with update taxonomy and added species. Schmitten-Oberreifenberg: Koeltz Botanical Books. p. 1-942.

CASA, V., MATALONI, G. & VAN DE VIJVER, B. 2018. Six new *Frustulia* species (Bacillariophyta) in Tierra del Fuego peatbogs, Patagonia, Argentina. Fottea. 18(1): 55-71. http://dx.doi.org/10.5507/fot.2017.016

CASARTELLI, M.R., LAVAGNOLLI, G.J. & FERRAGUT, C. 2016. Periphyton biomass accrual rate changes over the colonization process in a shallow mesotrophic reservoir. Acta Limnologica Brasiliensia 28: e9. http://dx.doi.org/10.1590/S2179-975X0116

CASCO, M.A., REYES, I., MARTÍN, G., FERNÁNDEZ, .R., SALA, S.E. & TOJA, J. 2018. Phycological flora of the Guadalquivir river basin. Its value in determining the ecoregionalization of the basin. Limnetica 37(2): 239-248. http://dx.doi.org/10.23818/limn.37.20

CINIGLIA, C., CENNAMO, P., DE STEFANO, M., PINTO, G., CAPUTO, P. & POLLIO, A. 2007. *Pinnularia obscura* Krasske (Bacillariophyceae, Bacillariophyta) from acidic environments: characterization and comparison with other acid-tolerant *Pinnularia* species. Fundamental and Applied Limnology Archiv für Hydrobiologie 170(1): 29-47. http://dx.doi.org/10.1127/1863-9135/2007/0170-0029

COSTA, L.F., WETZEL, C.E., LANGE-BERTALOT, H., ECTOR, L. & BICUDO, D.C. 2017. Taxonomy and ecology of *Eunotia* species (Bacillariophyta) in southeastern Brazilian reservoirs. Biblioth. Diatomol. 64: 1-302.

CREMER, H., GORE, D., HULTZSCH, N., MELLES, M. & WAGNER, B. 2004. The diatom flora and limnology of lakes in the Amery Oasis, East Antarctica. Polar Biol 27: 513-531. http://dx.doi.org/10.1007/s00300-004-0624-2

CUNÃ-RODRÍGUEZ, C., PIOVANO, E., DEL PUERTO, L., INDA, H. & GARCÍA-RODRÍGUEZ, F. 2018. On the relationship between holocene environmental variability and the diatomcomposition in the Peña Laggon, Se Uruguay. Ameghiniana 55(4): 423-436.

DARLING, J.P., GARLAND, D.D., STANISH, L.F., ESPOSITO, R.M.M., SOKOL, E.R. & MCKNIGHT, D.M. 2017. Thermal autecology describes the occurrence patterns of four benthic diatoms in McMurdo Dry Valley streams. Polar Biol 40: 2381-2396. https://doi.org/10.1007/s00300-017-2151-y

DELA-CRUZ, J., PRITCHARD, T., GORDON, G. & AJANI, P. 2006. The use of periphytic diatoms as a means of assessing impacts of point source inorganic nutrient pollution in south-eastern Australia. Freshwat. Biol. 51: 951-972. http://dx.doi.org/10.1111/j.1365-2427.2006.01537.x

DELGADO, C., NOVAIS, M.H., BLANCO, S. & ALMEIDA, S.P. 2015. Examination and comparison of *Fragilaria candidagilae* sp. nov. with type material of *Fragilaria recapitellata, F. capucina, F. perminuta, F. intermedia* and *F. neointermedia* (Fragilariales, Bacillariophyceae). Phytotaxa. 231(1): 1-18. http://dx.doi.org/ 10.11646/phytotaxa.231.1.1

DIXIT, S.S. & SMOL, J.P. 1995. Diatom evidence of past water quality changes in Adirondack Seepage Lakes (New York, U.S.A.). Diat. Res. 10(1): 113-129. http://dx.doi.org/10.1080/0269249X.1995.9705331

FARIA, D.M., GUIMARÃES, A.T.B. & LUDWIG, T.A.V. 2013. Responses of periphytic diatoms to mechanical removal to *Pistia stratiotes* L. in a hypereutrophic subtropical reservoir: dynammic and tolerance. Brazilian Journal of Biology 73(4): 681-689. http://dx.doi.org//10.1590/S1519-69842013000400002

FARIA, D.M., TREMARIN, P.I. & LUDWIG, T.A.V. 2010. Diatomáceas perifíticas da represa Itaqui, São José dos Pinhais, Paraná: Fragilariales, Eunotiales, Achnanthales, e *Gomphonema* Ehrenberg. Biota Neotropica 10(3): http://www.biotaneotropica.org.br/v10n3/pt/abstract?inventory+bn04110032010

FAUSTINO, S.B., FONTANA, L., BARTOZEK, E.C.R., BICUDO, C.E.M. & BICUDO, D.C. 2016. Composition and distribution of diatom assemblages from core and surface sediments of a water supply reservoir in Southeastern Brazil. Biota Neotropica: 16(2): e20150129. http://dx.doi.org/10.1590/1676-0611-BN-2015-0129

FELL, S.C., CARRIVICK, J.L., KELLY, M.G., FÜREDER, L. & BROWN, L.E. 2018. Declining glacier cover threatens the biodiversity of alpine river diatom assemblages. Global Change Biology 24: 5828-5840. http://dx.doi.org/ 10.1111/gcb.14454

FERRARI, F., PROCOPIAK, L.K., ALENCAR, Y.B. & LUDWIG, T.A.V. 2007. Eunotiaceae (Bacillariophyceae) em igarapés da Amazônia Central, Manaus e Presidente Figueiredo, Brasil. Acta Amaz. 37(1): 1-16. http://dx.doi.org/10.1590/S0044-59672007000100001

FRANÇA, A.A., DUNCK, B., RODRIGUES, L., FONSECA, B.M. & FELISBERTO, S.A. 2017. Periphytic diatoms (Bacillariophyta) in streams from three Conservation Units of centra Brazil: *Pinnularia* Ehrenberg. Hoehnea 44(4): 524-538. http://dx.doi.org/10.1590/2236-8906-107/2016

FRÁNKOVÁ, M., BOJKOVÁ, J., POULÍČKOVÁ, A. & HAJÉK, M. 2009. The structure and species richness of the diatom assemblages of the Western Carpathian pring fens long the gradient of mineral richness. Fottea 9(2): 355-368. http://dx.doi.org/10.5507/fot.2009.035

GOLDSBOROUGH, L.G. & ROBINSON, G.C.C. 1996. Pattern in wetlands. In: Algal ecology: freshwater benthic ecosystems (R.J. Stevenson, M.L. Bothwell & R.L. Lowe, eds). Academic Press, New York, p. 77-117.

GÓMÉZ, N. & LICURSI, M. 2001. The Pampean Diatom Index (IDP) for assessment of rivers and streams in Argentina. Aquatic Ecol. 5: 173-181. http://dx.doi.org/10.1023/A:1011415209445

GUIMARÃES, L.J.R. 2008. Levantamento das áreas potenciais ao assoreamento da barragem Piraquara I. Dissertação de Mestrado, Universidade Federal do Paraná Curitiba.

GUIRY, M.D. 2012. How many species of algae are there? Journal of Phycology 48: 1057-1063. http://dx.doi.org/10.1111/j.1529-8817.2012.01222.x

HÅKANSSON, S. 1993. Numerical methods for the inference of pH variations in mesotrophic and eutrophic lakes in Southern Sweden – a progress report. Diat. Res. 8(2): 349-370. http://dx.doi.org/10.1080/0269249X.1992.9705208

HOFMANN, G. 1994. Aufwuchs-Diatomeen in Seen und ihre Eignung als Indikatoren der Trophie. Biblioth. Diatomol. J. Cramer, Berlin – Stuttgart, 30.

HOFMANN, G., WERUM, M. & LANGE-BERTALOT, H. 2013. Diatomeen im Süßwasser - Benthos von Mitteleuropa. Bestimmungsflora Kieselalgen für die ökologische Praxis. Über 700 der häufigsten Arten und ihre Ökologie. Koeltz Scientific Books, Königstein.

HUSTEDT, F. 1927-1930. Die Kieselalgen. In Kryptogamen-Flora (L. Rabenhorst, ed). Akademische Verlagsgesellschaft, Leipzig, 7(1): 1-920.

HUSTEDT, F. 1931-1959. Die Kieselalgen. In Kryptogamen-Flora (L. Rabenhorst, ed). Akademische Verlagsgesellschaft, Leipzig, 7(2):1-845.

HUSTEDT, F. 1961-1966. Die Kieselalgen. In Kryptogamen-Flora (L. Rabenhorst, ed). Akademische Verlagsgesellschaft, Leipzig, 7(3): 1-816.

IVANOV, P. & KIRILOVA, E. 2004. Benthic diatoms assemblages from different substrates of the Iskar river, Bulgaria. In Proceeding of the Eighteenth International Diatom Symposium, Miedzyzdroje, Poland (A. Witkowski, ed). p. 107-124.

JAHN, R., KUSBER, W-H. & LANGE-BERTALOT, H. 2014. Typification and taxonomy of *Hantzschia amphioxys* (Ehrenberg) Grunow (Bacillariophyta): type of the genus name *Hantzschia* Grunow. Nova Hedwigia 143: 103-110. http://dx.doi.org/ 10.1127/1438-9134/2014/005

JOHANSEN, J., KOCIOLEK, P. & LOWE, R. 2008. *Spicaticribra kingstonii*, gen. nov. et. sp. nov. (Thalassiosirales, Bacillariophyta) from Great Smoky Mountains National Park, USA. Diat. Res. 23(2): 367-375. http://dx.doi.org/10.1080/0269249X.2008.9705763

JÚLIO JÚNIOR, H. F., THOMAZ, S. M., AOSTINHO, A. A., LATINI, J. D. 2005. Distribuição e caracterização dos reservatórios. In: RODRIGUES, L., THOMAZ, S. M., AGOSTINHO, A. A. & GOMES, L. C. (Eds). Biocenoses em reservatórios: padrões espaciais e temporais. São Carlos: Rima. cap. 1. p. 1-16.

KARTHICK, B. & KOCIOLEK, P. 2012. Reconsideration of the *Gomphonema* (Bacillariophyceae) species from Kholhapur, Northern Western Ghats, India: Taxonomy, typification and biogeography of the species reported by H. P. Gandhi. Phycological Research 60: 179-198. http://dx.doi.org/10.1111/j.1440-1835.2012.00649.x

KOCIOLEK, J.P. & STOERMER, E.F. 2009. Oligotrophy: the forgotten end of an ecological spectrum. Acta Bot. Croat. 68: 465-472.

KOPALOVÁ, K., KOCIOLEK, J.P., LOWE, R.L., ZIDAROVA, R. & VAN DE VIJVER, B. 2015. Five new species of the genus *Humidophila* (Bacillariophyta) from the Maritime Antarctic Region. Diatom Research 30(2): 117-131. http://dx.doi.org/10.1080/0269249X.2014.998714

KRAMMER, K. 1997a. Die cymbelloidean Diatomeen: eine Monographie der weltweit bekannten taxa. I Allgemeines und *Encyonema* part. Biblioth. Diatomol. 36: 1-382.

KRAMMER, K. 1997b. Die cymbelloidean Diatomeen: eine Monographie der weltweit bekannten taxa. II *Encyonema* part., *Encyonopsis* and *Cymbellopsis*. Biblioth. Diatomol. 37: 1-469. Verlag KG, Ruggell, v. 3, 584 p.

KRAMMER, K. 1992. *Pinnularia*, eine Monographie der europäischen Taxa. Bibliotheca Diatomologica, Stuttgart, 26.

KRAMMER, K. 2000. The genus *Pinnularia*. In Diatoms of Europe 1 (H. Lange-Bertalot) p. 1-703.

KRAMMER, K. & LANGE-BERTALOT, H. 1986. Bacillariophyceae: Naviculaceae. In Süsswasserflora von Mitteleuropa (H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer, eds). Gustav Fischer, Jena. v. 2, pars 1, 876 p.

KRAMMER, K. & LANGE-BERTALOT, H. 1988. Bacillariophyceae: Bacillariaceae, Epithemiaceae, Surirellaceae. In Süsswasserflora von Mitteleuropa (H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer, eds). Gustav Fischer, Stuttgart. v. 2,pars 2, 596 p.

KRAMMER, K. & LANGE-BERTALOT, H. 1991a. Bacillariophyceae: Centrales, Fragilariaceae, Eunotiaceae. In Süsswasserflora von Mitteleuropa (H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer, eds). Gustav Fischer, Stuttgart. v. 2, pars 3, 576 p.

KRAMMER, K. & LANGE-BERTALOT, H. 1991b. Bacillariophyceae: Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. In Süsswasserflora von Mitteleuropa (H. Ettl, J. Gerloff, H. Heynig, D. Mollenhauer, eds). Gustav Fischer, Stuttgart. v. 2, pars 4, 437 p.

KRASSKE, G. 1948. Diatomeen tropischer Moosrasen. Svensk Botanisk Tidskrift 42(4): 404-443.

KRSTIĆ, S.S., ZECH, W., OBREHT, I., SVIRČEV, Z. & MARCOVIĆ, S.B. 2012. Late quaternary environmental changes in Helambu Himal, Central Nepal, recorded in the diatom flora assemblage composition and geochemistry of Lake Panch Pokhari. J. Paleolimnol. 47: 113-124. https://doi.org/10.1007/s10933-011-9563-4

KRSTIĆ, S.S., PAVLOV, A., LEVKOV, Z. & JÜTTNER, I. 2013. New *Eunotia* taxa in core samples from Lake Panch Pokhari in the Nepalese Himalaya. Diat. Res. 28(2): 203-217. http://dx.doi.org/10.1080/0269249X.2013.782343

KULIKOVSKIY, M.S., LANGE-BERTALOT, H., WITKOWSKI, A., DOROFEYUK, N.I. & GENKAL, S.I. 2010. Diatom assemblages from Sphagnum bogs of the world. I. Nur bog in northern Mongolia. Biblio. Diatomol. 55: 1-326.

LANGE-BERTALOT, H. 1993. 85 Neue Taxa und über 100 weitere neu definierte Taxa ergänzend zur Süsswasserflora von Mitteleuropa. Bibliotheca Diatomologica 27: 1-164.

LANGE-BERTALOT, H. 1999. Neue Kombinationen von Taxa aus Achnanthes Bory (sensu lato). In Iconogr. Diatomol., Annotated Diatom Monographs (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 6:276-289.

LANGE-BERTALOT, H. 2001. *Navicula* sensu stricto. 10 Genera separated from *Navicula* sensu lato. *Frustulia*. Diatoms of Europe: diatoms of the European inland waters and comparable habitats. Ruggell: A.R.G. Gantner Verlag. K.G. 2:1-526.

LANGE-BERTALOT, H. & MOSER, G. 1994. *Brachysira* Monographie der Gattung. Wichtige Indikator Species für das Gewässermonitoring und *Naviculadicta* nov. gen. Biblio. Diatomol. 29: 1-212.

LANGE-BERTALOT, H. & METZELTIN, D. 1996. Indicators of oligotrophy-800 taxa representative of three ecologically distinct lake types, Carbonate buffered-Oligodystrophic-Weakly buffered soft water. In Iconogr. Diatomol. (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 2:1-390.

LANGE-BERTALOT, H. & METZELTIN, D. 2009. A dystrophic mountain lake in Panama – Hot spot of new and rare neotropical diatoms. Nova Hedwigia. 135: 137-166.

LANGE-BERTALOT, H., METZELTIN, D. & WITKOWSKI, A. 1996. *Hippodonta*gen. nov.-Umschreibung und begründung einer Gattung der Naviculaceae. In Iconogr.Diatomol (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 4: 247-275.

LANGE-BERTALOT, H.; BAK, M. & WITKOWSKI, A. 2011. *Eunotia* and some related genera. In: Diatoms of Europe (Lange-Bertalot, H., ed.). Königstein, Koeltz Scientific Books, 6.

LAMPARELLI, M.C. 2004. Grau de trofia em corpos d'água do estado de São Paulo: avaliação dos métodos de monitoramento. Tese de doutorado, Universidade de São Paulo, São Paulo.

LESKINEN, E. & HÄLLFORS, G. 1990. Community structure of epiphytic diatoms in relation to eutrophication on the Hanko Peninsula, south coast of Finland. In: Proceeding of the Thent International Diatom Symposium (H. Simola, ed.). Koeltz Scientific Books, Koenigstein, p. 323-333.

LEVKOV, Z., KRSTIC, S., METZELTIN, D. & NAKOV, T. 2007. Diatoms of Lakes Prespa and Ohrid. In Iconogr. Diatomol. (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 16: 1-613.

LOBO, E.A., CALLEGARO, V.L.M. & BENDER, E.P. 2002. Utilização de algas diatomáceas epilíticas como indicadores da qualidade da água em rios e arroios da região hidrográfica do Guaíba, RS, Brasil. EDUNISC, Santa Cruz do Sul, 127p.

LOWE, R.L. 1996. Periphyton patterns in lakes. In: Algal ecology: freshwater benthic ecosystems (R.J. Stevenson, M.L. Bothwell & R.L. Lowe, eds). Academic Press, New York, p. 57-76.

LOWE, R.L. & PAN, Y. 1996. Benthic algal communities as biological monitors. In: Algal ecology: freshwater benthic ecosystems (R.J. Stevenson, M.L. Bothwell & R.L. Lowe, eds). Academic Press, New York, p. 705-739.

LUDWIG, T.A.V., TREMARIN, P.I., BECKER, V. & TORGAN, L.C. 2008. *Thalassiosira rudis* sp. nov. (Coscinodiscophyceae): a new freshwater species. Diat. Res. 23(2): 389-400. http://dx.doi.org/10.1080/0269249X.2008.9705765

MA, Y., LI, G., LI, J., ZHOU, H. & JIANG, B. 2014. Seasonal succession of phytoplankton community and its relationship with environmental factors of North Temperate Zone water of the Zhalong wetland, in China. Ecotoxicology 23: 618-625. http://dx.doir.org/10.1007/s10646-014-1231-9

MANN, D.G. & VANORMELIGEN, P. 2013. An inordinate fondness? The number, distributions, and origins of diatom species. The Journal of Eukaryotic Microbiology 60: 414-420. http://dx.doi.org/10.1111/jeu.12047

MARRA, R.C., TREMARIN, P.I., ALGARTE, V.M. & LUDWIG, T.A. 2016. Epiphytic diatoms (Diatomeae) from Piraquara II urban reservoir, Paraná state. Biotaneotropica 16(4): e20160200. http://dx.doi.org/10.1590/1676-0611-BN-2016-0200

MARQUARDT, G.C. & BICUDO, C.E.M. 2014. Criptógamos do Parque Estadual das Fontes do Ipiranga, São Paulo, SP. Algas 36: Bacillariophyceae (Cymbellales). Hoehnea 41(2): 209-246. http://dx.doi.org/10.1590/S2236-89062014000200005

MARQUARDT, G.C., ROCHA, A.C.R., WETZEL, C.E., ECTOR, L. & BICUDO, C.E.M. 2016. *Encyonema aquasedis* sp. nov. and *Kurtkrammeria salesopolensis* sp. nov.: two new freshwater diatom species (Cymbellales, Bacillariophyceae) from an oligotrophic reservoir in southeastern Brazil. Phytotaxa 247(1): 62-74.

MARQUARDT, G.C., WENGRAT, S., BICUDO, D.C., WETZEL, C.E., ECTOR, L. & BICUDO, C.E.M. 2017. Morphology and distribution of *Encyonema angustecapitatum* Krammer species complex (Bacillariophyceae) with description of four new species from São Paulo, southeast Brazil. Fottea 17(2): 164-177. http://dx.doi.org/10.5507/fot.2017.008

MARQUARDT, G.C., BICUDO, C.E.M., LUDWIG, T.A.V., ECTOR, L. & WETZEL, C.E. 2018. Diatom assemblages (Bacillariophyta)in six tropical reservoirs from southeast Brazil: species composition and spatial and temporal variation patterns. Acta Limnologica Brasiliensia 30: e201. https://doi.org/10.1590/S2179-975X6417

MEDEIROS, G., AMARAL, M.W.W., FERREIRA, P.C., LUDWIG, T.A.V. & BUENO, N.C. 2018. *Gomphonema* Ehrenberg (Bacillariophyceae, Gomphonemataceae) of the São Francisco Falso River, Paraná, Brazil. Biota Neotropica 18(3): e20170495. http://dx.doi.org/10.1590/1676-0611-BN-2017-0495

MELO, E.F. 2008. Flora da Serra do Cipó, Minas Gerais: Polygonaceae. Bol. Bot. Univ. São Paulo. 26(2): 165-174. http://dx.doi.org/10.11606/issn.2316-9052.v26i2p165-174

METZELTIN, D. & LANGE-BERTALOT, H. 1998. Tropical diatoms of South America I. About 700 predominantly rarely known or new taxa representative of the neotropical flora. In Iconogr. Diatomol. (H. Lange-Bertalot, ed.) 5: 1-695.

METZELTIN, D. & LANGE-BERTALOT, H. 2002. Diatoms from the ″Island Continent″ Madagascar. In Iconogr. Diatomol (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 11: 1-286.

METZELTIN, D. & LANGE-BERTALOT, H. 2005. Diatoms of Uruguay. Compared with other taxa from South America and elsewhere. In Iconogr. Diatomol (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 15:1-736.

METZELTIN, D. & LANGE-BERTALOT, H. 2007. Tropical Diatoms of South America II. Special remarks on biogeographic disjunction. In Iconogr. Diatomol (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 18:1-877.

METZELTIN, D., LANGE-BERTALOT, H. & SONINKHISHING, N. 2009. Diatoms in Mongolia. In Iconogr.Diatomol (H. Lange-Bertalot, ed.). Gantner Verlag KG, Ruggell, 20: 3-686.

MONTOYA-MORENO, Y., SALA, S., VOUILLOUD, A. & AGUIRRE, N. 2011. *Capartogramma crucicula* (Grunow *ex* Cleve) Ross, primer registro del género para Colombia. Universitas Scientiarum 16(1): 70-76.

MOREIRA-FILHO, H. & VALENTE MOREIRA, I.M. 1981. Avaliação taxonômica e ecológica das diatomáceas (Bacillariophyceae) epífitas em algas pluricelulares obtidas nos litorais dos Estados do Paraná, Santa Catarina e São Paulo. Bol. Mus. Bot. Mun. 47: 1-17.

MOSER, G., LANGE-BERTALOT, H. & METZELTIN, D. 1998. Insel der Endemiten Geobotanisches Phänomen Neukaledonien (Island of endemics New Caledonia - a geobotanical phenomenon). Bibliotheca Diatomologica 38:464 pp.

MURDOCK, J.N. & DODDS, W.K. 2007. Linking benthic algal biomass to stream substratum topography. Journal of Phycological 43: 449-460. http://dx.doi.org/10.1111/j.1529-8817.2007.00357.x

NOGA, T., PESZEK, Ł., STANEK-TARKOWSKA, J. & PAJĄCZEK, A. 2014. The *Pinnularia* genus in south-eastern Poland with consideration of rare and new taxa to Poland. Oceanological and Hydrobiological Studies 43(1): 77-99. http://dx.doi.org/10.2478/s13545-014-0120-4

OSÓRIO, N.C., TREMARIN, P.I., LUDWIG, T.V. & RODRIGUES, L. 2017. *Gomphonema* Ehrenberg (Bacillariohyceae) in a lotic environment of the Upper Paraná River floodplain, Brazil. Acta Scientiarum. 39(2): 135-147. http://dx.doi.org/10.4025/actascibiolsci.v39i2.32134

PATRICK, R. & REIMER, C.W. 1966. The diatoms of United States: exclusive of Alaska and Hawaii. Acad. Nat. Sci. Philadelphia, Monographs, 13(1): 1-688.

PESTRYAKOVA, L.A., HERZSCHUH, U., GORODNICHEV, R. & WETTERICH, S. 2018. The sensitivity of diatom taxa from Yakutian lakes (north-eastern Siberia) to electoral conductivity nd other environmental variables. Polar Research 37:1485625. https://doi.org/10.1080/17518369.2018.1485625

POTAPOVA, M. & CHARLES, D.F. 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecol. Indic. 7: 48-70. http://dx.doi.org/10.1016/j.ecolind.2005.10.001

POULÍČKOVÁ, A., ŠPAČKOVÁ, J., KELLY, M.G., DUCHOSLAV, M. & MANN, D.G. 2008. Ecological variation within *Sellaphora* species complexes (Bacillariophyceae): specialists or generalists? Hydrobiologia 614: 373-386. http://dx.doi.org/10.1007/s10750-008-9521-y

REICHARDT, E. 1995. Die Diatomeen (Bacillariophyceae) in Ehrenberg´s material von Cayenne, Guyana Gallica (1843). In Iconogr. Diatomol. (H. Lange-Bertalot, ed.) 1: 1-107.

REICHARDT, E. 2015a. *Gomphonema gracile* Ehrenberg sensu stricto et sensu auct. (Bacillariophyceae): A taxonomic revision. Nova Hedwigia. 101(3-4): 367-393. http://dx.doi.org/10.1127/nova_hedwigia/2015/0275

REICHARDT, E. 2015b. Taxonomy and distribution of *Gomphonema subtile* Ehrenberg (Bacillariophyceae) and six related taxa. Fottea. 15(1): 27-38. http://dx.doi.org/10.5507/fot.2015.003

RIATO, L., LEIRA, M., DELLA BELLA, V. & OBERHOLSTER, P.J. 2017. Diatom-based models for inferring water chemistry and hydrology in temporary depressional wetlands. Hydrobiologia 797: 127-143. http://dx.doi.org/ 10.1007/s10750-017-3165-8

RIMET, F., BOUCHEZ, A. & MONTUELLE, B. 2015. Benthic diatoms and phytoplankton to assess nutrient in large lakes: complementarity of their use in Lake Geneva (France-Switzerland). Ecol. Indic. 53: 231-239. http://dx.doi.org/10.1016/j.ecolind.2015.02.008

RIVERA, P. & CRUCES, F. 2013. *Spicaticribra kingstonii* Johansen, Kociolek & Lowe (Thalassiosirales, Bacillariophyta): primera cita del género y de la especie para las aguas continentales de Chile. Gayana Bot. 70(2): 235-240. http://dx.doi.org/10.4067/S0717-66432013000200004

ROUND, F.E., CRAWFORD, R.M. & MANN, D.G. 1990. The diatoms. Biology and morphology of the genera. New York, Cambridge University Press, p.129.

RUCK, E.C., NAKOV, T., ALVERSON, A.J. & THERIOT, E.C. 2016. Nomenclatural transfers associated with the phylogenetic reclassification of the Surirellales and Rhopalodiales. Notulae Algarum 10:1-4.

SALA, S.E., PLATA-DÍAZ, Y., PEDRAZA, E. & PIMIENTA, A. 2015. Taxonomía y distribucíon de diatomeas epilíticas registradas por primera vez en Colombia. Caldasia 37(1): 125-141. http://dx.doi.org/10.15446/caldasia.v37n1.50814

SANTOS, E.M., TREMARIN, P.I. & LUDWIG, T.A.V. 2011. Diatomáceas perifíticas em *Potamogeton polygonus* Cham. & Schltdl.: citações pioneiras para o estado do Paraná. Biota Neotropica 11(3): http://www.biotaneotropica.org.br/v11n3/pt/abstract?inventory+bn01611032011

SANTOS, T.R. & FERRAGUT, C. 2018. Changes in the taxonomic structure of periphytic algae on free-loating crophyte (*Utricularia foliosa* L.) in relation to macrophyte richness over season. Acta Botanica Brasilica 32(4): 595-601. http://dx.doi.org/10.1590/0102-33062018abb0031

SAYER, C.D, ROBERTS, N., SADLER, J., DAVID, C. & WADE, P.M. 1999. Biodiversity changes in a shallow lake ecosystem: a multi-proxy palaeolimnological analysis. Journal of Biogeography 26(1): 97-114. http://dx.doi.org/10.1111/j.1365-2699.1999.00298.x

SIENKIEWICZ, E. 2005. Comparison of subfossil diatoms (Bacillariophyta) from two oligotrophic lakes: Maly Staw (Karkonosze MTS., Poland) and Somaslampi (Lapland, Finland). Polish Geological Institute Special Papers. 16: 109-115.

SILVA, A.M., TREMARIN, P.I., LUDWIG, T.A.V. & VERCELLINO, I.S. 2010. Diatomáceas perifíticas em um sistema eutrófico brasileiro (Reservatório do Iraí, estado do Paraná). Acta Botanica Brasilica. 24(4): 997-1016. http://dx.doi.org/10.1590/S0102-33062010000400015

SIMONSEN, R. 1974. The diatom plankton of the Indian Ocean Expedition of R/V "Meteor", 1964-65 Meteor. Forschungsergebnisse Reihe D-Biologie. 19: 1-66.

SIVER, P.A. & KLING, H. 1997. Morphological observations of *Aulacoseira* using scanning electron microscopy. Can. J. Bot. 75: 1807-1835. http://dx.doi.org/10.1139/b97-894

SOUZA, M.L. & FERRAGUT, C. 2012. Influence of substratum surface roughness on periphytic algal community structure in a shallow tropical reservoir. Acta Limnol. Bras. 24(4): 397-407. http://dx.doi.org/10.1590/S2179-975X2013005000004

SPAULDING, S. & EDLUND, M. 2009. *Pinnularia*. In Diatoms of the United States. Retrieved November 20, 2017, from http://westerndiatoms.colorado.edu/taxa/genus/Pinnularia

STENGER-KOVÁCS, C., BUCZKÓ, K., HAJNAL, E. & PADISÁK, J. 2007. Epiphytic, littoral diatoms as bioindicators of shallow lake trophic status: Trophic diatom index for lakes (TDIL) developed in Hungary. Hydrobiol. 589: 141-154. http://dx.doi.org/10.1007/s10750-007-0729-z

STRAUBE, A., TREMARIN, P.I. & LUDWIG, T.A.V. 2017. Species of *Luticola* D. G. Mann (Bacillariophyceae) in the Atlantic Forest rivers from southern Brazil. Diatom Research 32(4): 417-437. http://dx.doi.org/10.1080/0269249X.2017.1389771

SUDERHSA, 2007. http://www.aguasparana.pr.gov.br/modules/conteudo/conteudo.php?conteudo=80 (Acessed 06 September 2017).

SZULC, K., BESENYEI, L., SZULC, B. & RAKOWSKA, B. 2014. Diatomological aspects of the Fenn's and Whixall mosses complex (Shropshire, UK). Oceanological and Hydrobiological Studies 43(3): 274-282. http://dx.doi.org/10.2478/s13545-014-0142-y

TORRISI, M., SCURI, S., DELL´UOMO, A. & COCCHIONI, M. 2010. Comparative monitoring by means of diatoms, macroinvertebrates and chemical parameters of an Apennine watercourse of central Italy: The river Tenna. Ecol. Indic., 10: 910-913. http://dx.doi.org/10.1016/j.ecolind.2010.01.010

TREMARIN, P.I., MOREIRA-FILHO, H. & LUDWIG, T.A.V. 2010. Pinnulariaceae (Bacillaiophyceae) do rio Guaraguaçu, bacia litorânea paranaense, Brasil. Acta Botanica Brasilica 24(2): 335-353.

TREMARIN, P.I., FREIRE, E.G., BERTOLLI, L.M. & LUDWIG, T.A.V. 2009. Catálogo das diatomáceas (Ochrophyta-Diatomeae) continentais do estado do Paraná. Iheringia – Sér. Bot. 64(2): 79-107.

TUJI, A. & WILLIAMS, D.M. 2007. Type examination of Japanese diatoms described by Friedrich Meister (1913) from lakes Suwa. Bull. Natn. Sci. Mus. 33(2): 69-79.

TUNDISI, J.G. 2003. Água no século XXI: enfrentando a escassez. RiMa: São Carlos – SP, p. 1-248.

VAN DAM, H., MERTENS, A. & SINKELDAM, J. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. Neth. J. Aquatic Ecol. 28(1): 117-133. http://dx.doi.org/10.1007/BF02334251

VAN DE VIJVER, B., GREMMEN, N.J. & BEYENS, L. 2005. The genus *Stauroneis* (Bacillariophyceae) in the Antartic region. J. Biogeogr. 32: 1791-1798. http://dx.doi.org/10.1111/j.1365-2699.2005.01325.x

VAN DE VIJVER, B. & BEYES, L. 1997. The epiphytic diatom flora of mosses from Strømness Bay area, South Georgia. Polar Biol 17: 492-501. http://dx.doi.org/10.1007/s003000050148

VANHOUTTE, K., VERLEYEN, E., VYVERMAN, W., CHEPURNOV, V, & SABBE, K. 2004. The freshwater diatom genus *Kobaysiella* (Bacillariophyta) in Tasmania, Australia. Australian Systematic Botany 17:483-496. http://dx.doi.org/10.1071/SB03035

VESELÁ, J. 2009. Spatial heterogeneity and ecology of algal communities in an ephemeral sandstone stream in the Bohemian Switzerland National Park, Czech Republic. Nova Hedwigia 88(4-3): 531-547. http://dx.doi.org/10.1127/0029-5035/2009/0088-0531

WANG, R., XU, M., YANG, H., YANG, X., ZHANG, K., ZHANG, E. & SHEN, J. 2019. Ordered diatom species loss along a total phosphorous gradient in eutrophic lakes of the lower Yangtze River basin, China. Science of the Total Environment 650: 1688-1695. https://doi.org/10.1016/j.scitotenv.2018.09.328

WENGRAT, S., MARQUARDT, G.C., BICUDO, D.C., BICUDO, C.E.M., WETZEL, C.E. & ECTOR, L. 2015. Type analisys of *Cymbella schubartii* and two new *Encyonopsis* species (Bacillariophyceae) from southeastern Brazil. Phytotaxa 221(3): 247-264. http://dx.doi.org/10.11646/phytotaxa.221.3.3

WENGRAT, S., MORALES, E.A., WETZEL, C.E., ALMEIDA, P.D., ECTOR, L. & BICUDO, D.C. 2016. Taxonomy and ecology of *Fragilaria billingsii* sp. nov. and analysis of type material of *Synedra rumpens* var. *fusa* (Fragilariaceae, Bacillariophyta) from Brazil. Phytotaxa. 270(3): 191-202. http://dx.doi.org/10.11646/phytotaxa.270.3.3

WENGRAT, S., PADIAL, A.A., JEPPESEN, E., DAVIDSON, T.A., FONTANA, L., COSTA-BÖDDEKER & BICUDO, D.C. 2017. Paleolimnological records reveal a biotic homogenization driven by eutrophication in tropical reservoirs. J. Paleolimnol https://doi.org/10.1007/s10933-017-9997-4

WETZEL, C.E., VAN DE VIJVER, B., HOFFMANN, L. & ECTOR, L. 2013. *Planothidium incuriatum*sp. nov. a widely distributed diatom species (Bacillariophyta) and type analysis of *Planothidium biporomum*. Phytotaxa. 138(1): 43-57. http://dx.doi.org/10.11646/phytotaxa.138.1.6

WOLFE, A.P. & KLING, H.J. 2015. A consideration of some North American soft-water *Brachysira* taxa and description of *B. arctoborealis* sp. nov. Studies on Diatoms. 243-264.

YANG, J.R. & DICKMAN, M. 1993. Diatoms as indicators of lake trophic status in central Ontario, Canada. Diat. Res. 8(1): 179-193. http://dx.doi.org/10.1080/0269249X.1993.9705249

ZORZAL-ALMEIDA, S., BICUDO, D.C., LAMPARELLI, M.C., FERRAGUT, C. & BICUDO, C.E.M. 2017. Avaliação do índice de estado trófico e sua aplicação na represa Guarapiranga em longa série temporal. In: 100 Anos da represa Guarapiranga: lições e desafios (Bicudo, C.E.M. & Bicudo, D.C., eds). Editora CRV, Curitiba-PR, Brasil. p. 401-428.

Received: 25/04/2018
Revised: 16/12/2018
Accepted: 21/02/2019