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article

Agroforestry system as a buffer zone in a Brazilian Atlantic Forest conservation unit: an artificial nest predation perspective

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Abstract: Here we inferred about the effectiveness of a buffer zone composed by an Agroforestry System (AS) for providing reproductive conditions for Atlantic Forest birds by comparing artificial nest predation rates between a buffer zone, a primary forest area, and a set of small and isolated Atlantic Forest fragments. We have used 237 nests throughout the three areas, of which 100 (42.2%) were depredated. Predation frequencies were 28.6% in the continuous forest, 100% in the AS, and 51.9% in the fragments, differing significantly. We could detect nest predators in 48 of the 100 depredated nests (48%) using camera traps. Differences between areas may be attributed to changes in nest predator species. We support previous evidences that the conservation of bird communities may not rely on buffer zones as an extension of their breeding habitats.

Keywords: *Protected areas, management, mesopredators, birds, camera traps*

Sistema Agroflorestal como zona de amortecimento em uma Unidade de Conservação da Mata Atlântica brasileira: uma perspectiva de predação de ninho artificial

Resumo: O presente estudo teve como objetivo avaliar a efetividade de uma zona tampão composta por um sistema agroflorestal (SA) para a reprodução de aves da Mata Atlântica. Para isto, as porcentagens de predação em ninhos artificiais foram comparadas entre as zonas tampão, uma área de floresta primária e em um conjunto de fragmentos de Mata Atlântica pequenos e isolados. Um total de 237 ninhos foi utilizado nas três áreas e 100 deles (42,2%) foram predados. As porcentagens de predação foram 28,6% na área de floresta primária, 100% no SA e 51,9% nos fragmentos, havendo diferença significativa. Os predadores puderam ser identificados em 48 das 100 predações (48%) com o uso de câmeras *trap*, o que gerou evidências de que as diferenças possam ser atribuídas a mudanças na composição de espécies de predadores entre as áreas. Esta análise dá suporte a estudos prévios de que as zonas tampão não devam ser consideradas como extensão dos hábitats naturais para a conservação de comunidades de aves.

Palavras-chave*: áreas protegidas, gestão, mesopredadores, aves, câmeras trap*

Introduction

Buffer zones correspond to areas surrounding conservation units where human activities are subject to rules and restrictions, in order to minimize negative impacts on protected areas. They are generally complex and some of the practices often forbidden in these areas include burning vegetation, logging, and the use of conventional agriculture (Sayer 1991, Bennet & Mulongoy 2006). Over the last few decades a number of works have provided mixed evidences for the effectiveness of buffer zones to the conservation of faunal components, and this is still an important open debate for the management of protected areas (Goodale et al. 2014, Mammides et al. 2015). The Agroforestry Systems (ASs) are agricultural systems that incorporate food production together with environmental conservation (Schroth et al. 2004). They are believed to hold more biodiversity than mono-crops, and for this reason are considered good buffer zones (Goodale et al. 2014), but they have never been evaluated under the faunal perspective in Brazil.

Nest predation rates are often believed to increase with habitat disturbance (Oniki 1979, Robinson et al. 2000), and this can be a valuable indicative of habitat quality for birds (Alvarez & Galetti 2007). The main hypothesis that attempt to explain the inflated nest predation rates in impacted areas imply that in this condition, with the extinction of top predators, middle-sized animals, that are the main nest predators, increase their densities putting a lot more pressure over the nests (mesopredator-release hypothesis) (Terborgh 1974, Oniki 1979). Artificial nests are known to result in different predation rates compared with natural nests because they lack the anti-predatory strategies adopted by the different species of birds, and eggs are not similar (Buler & Hamilton 2000). On the other hand they have standardized format, they permit prompt evaluations using large samples, and nest predation rates in artificial nests are often indexes of predation rates of natural nests (Buler & Hamilton 2000). Then, it can be considered as a useful tool for comparative purposes of habitat conditions (Oja et al. 2015).

Here we aim to infer about the effectiveness of a buffer zone composed by an AS for providing reproductive conditions for birds, by addressing predation rates in artificial nests. Specifically, we have compared nest predation rates between an AS buffer zone and a well-preserved primary forest area, that has served as a positive control, and a set of small and isolated Atlantic Forest fragments that have served as a negative control. We predict that the AS should present intermediate nest predation rates between the continuous forest and the isolated fragments. We also tested the hypothesis that variations in nest predation rates can be caused by changes in the composition of nest predators communities in these different habitats. To achieve this purpose, we have used a set of camera traps to identify nest predators.

Materials and Methods

1. Study areas

We assessed artificial nest predation frequency in undisturbed areas from "Carlos Botelho" State Park (CBSP), São Paulo State, Brazil, in an AS adjacent to the Park, and in a set of isolated fragments (Figure 1). CBSP (24º06'55"S, 47º47'18"W) holds 37.644 ha of Atlantic Forest and is among the few areas that still preserve original faunal elements such as jaguars, tapirs, and large primates. The elevation ranges from 20 to 1.000 m above sea level, and annual mean temperature varies between 18 and 20ºC, with annual precipitation from 1.500 to 2.200 mm (Ferraz & Varjabedian 1999). Our work was conducted at an altitude of 714 to 837 m, where the vegetation is classified as submontane rain forest.

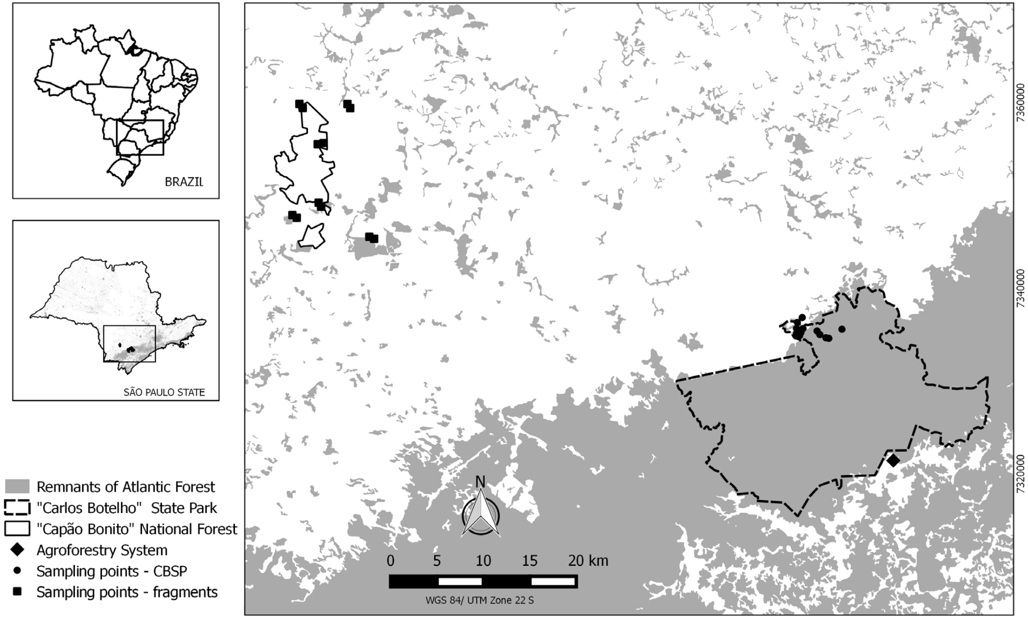
The AS is a private property located in the city of "Sete Barras" (24°11'19"S, 47°53'19"W; altitude 35 m), adjacent to CBSP, with a total area of approximately 200 ha. Formerly, the whole area was used for traditional agriculture, but these practices have been abandoned about 20 years ago, when an AS of mixed shrub-crops (including coffee, banana, cocoa, guava, and pupunha palm heart) has initiated. In this system, patches of Coastal Atlantic Forest were permitted to regenerate to provide organic matter, soil protection, pollinators, and parasitism control for the intermixed crops. We have placed the nests in a area where mixed cultures of banana, cocoa, guava, and pupunha were managed in the middle of approximately 5 to 10 ha secondary Atlantic Forest fragments.

The Atlantic Forests fragments (n = 12) were located in private areas nearby "Capão Bonito" National Forest (CPNF) (23°54'29" S, 48°30'38" W; altitude 670 m), which is about 60 km far from CBSP. These fragments varied from 2 to 50 ha, and they were 360 m to 15 km distant from one another. They were imbedded in a complex mosaic of mono-crops that included corn, soy bean, wheat, sylvicultures of *Araucaria angustifolia*, and *Pinus spp*., and pasturelands.

2. Artificial nests experiment

To infer about the relative habitat quality for birds reproduction, we have set a number of artificial nests in each study site, during 2014 and 2015, from August to February. We used cup nests commercially available for domestic canaries, each containing two eggs of quail (*Coturnix coturnix*). Latex gloves were used to manipulate the nests and eggs to avoid predators attraction by human odor. Nests were placed at least 30 m apart in transects. We have alternated nests placed on the ground with nests attached to tree branches, 1-2 m above ground, and transects were at least 100 m far from each other. Each nest was exposed for 15 days which represents the incubation period of many birds occurring in the study areas (Sick 1997), and nests in which only one egg has been damaged or removed were also considered as depredated.

At CBSP we have set 14 transects of 10 nests each, totaling 140 nests, and at AS we used 2 transects of 10 nests each, totaling 20 nests, being this number proportional to the size of the area. In the fragments we have set 12 transects (one in each fragment), where the number of nests in each fragment and fragment sizes were 3 (2 ha), 4 (2 ha), 5 (20 ha), 5 (20 ha), 5 (30 ha), 5 (30 ha), 5 (10 ha), 5 (10 ha), 10 (50 ha), 10 (20 ha), and 10 (30 ha), 10 (30 ha), totaling 77 nests. In the AS, in which habitats are heterogeneous,



Modified from: IBGE (2010); SOS MATA ATLÂNTICA (2012); OBSERVATÓRIO DE UCS - WWF (2015)
Org: Galvão CA

Figure 1. Study areas: Carlos Botelho State Park (CBSP); fragments in the region of Capão Bonito National Forest (CBNF), and the private property containing the Agroforestry System (AS). Markings indicate the study sites.

we established transects randomly crossing the area in its longer extension, in such a way that some nests fell in forest fragments and others in crop plants.

3. Predators identification using camera traps

In each nest we have used a camera trap Bushnell Trophy Cam HD in an attempt to identify nest predators. These cameras were placed 1 to 3 m from the nests, depending on the availability of nearby branches to attach them. Cameras were programmed for recording 30s videos, with 3s intervals. To avoid over-exposure of the films, infra-red leds were programmed to low exposure, and we have used the maximum level of sensitivity of the movement sensor. Videos were recorded in 2GB memory cards and were analyzed in laboratory.

4. Data analyses

The proportions of depredated nests between the areas, and the proportions of depredated nests on trees and on the ground were compared through contingence tables using the *G*-test, and the data from the fragments surrounding Capão Bonito National Forest were pooled together. To compare the proportions of predator species across the areas, we also performed a *G*-test by constructing a contingency table in which rows represented the species detected by the cameras depredating the nests, the columns represented the study sites, and the cells contained the number of nests depredated by each species in each area. All of the statistical analyses were performed using the Program BioEstat 5.0 (Ayres et al.2007), with 5% significance level.

Results

We have used a total of 237 nests throughout the three areas, of which 100 (42.2%) were depredated. Predation frequencies were 28.6% (40 out of 140 nests) in the CBSP, 100% in the AS (20 out of 20 nests), and 51.9% in the fragments (40 out of 77 nests). The proportions of nest predations have differed significantly between the study areas (G = 48.6, P = 0.001).

Overall, 59 out of 119 (49.6%) nests placed on the ground were depredated, against 41 out of 118 (34.7%) nests placed on tree branches, and the proportion of nest predations was significantly higher on the ground (G = 5.36, P = 0.02). Considering each specific area, predation was significantly higher on the ground in CBSP, and no significant differences were detected in AS and in the fragments (Table 1).

Pooling the three areas together, our camera effort has corresponded to 3.555 cameras/day and 85.320 hrs/camera, distributed in 50.400 hr/camera in CBSP, 7.200 in the AS, and 27.720 in the fragments. We could detect nest predators in 48 out of the 100 depredated nests (48%), being 18 in the CBSP (45%), six in the AS (30%), and 24 in the fragments (58.5%). Overall, we detected 19 predator species, being 10 bird species, eight mammals, and one reptile (Figure 2). Birds were responsible for 50% of the observed predations, followed by mammals (48%), and reptiles (2%). Most of the recorded predations caused by birds were on nests placed on tree branches (15 out of 24; 62.5%), whereas most of the detected predations caused by mammals were on nests placed on the

Table 1 – Numbers and percentages of predations on artificial nests placed on the ground and on nests placed on tree branches. Proportions of predations in each substrate were compared using *G*-test for each of three study areas: Carlos Botelho State Park (CBSP), Agroforestry System (AS), and a set of fragments located in an anthropic matrix represented by Capão Bonito National Forest (Fragments). *P* represent G-tests significance levels.

	Ground	Tree branches	Significance
CBSP	26 out of 70 (37.1%)	14 out of 70 (20%)	G = 5.09, P = 0.02
AS	9 out of 9 (100%)	11 out of 11 (100%)	G = 0, P = 1.0
Fragments	24 out of 39 (61.5%)	16 out of 38 (41.0%)	G = 2.93, P = 0.08

Figure 2. Images obtained from camera trap videos depicting predations by: (A) Wild Boar (*Sus scrofa*) in the fragments, (B) Azure Jay (*Cyanocorax caeruleus*) in the AS, (C) Collared Forest-Falcon (*Micrastur semitorquatus*) in CBSP, and (D) Red-ruffed Fruitcrow (*Pyroderus scutatus*) in the fragments.

ground (21 out of 23; 91%), so was the only predation performed by a reptile. Species of predators observed in each area and their impact on predation are presented in Table 2. The *G*-test revealed a significant difference in the proportion of the identified predator species between the study areas ($G = 72.9$, $P = 0.0001$).

Table 2 - Number of artificial nest predations caused by each species identified using camera traps in each study area: Carlos Botelho State Park (CBSP), Agroforestry System (AS), and a set of fragments located in an anthropic matrix represented by Capão Bonito National Forest (Fragments).

Species	Sampling areas			Total
	CBSP	AS	Fragments	
Birds				
Order Tinamiformes				
Family Tinamidae				
Tinamus solitarius	2	0	0	2
Order Galliformes				
Family Cracidae				
Penelope obscura	1	0	2	3
Family Odontophoridae				
Odontophorus capueira	1	0	0	1
Order Falconiformes				
Family Falconidae				
Micrastur semitorquatus	1	0	0	1
Order Passeriformes				
Family Dendrocolaptidae				
Dendrocolaptes platyrostris	3	0	0	3
Family Cotingidae				
Pyroderus scutatus	0	0	1	1
Family Vireonidae				
Cyclarhis gujanensis	0	0	1	1
Family Corvidae				
Cyanocorax chrysops	0	0	7	7
Cyanocorax caeruleus	0	4	0	4
Family Thraupidae				
Lanio melanops	1	0	0	1
Mammals				
Order Didelphimorphia				
Family Didelphidae				
Philander frenatus	4	0	0	4
Didelphis aurita	0	2	4	6
Order Rodentia				
Family Sciuridae				
Guerlinguetus brasiliensis ingrami	0	0	1	1
Family Cricetidae				
unidentified*	1	0	0	1
Order Carnivora				
Family Procyonidae				
Nasua nasua	1	0	3	4
Family Mustelidae				
Eira barbara	2	0	1	3
Order Artiodactyla				
Family Suidae				
Sus scrofa	0	0	4	4
Reptiles				
Order Squamata				
Family Teiidae				
Tupinambis merianae	1	0	0	1
TOTAL	18	6	24	**48**

(*rodent that could not be identified at the species level).

Discussion

Our main finding was that artificial nest predation rate in a buffer zone composed by an AS was extremely high. Our data corroborated the premise that nest predation rate should be higher in a set of fragments embedded in anthropogenic habitats than in continuous forests, but not that the AS should present intermediate nest predation levels. In CBSP seven of the 18 predations recorded on camera were performed by species that rely on large tracts of forest to survive, i.e. *T. solitarius*, *O. capueira*, *M. semitorquatus*, and *D. platyrostris* (Willis 1979, Ribon et al. 2003). Of 24 predations recorded in the fragments, seven were caused by *C. chrysops*, a bird typical of forest borders and secondary habitats (Sick 1997), and four were caused by *Sus scrofa*, an alien species typically associated with crops (Pedrosa et al. 2015). Then, these species may not be involved in mesopredator-release adjustments. On the other hand, all of the other detected predators can occur in both continuous forests and in anthropic habitats (Willis 1979, Sick 1997, Ribon et al. 2003, Reis et al. 2006), and could potentially have their densities inflated in the disturbed areas due to the lack of top predators. Testing the mesopredator-release effect is beyond the scope of this work, but here we corroborate the hypothesis that changes in predator types may also govern nest predation variations. Although other nest predator species may occur in our study sites (see for instance Menezes & Marini 2017), especially those that can be eventual nest predators, our hypothesis was based on the assumption that our cameras have registered the species that are more willing to depredate nest's contents in each area. The fact that all of the predator species detected by the cameras occur from sea level to higher altitudes (Ridgely & Tudor 1994, Sick 1997, Reis et al. 2006) also suggests that altitudinal variation between our study areas alone may not have played a significant role in our predator species survey.

The higher predation level on ground nests in CBSP corroborated the conclusions of a wide review for tropical forests (Söderström 1999). As most of the predators we detected (birds and mammals) could potentially forage both on the ground and on tree branches, lower nest concealment on the ground than in tree branches may explain this difference (see also Söderström 1999). In the AS and in the fragments, inflated nest predation rates in general may have contributed to uncover this effect.

The 100% predation in the AS was unexpected and one of the reasons may be the presence of *C. caeruleus* in the area. Jays are omnivorous birds (Sick 1997) that commonly visit orchards to consume fruits (personal observation), and the concentration of this type of resource in the AS is what may have attracted them. Notably, corvids are also typical predators of nests of smaller birds (Martin & Joron 2003), and we provide evidences that this context may be unfavorable to birds willing to reproduce in the AS we studied. The vegetation cover around the nests is another important parameter determining the risks of predation by visually oriented predators such as birds, and there is at least one good evidence that nest predation by corvids can increase with decreasing vegetation cover (Martin & Joron 2003). In both CBSP and in the fragments nests were placed in forested habitats, while in the AS part of the nests has been placed in crop plants, which may have favored nest uncover. We believe, however, that placing nests randomly across Atlantic Forest patches and crop plants in the AS did not represent an unrealistic situation, because various tropical passerine species build their nests in crop trees (Skutch 1954).

In conclusion, here we depict one case in which an AS could potentially work as reproductive trap for birds. ASs are certainly one of the types of buffer zones that are structurally more similar to the original habitats (Schroth et al. 2004, Mcneely & Schroth 2006), and we provide new evidences that benefits envisioned for ASs as buffer zones must focus on avoiding soil erosion, reducing physical edge effects, or on preserving the diversity of fauna from the soil (Schroth et al. 2004, Junqueira et al. 2013), but the conservation of bird communities, especially endangered species, may not rely on buffer zones as an extension of their breeding habitats.

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Author contributions

Camila André Galvão: 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.

Mercival Roberto Francisco: 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 intelectual content.

Marcelo Nivert Schlindwein: 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.

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Changes in vegetation cover of the Pantanal wetland detected by Vegetation Index: a strategy for conservation

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Abstract: The Brazilian Pantanal wetland undergoes landscape alterations that can cause impacts on hydrological processes, changing the flood pulse. The objective of this work is to analyse the vegetation cover of the Pantanal in the period of 2000, 2008 and 2015, and to make a projection for 2030. Therefore, NDVI from the sensor MODIS was analysed and the transition matrix was calculated by the DINAMICA EGO. The methods adopted were open sources. The results were worrisome, indicating alterations of the vegetation cover of the Pantanal, with an increase of short vegetation (grasslands or pastures) in the evaluated period. The projection pointed out that in 2030 the Brazilian Pantanal wetland area will be covered by 78% of short vegetation and only 14% of dense (arboreal-shrubby) vegetation. The approach can be a useful tool for conservation of the Brazilian Pantanal wetland.

Keywords: *EGO DYNAMICS, wetland, geoprocessing, NDVI, Transition rate.*

Mudanças na cobertura da vegetação do Pantanal dectadas por Índice de Vegetação: uma estratégia de conservação

Resumo: O Pantanal brasileiro sofre alterações em sua paisagem que podem provocar impactos sobre os processos hidrológicos, afetando os pulsos de inundação. O objetivo do trabalho é analisar a cobertura vegetal do Pantanal no período de 2000, 2008 e 2015 e realizar a projeção quantitativa para 2030. Portanto, foram analisados dados NDVI do sensor MODIS e a análise da matriz de transição foi calculada pelo DINAMICA EGO. Os métodos utilizados foram todos em softwares livres. Os resultados foram preocupantes, indicando alteração da cobertura vegetal do Pantanal, com o aumento da vegetação rasteira (campos ou pastagens) no período avaliado. A projeção apontou que em 2030 a área do Pantanal será coberta por 78% de vegetação rasteira e apenas 14% de vegetação densa (arbóreo-arbustiva). A abordagem apresentada pode ser uma ferramenta útil para a conservação do Pantanal.

Palavra-chave: *Dinamica EGO, área úmida, geoprocessamento, NDVI, taxa de transição.*

Introduction

The structure of ecosystems has changed, mostly in the second half of the 20th century, and mostly all ecosystems were modified by human actions (Hassan et al. 2005). Therefore, many studies and tools that contributes to monitor of ecosystems. Thus, creation of production alternatives towards the valoration of the ecosystems is a strategy for their preservation (Lovins et al. 1999). Wetlands are important natural areas, since they provide services as retention of pluvial waters, improvement of water quality, maintenance of biodiversity, carbon storage and climate regulation (Hassan et al. 2005).

The Brazilian Pantanal wetland has altitudes between 80 and 190 meters above sea level, inside the hydrographic basin of the Upper Paraguay River. Due to its phytogeographic location, the vegetation has elements of four different domains: the Amazon forest (northwest), the Cerrado tropical savanna (east), the Chaco stepic savanna (southwest) and the Atlantic Forest (Pott & Pott, 2004). It is a complex of floodplains inserted in a large Quaternary sedimentary lowland filled by deposition of alluvial fans of various affluents (Hamilton 2012, Assine 2015). For its particular characteristics, biodiversity and unique landscapes, the Pantanal brings special environmental interest and has outstanding position in the international scenario, besides being one of the largest freshwater wetlands on Earth.

Wetlands such as the Pantanal are being converted into anthropized areas to meet the growing demand for food production (Verhoeven et al. 2010). Such land use changes have a large impact upon the hydrologic processes, affecting the energy and water balances, and the flood pulse in the Brazilian Pantanal wetland (Harris et al. 2006, Goulden et al. 2007; Silva et al. 2011). The traditional fishing and cattle ranching are being replaced by intensive models, with deforestation and alteration of natural areas (MMA 2002).

The analysis and the monitoring of alterations in vegetation cover are tools that can help to understand the spatial dynamics of the Brazilian Pantanal wetland and offer relevant information to public and private decision makers, who could adopt efficient measures through public policies, programs, projects and others, aiming preservation of the region's landscape and biodiversity. However, monitoring those areas is a difficult task, because of the large size and its difficult access (Pott & Pott 2004). Thus, the use of remote sensing is essential for monitoring and, consequently, for preservation of the Brazilian Pantanal wetland. Due to the spectral response characteristics of the vegetation it is possible to utilize geoprocessing techniques for its identification and evaluation. An example of such techniques is the vegetation index. The Normalized Difference Vegetation Index (NDVI) is widely utilized in the assessment of several biophysical parameters, such as vegetation coverage, biomass, fraction of the photosynthetically active radiation and phenological variations (Huete et al. 2002; Prabakaran et al. 2013). Furthermore, it is a classic example of vegetation mapping utilizing remote sensing, as it deals with the information from the reflectance in the spectrum range of red and near-infrared wavelengths (Xie et al. 2008).

In this context, our work aims to make a multitemporal analysis of the vegetation cover of the Brazilian Pantanal wetland in the last 15 years, and a quantitative projection for 2030.We emphasize, yet, that to carry out our work, we applied only freely available geotechnologies, to allow the replication of methods proposed in other periods and regions.

Material and Methods

1. Study Area

The study area is the Brazilian Pantanal wetlend, with an area of 138,183 Km^2, occupying around 40% of the hydrographic basin of the Upper Paraguay River, located in the states of Mato Grosso and Mato Grosso do Sul, in Central-West region of Brazil (Silva & Abdon 1998) (figure 1).

2. Normalized Difference Vegetation Index

The Normalized Difference Vegetation Index (NDVI), proposed by Rouse et al. (1974), is calculated by the difference of the vegetation reflectances in the near-infrared wavelengths (NIR) and the red wavelengths (equation 1). This difference is then normalized. For being the result of the combination of two bands, the NDVI partially covers-up the effects of atmospheric components and of radiometric and geometric disturbances. The NDVI value varies from -1 to +1.

$$\mathrm{NDVI} = \left(\mathrm{NIR} - \mathrm{R}\right) / \left(\mathrm{NIR} + \mathrm{R}\right) \qquad (1)$$

where, NIR: Reflectance of the vegetation in the near-infrared wavelength, and R: Reflectance of the vegetation in the red wavelength.

3. Acquisition of the data base

To perform our work, we obtained images from the sensor MODIS of the satellite TERRA, product MOD13Q1, quadrants h12v10 and h12v11, available in a 16-day composite of cloud-free images, spatial resolution of 250 m, on the website of the USGS – United States Geological Survey (https://mrtweb.cr.usgs.gov). Among the available scenes, we chose the spectral bands of red (R), near-infrared wavelengths infrared (NIR), and mean infrared (MIR), for elaboration of the false-color compositions, plus the available NDVI (product MOD13Q1). It is interesting to point out that the MODIS products include the geometric and radiometric correction already, which facilitate its utilization. It is also worth mentioning that such images are capable to cover the whole studied region in a single day, preventing seasonal and phenological variations (Paranhos-Filho et al. 2014).

The selected dates were August 12, 2000, August 12, 2008 and August 13, 2015 (USGS, 2000; 2008; 2015), corresponding to the Brazilian Pantanal wetland dry season (from June to September). We performed the conversion of the projection of the acquired scenes to UTM cartographic projection, Datum WGS1984, 21S zone.

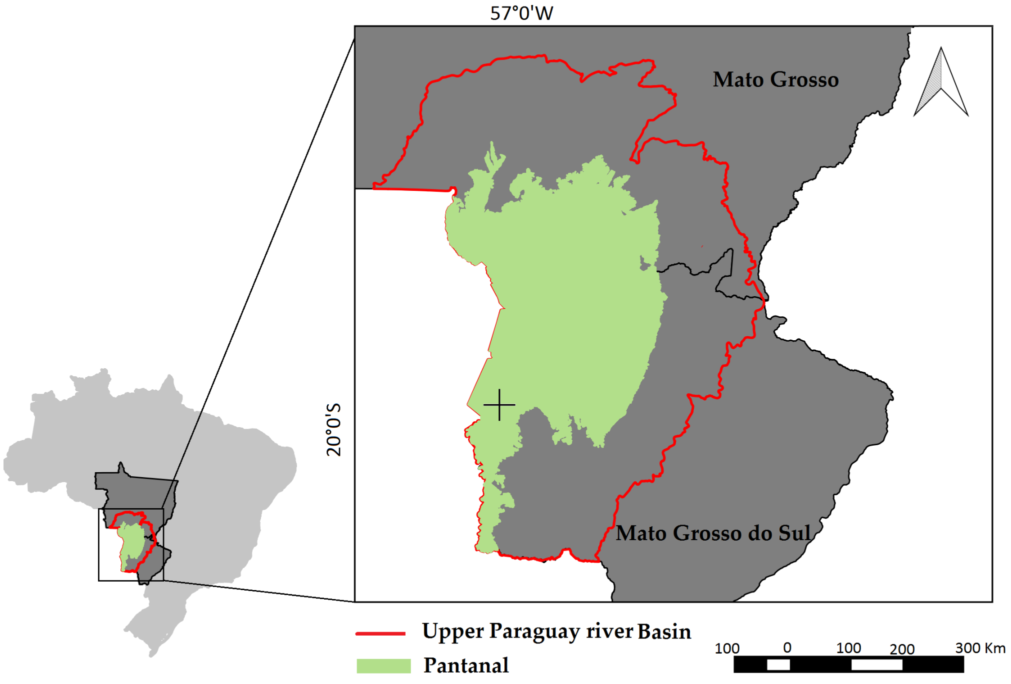

Figure 1. Location of the Brazilian Pantanal plain (in green) inserted in the Upper Paraguay River basin (red limit) (Silva e Abdon, 1998).

To delimit the vegetation classes, we utilized the vectors of the "Monitoring of Alterations of the Vegetation Cover and Land Use in the Hydrographic Basin of the Upper Paraguay – BAP (Brazilian portion)", which covers the Pantanal, carried out by the Instituto SOS Pantanal and by WWF-Brazil (2015). We looked for support from polygons which would represent the class of interest and did not undergo alteration along the analyzed period in the above-mentioned study.

4. Analysis of vegetation cover

We separated the vegetation in three different classes to detect the landscape dynamics: 1) dense vegetation, representing the arboreal/shrubby vegetation, such as woody and forested savanna, formations with fluvial influence, like areas with spectral patterns of tall vegetation without signs of human influence; 2) short vegetation, that indicates the formations of grassy savanna, grasslands, areas with anthropic activities signs and native and tame pastures; 3) areas without vegetation like the large water bodies, wet areas, areas with spectral response of bare soil, characteristic of agriculture. Those classes were proposed before by Peres et al. (2016). During the dry season, in August, sandy areas can become overgrazed, with much exposed soil, while near the Paraguay river the lower areas can still be under its delayed flooding (Pott & Pott, 2004; Hamilton et al. 2002).

The collection of samples of each class was made by interpreting MODIS images from 2000, 2008 and 2015, in the RGB false-color composition, where R – MIR (mean-infrared), G – NIR (near-infrared wavelengths) and B - R (red), considering elements such as texture, color and pattern (Abdon et al., 2007), as well as the polygons derived from the Monitoring of Alterations of the Vegetation Cover and Land Use in the Hydrographic Basin of the Upper Paraguay – BAP (Brazilian portion) (Instituto SOS Pantanal and WWF-Brazil, 2015). After the selection of 7 to 10 samples of each interest class, the samples were overlayed by the NDVI and cropped to obtain its minimum and maximum values, as well as its range index. The NDVI values were reclassified according to the intervals obtained for each encompassed class – the index slicing (Peres et al. 2016). Groupment of the NDVI values in certain intervals were made to represent the evaluated categories for each studied year to obtain distinct and independent values regarding its time basis (Benedetti et al. 2013). All the cited geoprocessing procedures were performed in the open and free GIS, QGIS 2.8 (QGIS Development Team 2015). This work utilized Landsat TM and Landsat 8 images with spatial resolution of 30 m, plus Resource-Sat-1 LISS III images, with spatial resolution of 23.5 m in several temporal compositions, which provides refined samples of vegetation classes at geographic scale.

We made the calculation of transition matrices between classes with the simulator Dynamic Environment for Geoprocessing Objects – EGO – (2015), a free software, which can model various types of data and evaluate different probabilities of transition with the use of the temporal variable in any number of steps regarding time with a pre-defined transition rate from the transition matrix (Soares-Filho et al. 2006, Soares-Filho et al. 2009, Wang et al. 2016). The calculations were done for the periods 2000-2008, 2008-2015 and 2000-2015, the latter applied in the projection of the changes between classes for 2030.

The transition rate is one of the keys for modelling, because it determines each soil cover's area undergoing alteration (Elz et al. 2015). The modelling work is a necessary step to verify any possible errors. For that, the values for the classes are confronted with the ones projected by the transition matrix, estimating errors in the modelled transition rates.

Results

The result of the multitemporal mapping of the vegetation cover derived from NDVI slicing, using maximum and minimum digital value for each class, is shown in figure 2.

The ranges of values utilized in the identification of the classes are presented in table 1. We pointed out that the maximum and minimum

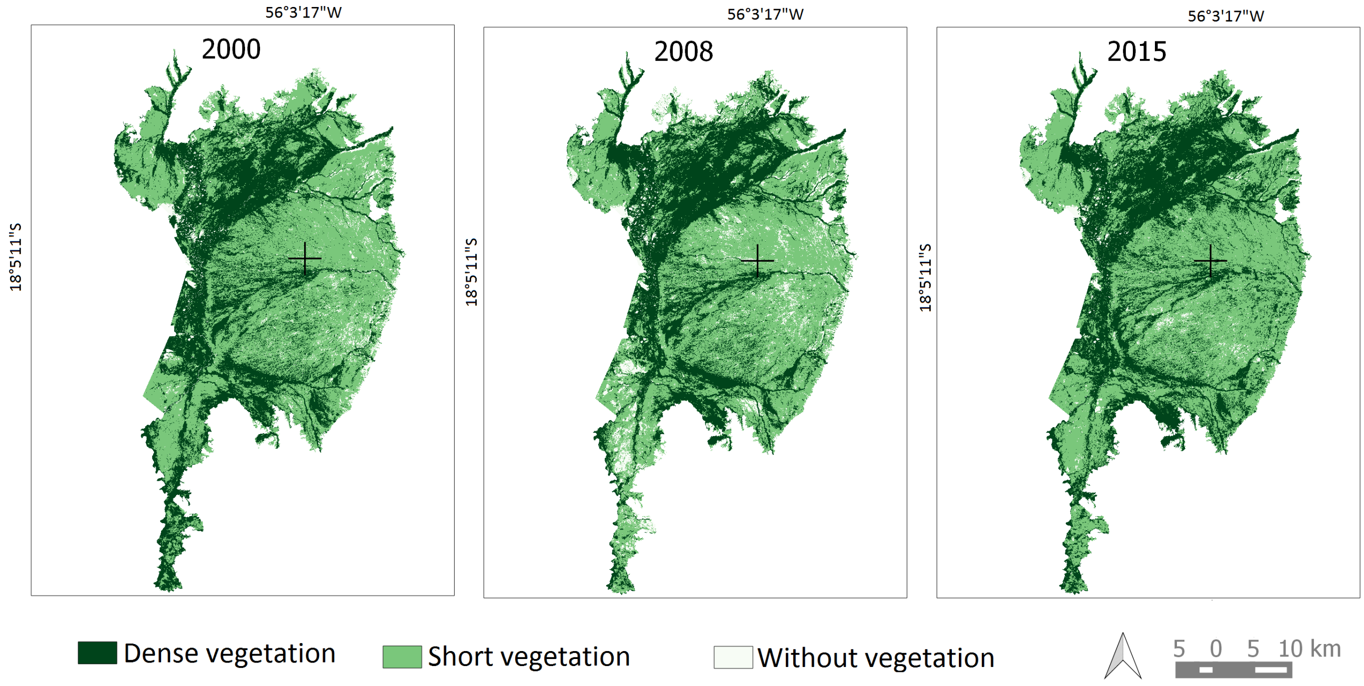

Figure 2. Mapping of the vegetation cover of the Brazilian Pantanal wetland using NDVI recorded in 12/08/2000, 12/08/2008 and 13/08/2015. The darker areas have the highest index value and represent regions covered by denser vegetation. In green, the areas covered by short vegetation, and in white are areas without vegetation.

Table 1. Maximum and minimum values of NDVI for each class of vegetation cover for the years 2000, 2008 and 2015 in the Brazilian Pantanal wetland.

	2000		**2008**		**2015**	
	Minimum	**Maximum**	**Minimum**	**Maximum**	**Minimum**	**Maximum**
Dense vegetation	0.6028	1	0.5962	1	0.6643	1
Short vegetation	0.4198	0.6027	0.4402	0.5961	0.4429	0.6642
Without vegetation	-1	0.4197	-1	0.4401	-1	0.4428

Table 2. Areas (Km^2) of conversion between classes of vegetation cover from 2000 to 2008 and from 2008 to2015 in the Brazilian Pantanal wetland.

	2000-2008		
From/To	**Dense vegetation**	**Short vegetation**	**Without vegetation**
Dense vegetation		11,503	697
Short vegetation	7,448		7,449
Without vegetation	290	3,111	
	2008-2015		
From/To	**Dense vegetation**	**Short vegetation**	**Without vegetation**
Dense vegetation		21,979	313
Short vegetation	1,896		4,911
Without vegetation	62	7,654	

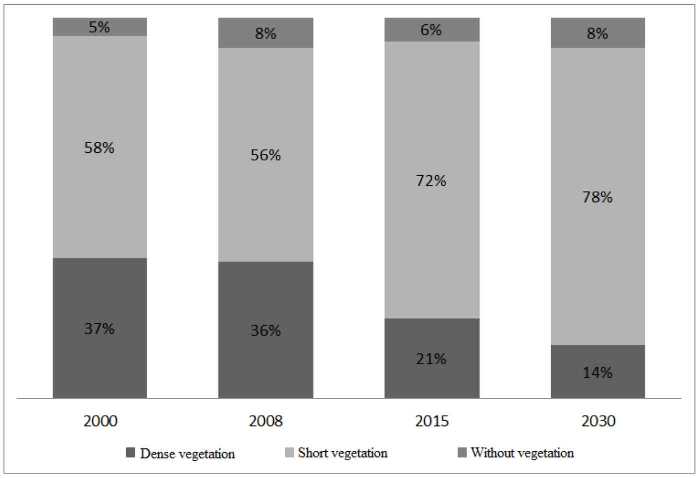

Figure 3. Comparison of the classes of vegetation cover mapped in the Brazilian Pantanal wetland in the years 2000, 2008 and 2015, plus the projection of the behavior of the classes for the year 2030.

values presented in the table 1 are different, since they were obtained from images of different years (Peres et al. 2016; Benedetti et al. 2013).

The results allowed the quantification of the classes of vegetation cover and the comparison between the years 2000, 2008 and 2015, according to the slicing process (table 1). The calculation of the transition rate of 15 years from 2000 to 2015 produced the projection for 2030.The comparison between the area percentage of the vegetation cover classes in the Brazilian Pantanal wetland is shown in figure 3.

Table 2 shows the value in area (Km^2) regarding conversion processes, reduction and increase between classes, during the period of 2000 to 2008, and 2008 to 2015, derived from the rates of the transition matrices generated in the software Dinamica EGO.

The calculations indicated the transition rate of dense to short vegetation and vice-versa of 22.55% and 13%, respectively, in the period of 2000-2008. In the same period, there was a loss of 1.37% of dense vegetation to areas without vegetation. The recovery from areas without vegetation to short vegetation and to dense vegetation was of 47.10% and 4.40%, respectively. In the period of 2008-2015 the transitions were more expressive if compared with the previous period. The reduction of areas of dense to short vegetation was 44.34%, while the recovery from short to dense vegetation was only 2.46%. The conversion of areas without vegetation to short vegetation was 67.40%. The analysis of the whole period, from 2000 to 2015, shows that the most expressive rates are those of from dense to short vegetation at 4.75% a year and from an area without vegetation to short vegetation at 6.65% a year. Elz et al. (2015) recommend a test concerning the error of the calculated rates. The highest difference found was an underestimation of 3%.

Discussion

The use of geotechnologies allows a holistic analysis in continental wetlands, bringing practical approaches for control, land planning, public policies and conservation practices (Elz et al. 2015). Beside those technologies being used to observe and quantify changes of vegetation cover, it can also monitor the provision of ecosystem services, and a more general utilization can cause positive effect on implementing programs of compensation for ecosystem services, since they provide evidences of the effective supply of services from the Brazilian Pantanal wetland (Alston et al. 2013; Schulz et al. 2015).

In the class of areas "without vegetation" the selected intervals presented values above 0.4 of NDVI for all studied periods, due to the water bodies presenting a wide variation, so the presence of aquatic vegetation could have caused this behavior (Andrade et al. 2012). The flood of the Paraguay River has a delay of 2-3 month in relation to the rainy season, and in August some western areas may be still flooded (Hamilton et al. 2002). Furthermore, the areas interpreted as bare soil are also framed as agricultural areas (Instituto SOS Pantanal & WWF-Brazil 2015), that in August appear as dry vegetation and exposed soil, what justifies the high values for the category assessed as without vegetation (Andrade et al. 2012).

Our most striking observation in the Brazilian Pantanal wetland was the conversion of the class dense vegetation into short vegetation, a result like the reported by Paranhos et al. (2014) and Peres et al. (2016). Abdon et al. (2007) pointed out the replacement of native grassland by cultivated pasture, in addition to loss of high biomass vegetation, what means alterations of the natural environment. Cardoso et al. (2010) studied the Brazilian Pantanal wetland sub region Nhecolândia and said that the conversion of native forest into cultivated pasture and the continuous grazing native grassland reduce stocks of organic and microbial carbon in the soil, notably depleted under older cultivated pastures.

The degradation of the original vegetation in the studied area can be explained by the new tendencies of economic development towards more intensive land use, with deforestations and alteration of natural areas (MMA 2002). Harris et al. (2006) assessed the period of 2000-2004 and estimated a yearly deforestation rate of 2.3%, concluding that in 2051 the original vegetation cover of the Brazilian Pantanal wetland would be completely lost or modified. In their study the suppression projection used mean geometric growth, Silva et al. (2011) estimated that the vegetation cover of the Brazilian Pantanal wetland could be totally lost until 2045.

The change of the vegetation cover in the whole study area (Table 2 and Figure 3) recorded in the period of 2000-2008 points to a reduction and increase between classes in a more balanced way, as we estimated that 12200 Km^2 of dense vegetation were lost, but 7738 Km^2 were recovered. The transition of the class without vegetation to dense vegetation between years is observed in areas where previous limits of water bodies were covered by vegetation.

The recovery from short to dense vegetation represents a gain of biomass, probably because of two management systems of cattle ranching: cattle stays year-round (the most common) or the cattle only stays during the dry season and it is removed when flooding begins (Pott, 1994), which allows the recovery of the flooded area. Such management induced changes can be observed on contrasting sides of fence lines. Furthermore, invasive of woody plants, e.g., *Vochysia divergens*, occurred over grasslands, particularly in the northern part of the Brazilian Pantanal wetland (Junk & Nunes 2012).

During the period of 2008-2015 the biggest reduction of dense vegetation occurred, summing up 22292 Km^2. Figure 3 shows the evolution of the short vegetation over the plain, which corroborates numerous studies in the region (Abdon et al. 2007, MMA 2011, Silva et al. 2011, Paranhos et al. 2014, Peres et al. 2016). In our work the projection of the transition between classes shows that in 2030 the Brazilian Pantanal wetland will have 78% of its area covered by short vegetation and only 14% by dense vegetation. Such information is an additional justification for more conservative actions on vegetation suppression control in the Brazilian Pantanal wetland. The decisions made by public managers and other decision makers shall be directed to design more effective public policies in controlling and monitoring the Brazilian Pantanal wetland and its basin (Silva et al. 2011). Therefore, the challenge for conservation of the Pantanal consists in new socioeconomic model to compromise environmental protection and land use, with evaluation of ecosystem services, organic production, as well as the valorization of the genetic diversity patrimony (Tocantins et al. 2006, Neves 2009).

Conclusion

Our work ratifies the alteration of the vegetation cover in the Brazilian Pantanal wetland. If the present pattern continues, the natural vegetation of the Brazilian Pantanal wetland will be mostly lost or modified, in medium time interval, which could seriously affect its climatic-hydrologic dynamics, like change the cycles of floods and droughts (flood pulse), the exuberance of waters and its biological biodiversity.

The contribution of the present work is relevant, beyond the generated products and the applied methods. The results alerts for the need of stricter public policies aiming the conservation and the sustainable use of biodiversity at several administration levels. For being an important ecological area, our study points out the need for preservation of the Brazilian Pantanal wetland biodiversity. We highlight the challenge to transform this biodiversity into opportunity of sustainable development. Thus, this article offers a distinct picture for analysis of the approaches for control, land use planning, public policies and conservation practices.

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Author Contributions

Arnildo Pott: substantial contribution to data analysis and interpretation, manuscript critical revision and translated of manuscript.

Antonio Conceição Paranhos: contribution to conceived and planned the experiments.

Ciomara de Souza Miranda: contribution to data collection and data analysis. Planned and carried out the simulations and manuscript preparation.

All authors provided critical feedback and helped shape the research, analysis and manuscript.

Conflicts of interest

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

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Ichthyofauna of Marinheiros Island, Patos Lagoon estuary, southern Brazil

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Abstract: Marinheiros is the largest island of the estuarine archipelago of Patos lagoon and is considered an area of biological relevance in the coastal region of the extreme south of Brazil. Aiming to contribute to the knowledge on the biodiversity of this important area, we conducted an ichthyofaunistic inventory in the limnic environments of the island. Twenty-seven field trips were performed between April 2015 and May 2017. A total of 12 sites representative of habitats of an intermittent shallow lagoon, intermittent pools and a perennial artificial channel were sampled. Sampling of 2,436 specimens revealed the occurrence of 30 species, including the non-native piscivorous *Acestrorhynchus pantaneiro*. Characiformes was the most diverse order (18 species), followed by Cichliformes (four species) and Cyprinodontiformes (three species). Characiformes was also the most numerically abundant order (77%) followed by Cichliformes (17.6%) and Cyprinodontiformes (5.1%). The species richness recorded in Marinheiros Island is is comparable to the richness observed for lotic systems of southernmost Brazilian coastal plain. The fish assemblage is composed mainly of species of limnic habits whereas few taxa of estuarine-marine-limnic and estuarine-limnic habits were recorded. In view of the presence of allochtonous *A. pantaneiro* and its potential to impact native species, monitoring is recommended to the ichthyofauna of Marinheiros Island and the other estuarine islands, as well as peninsular systems connected to Patos lagoon estuary.

Keywords: *Acestrorhynchus pantaneiro, ictiocenose, invasive species, Neotropical region*

Ictiofauna da Ilha dos Marinheiros, estuário da Lagoa do Patos, sul do Brasil

Resumo: Marinheiros é a maior ilha do arquipélago estuarino da Lagoa dos Patos e é considerada uma área de relevância biológica na região costeira do extremo sul do Brasil. Com o objetivo de contribuir para o conhecimento sobre a biodiversidade desta importante área, realizamos um inventário ictiofaunístico nos ambientes limnicos da ilha. Vinte e sete visitas foram realizadas entre abril de 2015 e maio de 2017. Um total de 12 locais representativos dos habitats de uma lagoa rasa intermitente, poções intermitentes e um canal artificial perene foram amostrados. A amostragem de 2.436 espécimes revelou a ocorrência de 30 espécies, incluindo o piscívoro não-nativo *Acestrorhynchus pantaneiro*. Characiformes foi a ordem mais diversa (18 espécies), seguido por Cichliformes (quatro espécies) e Cyprinodontiformes (três espécies). Characiformes foi também a ordem mais numericamente abundante (77%), seguida po Cichliformes (17,6%) e Cyprinodontiformes (5,1%). A riqueza de espécies registrada na Ilha dos Marinheiros é comparável à riqueza observada para sistemas lóticos da planície costeira do extremo sul do Brasil. A assembleia de peixes é composta principalmente por espécies de hábitos límnicos, enquanto que poucos taxa de hábitos estuarino-marinho-límnico e estuarino-límnico foram registrados. Em vista da presença do alóctono *A. pantaneiro* e seu potencial para impactar espécies nativas, é recomendado o monitoramento da ictiofauna da Ilha dos Marinheiros e de outras ilhas estuarinas, bem como dos sistemas peninsulares conectados ao estuário da Lagoa dos Patos.

Keywords: *Acestrorhynchus pantaneiro, ictiocenose, espécie invasora, região Neotropical*

Introduction

Patos lagoon is the world's largest choked lagoon, extending over 240 km on the southernmost Brazilian coastal plain (Kjerfve 1986). The estuarine zone of Patos lagoon is characterized by the presence an archipelago composed by eight main islands and several islets emerged during the Holocene. Marinheiros is the largest island, covering an area of 62 km^2. It is also the first to emerge from the estuarine submerged flats, at about 5,000 years ago, in a process of lagoon sediment deposition (Vieira 1983).

Studies on vertebrate fauna in Marinheiros Island date back only to the last decade and have already highlighted the island as an important area from the biogeographical and conservation perspectives in southern

Brazilian coast. Sampling efforts on anuran (Quintela et al. 2007, 2009, Bernardo-Silva et al. 2012, Dalmolin et al. 2017), reptilian (Quintela et al. 2011) and avian fauna (Gianuca et al. 2007, 2008, 2012) in the insular environments have identified the occurrence of some unexpected taxa for the region, including the southernmost and isolated population of the threatened red-bellied toad *Melanophryniscus dorsalis* (Mertens, 1933) (Quintela et al. 2006, Bernardo-Silva et al. 2012). While some groups such as herpetofauna and avifauna are relatively well-known in the island, the ichthyofauna remained practically unexplored. Meanwhile, Marinheiros shelters a peculiar complex of intermittent shallow lagoons and pools, which consist of potential habitats for the fish fauna.

Marinheiros Island is separated from the mainland by approximately 1.7 km and remained isolated until the year 2004, when the establishment of a bridge linked it to an adjacent island (Leonídeo), which is in turn connected to Rio Grande peninsula. The easy access to Marinheiros resulted in the increase of anthropogenic impacts due to the expansion of settlements and a large increment on tourism and visitation. The insular limnic ecosystems, in particular, also face the threat of pesticides, used in a wide range in the local agricultural production (Quintela et al. 2009, 2011). Thus, pollution and contamination represent potential risks to Marinheiros waterbodies and its associated biota.

Despite the aforementioned importance of Marinheiros for the local biodiversity, the island still lacks an effective plan for the management and conservation of ecosystems, in part due to the gap of knowledge on its biota. Herein, we present the first list of the ichthyofauna occurring in freshwater systems of Marinheiros Island, aiming to contribute to the knowledge on the faunistic diversity in this important area.

Material and Methods

1. Study area

Marinheiros Island (31º58’- 32º02’S; 52º05’-52º12’W) is located in the southern portion of Patos lagoon estuary, Rio Grande municipality, Rio Grande do Sul state, southern Brazil. The island is distant about 1.4 km from the adjacent Leonídeo island, 1.7 km from peninsular mainland (Rio Grande), and about 16 km from the estuarine mouth (Figure 1). The climate in Rio Grande municipality is classified as subtropical temperate (*Cfa* of Köppen), with temperatures varying from 9.5ºC (mean minimum) in midwinter to 27.2ºC (mean maximum) in midsummer. The average annual rainfall is 1,252 mm and the rainiest period is from July to September (Vieira 1983).

Hydrographically, Marinheiros Island is characterized mainly by a set of shallow lagoons and pools, strongly influenced by rainfall regime. Some systems can dry completely during periods of high evaporation although heavy rainfall periods can promote the interconnection of systems. Heavy rainfall may also result in the interconnection between internal systems and estuarine waters due to flooding of the peripheral areas of the island. The largest lagoon, locally know as “Lagoa do Rey”, can extended over 3.3 km during rainy period while smaller lagoons range from ca. 150 to 700 m long. The coverage of marginal areas varies from sandy bottom to dense patches of emergent macrophytes *Typha dominguensis* Pers., *Schoenoplectus californicus* (C.A. Mey.) Soják and *Scirpus* spp. and deeper sections are colonized by the submerged rooted *Cabomba caroliniana* A. Gray. Bottom varies from sandy in marginal areas to muddy in deeper sections. A set of around 20 pools ranging from 7 to 20 m in length is located in the eastern portion of the island, know as “Marambaia”. The marginal coverage of pools varies from sandy bottom to dense patches of emergent vegetation (mainly *T. dominguensis* and *Scirpus* spp.) and some systems are colonized by *Nymphoides indica* (L.) Kuntzae, *Myriophyllum* sp. and *C. caroliniana*. Both pools and lakes do not exceed 2.5 m in depth and have bottoms varying from sandy to muddy. In addition to these natural hydrographic elements, a pluvial channel extends along the 28 km of the island perimeter. This artificial system has an average depth of 0.8 m, muddy bottom, and can be densely covered by floating macrophytes (*Azolla filiculoides* Lam., *Eichornia* spp., *Salvinia* spp.) mainly in the warmer periods.

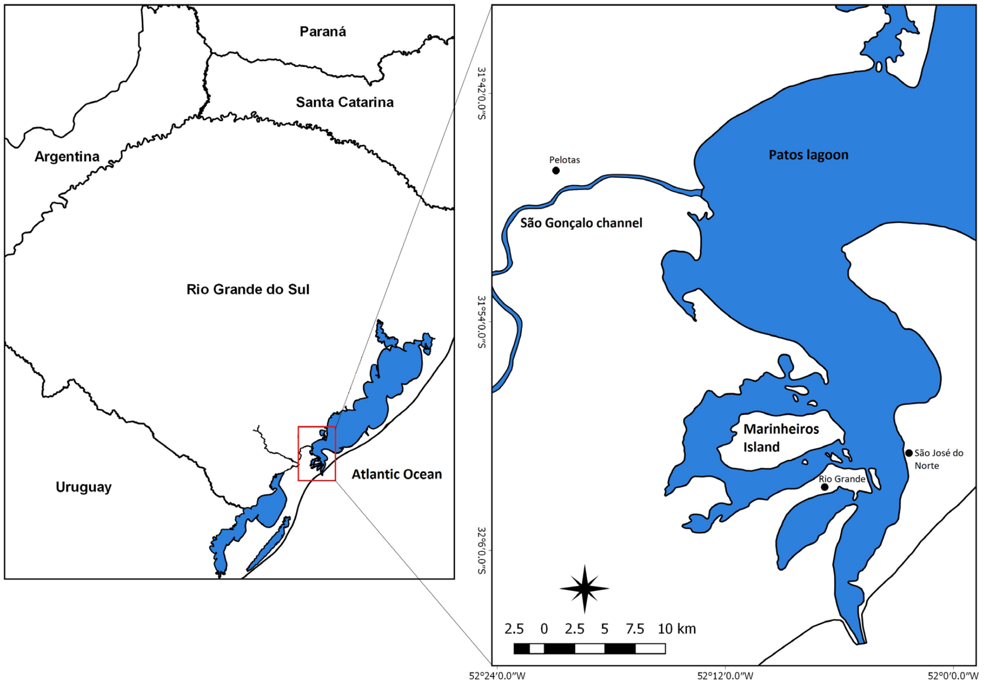

Figure 1. Location of Marinheiros Island, and the sites of collection of the ichthyofauna, Patos Lagoon estuary, southern Brazil.

2. Sampling

Twenty-seven random samplings were conducted between April 2015 and May 2017. A total of 12 sites were sampled, covering environments of the shallow lagoon “Lagoa do Rey”, the set of pools “Marambaia” and the pluvial channel (Table 1, Figure 2). Fishes were captured using the following gear: seine net 5 x 2 m, mesh size 5 mm; gillnet 20 x 2.5 m, mesh size 7 mm; hand net 0.60 x 0.40 m, mesh size 2 mm; funnel traps (length 0.74 m, diameter 0.38 m, mesh size 25 mm; length 0.60 m, diameter 0.32 m, mesh size 15 mm). Captured individuals were euthanized in clove oil solution, fixed in 10% formalin and preserved in 70% ethanol in the Fish Reference Collection of the Instituto de Ciências Biológicas at the Universidade Federal do Rio Grande (CIFURG) (Appendix). Specimens were identified with the help of specialized literature (Reis et al. 2003, Lucinda 2008, Malabarba et al. 2013). Collection was authorized by the Brazilian environmental agency ICMBio through license number 56947-1. Nomenclature of species and higher taxa follows Bertaco et al. (2016).

Table 1. Characteristics and locations of the sites sampled in Marinheiros Island, Patos Lagoon estuary, southern Brazil, and fishing gear applied in each site.

Site	System	Coordinates	Vegetation	Fish gear
1	shallow lagoon	32º01’20”S; 52º09’28”W	submersed/emergent	seine, gill
2	shallow lagoon	32º00’58”S; 52º08’29”W	submersed/emergent	seine, gill
3	shallow lagoon	32º00’48”S; 52º08’11”W	submersed/emergent	seine, gill
4	pool	31º59’54”S; 52º06’10”W	submersed/emergent	seine, gill, funnel
5	pool	31º59’54”S; 52º06’09”W	submersed/emergent	seine, funnel
6	pool	31º59’54”S; 52º06’07”W	submersed/emergent	funnel, handnet
7	pool	31°59’53”S; 52º06’06”W	submersed/emergent	seine, funnel
8	pool	31º59’52”S; 52º06’06”W	submersed/emergent	seine, funnel
9	pool	31º59’50”S; 52º06’04”W	submersed/emergent	seine, funnel
10	pool	31º59’49”S; 52º06’03”W	submersed/emergent	seine, funnel
11	channel	31º59’15”S; 52º06’58”W	emergent	gill, funnel, handnet
12	channel	31º59’09”S; 52º07’06”W	floating/emergent	funnel, handnet

Figure 2. Limnic systems sampled in Marinheiros Island, Patos Lagoon estuary, southern Brazil: shallow lagoon “Lagoa do Rey” (above), set of pools of “Marambaia” (below, left); pluvial channel (below, right).

Results

A total of 2,436 specimens belonging to 30 species, 21 genera, 11 families and six orders was collected (Table 2). Characiformes was the order with the highest species richness (18 species), followed by Cichliformes (four species) and Cyprinodontiformes (three species). Characiformes was the most numerically abundant order (77%), followed by Cichliformes (17.6%) and Cyprinodontiformes (5.1%). Characidae was the most rich family (14 species) and also the most abundant family (74.8%). Cichlidae was the second most diverse (four species) and abundant family (17.6%). All species except *Acestrorhynchus pantaneiro* Menezes, 1992 (Figure 3) are native to Patos lagoon basin (Saccol-Pereira et al. 2006, Assumpção et al. 2016).

Table 2. Number of collected specimens and relative abundance (in parentheses) of fish species recorded in three types of limnic systems in Marinheiros Island, Patos Lagoon estuary, southern Brazil.

Taxon	Pools	Shallow lagoon	Channel	Subtotal
Characiformes				
Acestrorhynchidae				
Acestrorhynchus pantaneiro Menezes, 1992	2 (0.13)			2 (0.08)
Characidae				
Astyanax eigenmanniorum (Cope, 1894)	649 (43.44)	76 (9.48)		725 (29.76)
Astyanax lacustris (Lütken, 1875)	4 (0.26)			4 (0.16)
Astyanax henseli Melo & Buckup, 2006	1 (0.06)			1 (0.04)
Charax stenopterus (Cope, 1894)	2 (0.13)			2 (0.08)
Cheirodon ibicuhiensis Eigenmann, 1915	8 (0.53)			8 (0.3)
Cheirodon interruptus (Jenyns, 1842)	99 (6.62)	195 (24.34)		294 (12.06)
Hyphessobrycon anisitsi (Eigenmann, 1907)		21 (2.62)		21 (0.86)
Hyphessobrycon boulengeri (Eigenmann, 1907)	2 (0.13)		28 (19.85)	30 (1.23)
Hyphessobrycon luetkenii (Boulenger, 1887)	79 (5.28)	470 (58.67)	12 (8.51)	561 (23.02)
Hyphessobrycon igneus Miquelarena, Menni, López & Casciotta, 1980	87 (5.82)	7 (0.87)	12 (8.51)	106 (4.35)
Hyphessobrycon meridionalis Ringuelet, Miquelarena & Menni, 1978	2 (0.13)			2 (0.08)
Hyphessobrycon togoi Miquelarena & López, 2006	1 (0.06)			1 (0.04)
Oligosarcus jenynsii (Günther, 1864)	29 (1.94)		1 (0.71)	30 (1.23)
Oligosarcus robustus Menezes, 1969	38 (2.54)			38 (1.55)
Erythrinidae				
Hoplias malabaricus (Bloch, 1794)	6 (0.40)	3 (0.37)	1 (0.71)	10 (0.41)
Curimatidae				
Cyphocharax saladensis (Meinken, 1933)		3 (0.37)		3 (0.12)
Cyphocharax voga (Hensel, 1870)	38 (2.54)		1 (0.71)	39 (1.60)
Cichliformes				
Cichlidae				
Australoheros acaroides (Hensel, 1870)	6 (0.40)			6 (0.24)
Cichlasoma portalegrense (Hensel, 1870)	194 (12.98)	3 (0.37)	1 (0.71)	198 (8.12)
Crenicichla lepidota Heckel, 1840	107 (7.16)	1 (0.12)	2 (1.41)	110 (4.51)
Geophagus brasiliensis (Quoy & Gaimard, 1824)	90 (6.02)	2 (0.24)	24 (17.02)	116 (4.76)
Gobiiformes				
Eleotridae				
Dormitator maculatus (Bloch, 1792)			1 (0.71)	1 (0.04)
Gobiidae				
Ctenogobius shufeldti (Jordan & Eigenmann, 1887)	1 (0.06)			1 (0.04)
Siluriformes				
Heptapteridae				
Pimelodella australis Eigenmann, 1917	1 (0.06)			1 (0.04)
Rhamdia aff. quelen (Quoy & Gaimard, 1824)	2 (0.13)			2 (0.08)
Cyprinodontiformes				
Anablepidae				
Jenynsia multidentata (Jenyns, 1842)	44 (2.94)	20 (2.49)		64 (2.62)
Poeciliidae				
Phalloceros caudimaculatus (Hensel, 1868)	4 (0.26)		55 (39.00)	59 (2.42)
Poecilia vivipara Bloch & Schneider, 1801			1 (0.71)	1 (0.04)
Synbranchiformes				
Synbranchidae				
Synbranchus marmoratus Bloch, 1795			2 (1.4)	2 (0.08)
Total	1494	801	141	2436

Figure 3. Specimens of *Acestrorhynchus pantaneiro* (above CIFURG 200, below CIFURG 201) collected in Marinheiros Island, Patos Lagoon estuary, southern Brazil.

None of the recorded species is considered as threatened in Rio Grande do Sul (FZB 2014) and Brazilian (MMA 2014) lists.

The set of pools was the systems with the highest richness recorded (25 species), followed by the pluvial channel (13 species) and the shallow lagoon "Lagoa do Rey" (11 species). The characids *Astyanax eigenmanniorum* (Cope, 1894) and *Hyphessobrycon luetkenii* (Boulenger, 1887) were the most abundant species in the pools set and the shallow lagoon, respectively. The poeciliid *Phalloceros caudimaculatus* (Hensel, 1868) was the most abundant species in the pluvial channel. The number of collected specimens and the relative abundance of recorded species in each sampled system are given in Table 2.

Discussion

The limnic enviroments of Marinheiros Island house an ichthyofaunistic diversity within the range observed for lotic systems in southernmost Brazilian coastal plain, which is between 22 (Fortaleza lagoon; Schifino et al., 2004) and 67 species (Peixe lagoon; Loebmann & Vieira 2005). It is clear the taxonomic and numerical dominance of order Characiformes, with family Characidae encompassing around 46% of the species richness and 75% of all specimens collected. The high diversity of Characiformes corroborates the general pattern found in Neotropical region (Reis et al. 2003), as well as the regional patterns observed in all of the limnic systems of the southernmost Brazilian coastal plain studied so far, where Characidae stands out as the richest family (Assumpção et al. 2016). The shallow depths of island systems are favorable to the occurrence of characids, which are referred as inhabitants mainly of shallow waters (Lowe-McConnell 1987). However, the most speciose order in Rio Grande do Sul state (Bertaco et al. 2016), Siluriformes, was poorly represented in the insular systems, with only two recorded species (*Pimelodella australis* Eigenmann, 1917 and *Rhamdia* aff. *quelen* Quoy & Gaimard, 1824). It is possible that the great predominance of shallow zones in the systems acts limiting the occurrence of species of this order, usually benthic and inhabitants of deeper zones (Malabarba et al. 2013). Cichlidae, the second most diverse family in Marinheiros, is also among the most rich fish groups in Neotropical region (Kullander 2003) and it is well represented in limnic environments of Rio Grande do Sul coastal plain (Assumpção et al. 2016). Moreover, the insular systems of Marinheiros present physiognomic characteristics such low depths, abundance of aquatic macrophytes and sandy bottoms, which are considered suitable for the cichlids occurrence (Malabarba et al. 2013).

Except by *Jenynsia multidentata* (Jenyns, 1824)*, Ctenogobius shufeldti* (Jordan & Eigenmann, 1887) (both estuarine-limnic) and *Dormitator maculatus* (Bloch, 1792) (marine-estuarine-limnic), all the remain recorded species exhibit limnic habits (see Assumpção et al. 2016). Marinheiros Island, as well as the other estuarine islands in Patos lagoon estuary, is strongly subject to flooding during the periods of high precipitation and consequent high discharge of Patos-Mirim basin. Under this condition, estuarine waters overrun a considerable stretch of the borders of the island, including the peripheral freshwater systems. Thus, it was expected a higher representativeness of coastal marine and estuarine-related species which also inhabit freshwater coastal environments of the region, such as *Atherinella brasiliensis* (Quoy & Gaimard, 1825), *Mugil* spp., *Micropogonias furnieri* (Desmarest, 1823), *Odontesthes argentinensis* (Valenciennes, 1835), *O. bonariensis* (Valenciennes, 1835), *Platanichthys platana* (Regan,1917) and others (see Tagliani 1994, Loebmann & Vieira 2005, Garcia et al. 2006, Artioli et al. 2009, Bastos et al. 2013), were absent in our sampling. It should be noted that the insular systems were also sampled subsequently to the heavy rainfall period associated to last El Niño (2015-2016), when Patos Lagoon estuarine archipelago experienced severe flooding events. This fact can be related to the strong influence of the precipitation in the insular systems, which may decrease the salinity in short time after estuarine floods, making the environments little attractive for the coastal and estuarine-related species.

The capture of two individuals of *Acestrorhynchus pantaneiro*, an invasive species in Patos lagoon basin (Saccol-Pereira et al. 2006), is remarkable. *Acestrorhynchus pantaneiro* is a piscivorous medium-sized species (maximum total length 35 cm) (Zaniboni et al. 2004), native to Mamoré, Paraguay, Paraná and Uruguay rivers basins (Menezes 2003). In the southernmost Brazilian state of Rio Grande do Sul, *A. pantaneiro* is considered autochthonous solely for Uruguay basin (Menezes 2003, Saccol-Pereira et al. 2006). About a decade, it also has been found in Patos-Mirim and Tramandaí basins (Saccol-Pereira et al. 2006, Leal et al. 2009, Artioli et al. 2013, Rocha and Hartz 2013, Einhardt et al. 2014, Corrêa et al. 2015, Neuhaus et al. 2016), where it is considered an invasive species in rapid expansion (Neuhaus et al. 2016). Nevertheless, our record is the first in the estuarine zone of Patos lagoon. Considering that Patos lagoon estuary was systematically sampled in the last decade (Garcia et al. 2003, Garcia et al. 2004, Burns et al. 2006), it is possible that the species settled this area only recently.

Piscivorous invasive fish species show a tendency for establishment, promoting disturbances in trophic cascades and populations of native species (Pusey et al. 2006). Neuhaus et al. (2016) verified a high food niche overlap between invasive *A. pantaneiro* and native *Oligosarcus robustus* Menezes, 1969 in Jacuí and Sinos rivers, northern Patos Lagoon basin, were both species predated predominantly on small characids. Thus, if *A. pantaneiro* became an established invasive species in Marinheiros Island, populations of *O. robustus* and other native species may be directly affected by resource competition and predation. In view of this, we recommend the monitoring of *A. pantaneiro* and the ichthyofauna of limnic systems of Marinheiros in general, as well as other estuarine islands and systems of the peninsular systems of the coastal plain connected to the estuary of Patos lagoon. This procedure could detect new areas of occurrence of *A. pantaneiro* and collect data for the analysis on the relationships between this invasive species and the native ichthyofauna.

Supplementary material

The following online material is available for this article:

Appendix 1: List of vouchers housed in the Fish Reference Collection of FURG (CIFURG).

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Author Contributions

Fernando Marques Quintela: contribution to conception and design of the study; contribution to data sampling and analysis; contribution to manuscript preparation.

Fabiano Corrêa: contribution to data analysis; contribution to manuscript preparation.

Rafael Martins Pinheiro: contribution to data sampling; contribution to manuscript preparation.

Daniel Loebmann: contribution to manuscript preparation and critical revision.

Conflicts of interest

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

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Predicting the distribution of *Omalonyx* (Mollusca: Pulmonata: Succineidae) species from literature review, museum databases and new sampling efforts in Brazil

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Abstract: Accurate distributional information is crucial for studies on systematics, biodiversity and conservation. To improve the knowledge regarding the geographical distribution of *Omalonyx* in South America, we present updated information based on data from a literature review, institutional collections and malacological surveys. All this information composed the dataset used to predict species distribution employing the Maximum Entropy Algorithm (MaxEnt). The model was run using data on species distribution, altitude and bioclimatic variables (WorldClim database). The model had consistent performance, and areas presenting similar conditions to areas where the species were recorded were considered areas of occurrence. The predicted occurrence areas included those that were already surveyed and those that are considered potential occurrence areas. The results demonstrate that the genus has widespread distribution in the Neotropical region and occurs in the tropical, temperate and arid regions of South America and Lesser Antilles. *Omalonyx* spp. were recorded in all South American countries and hydrographic regions. However, in some countries, there were only isolated records (ex: Colombia and Ecuador). Here, we also present the first record of *Omalonyx* spp. in four Brazilian States (Acre, Rondônia, Piaui, and Amapá). The genus was found in all hydrographic regions within Brazil and among 27 federative unities; it was absent from only two unities (Roraima State and Distrito Federal). This work contributes to the knowledge on *Omalonyx* spp. distribution and provides an important basis for the work of ecologists and taxonomists.

Keywords: *MaxEnt, niche model, species distribution, Modeling, Succineids, Neotropic.*

Predição da distribuição de *Omalonyx* (Mollusca: Pulmonata: Succineidae) a partir da revisão da literatura, dados de museu e novo esforço amostral no Brasil

Resumo: A informação precisa sobre a distribuição é crucial para os estudos sobre sistemática, biodiversidade e conservação. Para melhorar o conhecimento sobre a distribuição geográfica de *Omalonyx* na América do Sul, apresentamos informações atualizadas com base em dados de revisão de literatura, coleções institucionais e pesquisas malacológicas. Toda essa informação compôs o conjunto de dados usado para predição da distribuição de espécies empregando o Algoritmo de Entropia Máxima (MaxEnt). O modelo foi executado usando dados de distribuição de espécies, altitude e variáveis bioclimáticas (banco de dados WorldClim). O modelo apresentou um desempenho consistente e as áreas que apresentaram condições semelhantes às áreas onde as espécies foram registradas, foram consideradas áreas de ocorrência. As áreas de ocorrências previstas incluíram aquelas que já foram pesquisadas e aquelas que são consideradas áreas de ocorrência potencial. Os resultados demonstram que o gênero tem uma distribuição Neotropical ampla e que ocorre nas regiões tropical, temperada e árida da América do Sul e nas Pequenas Antilhas. *Omalonyx* spp. foram registradas em todos os países e bacias sul-americanas. No entanto, em alguns países, apenas registros isolados foram encontrados (ex: Colômbia e Equador). Aqui, também apresentamos o primeiro registro de *Omalonyx* spp. em quatro estados brasileiros (Acre, Rondônia, Piauí e Amapá). O gênero foi encontrado em todas as regiões hidrográficas no

Brasil e nas 27 unidades federativas; sendo ausente em apenas duas unidades federativas (Estado de Roraima e Distrito Federal). Esse trabalho contribui para o conhecimento da distribuição das espécies de *Omalonyx* e fornece uma importante base para trabalhos de ecólogos e taxonomistas.

Palavras chave: *MaxEnt, modelagem de nichos, distribuição de espécies, Modelagem, Succineídeos, Neotrópico.*

Introduction

The genus *Omalonyx* d'Orbigny, 1837 presents a reduced shell and represents one of the slug-like lineages within the family Succineidae (Patterson 1971, Tillier 1981, Barker 2001). The earliest fossil records of Succineidae were from the Tertiary of Europe and, currently, most of the diversity within the family occurs in the islands of the Pacific, in the Indian subcontinent and the Americas (Barker 2001). These Neotropical slugs are terrestrial, live on aquatic macrophytes or riparian vegetation and can also be found in humid soil (Garcia et al. 2012). They can act as agricultural plagues (Olazarri 1979, Garcia et al. 2012) and are agents in the control of water-cress (Poi de Neiff et al. 1977). They can also act as intermediate hosts of an avian trematode, *Leucochloridium* (Lutz 1921, Travassos 1928), in the wild and of the nematodes *Angiostrongylus costaricensis* and *A. vasorum* in the laboratory (Montresor et al. 2008, Mozzer et al. 2011).

Currently, it is assumed that the genus comprises six recognized species (Tillier 1980, 1981, Martínez 1993, Arruda & Thomé 2008a, b, Arruda et al. 2009, Coscarelli & Vidigal 2011): *Omalonyx convexus* (Martens, 1868), *Omalonyx unguis* (d'Orbigny, 1837), *Omalonyx brasiliensis* (Simroth, 1896), *Omalonyx matheroni* (Pontiez & Michaud, 1835), *Omalonyx geayi* Tillier, 1980, and *Omalonyx pattersonae* Tillier, 1981. However, identification of the species within this genus is greatly complicated by intraspecific morphological variation in the diagnostic characteristics such as the papillae on the internal surface of the penis, the insertion of the retractor muscle of the penis, the shape of papillae on the inner surface of the vagina and others (Tillier 1980, 1981, Martínez 1993, Arruda & Thomé 2008a, b, Arruda et al. 2009, Coscarelli & Vidigal 2011).

To improve taxonomic knowledge on *Omalonyx* species, studies encompassing morphological and molecular data are essential. However, the first step to elucidating these questions is to acquire knowledge on species geographical distribution. Accurate geographical distributional information is crucial to solve systematics and biogeographic problems and to drive conservation efforts. Species-distribution models infer potential distribution from data on observed distribution and from environmental variables related to the sites where occurrences were recorded (Bradie & Leung 2017). These models assume that climate ultimately restricts a species' distribution and summarize it with a number of climatic variables within the known range of the species, generating a bioclimatic profile. They play an important role as first filters in assessing the potential distribution of each species, improving the success of future sampling efforts and providing initial insight into the bioclimatic tolerance of different species (Beaumont et al. 2005, Rubio & Acosta 2011). These tools have also been applied in several conservation biology studies to identify areas with high species richness and to predict effects of climate change on species' distributions (Araújo & Rahbek 2006, Costa et al. 2010, Vogler et al. 2013).

There are several methods to model species distribution, and they may use presence/absence, abundance or presence-only data. Usually, species records have limited coverage and consist of presence-only data (Elith et al. 2011). The Maximum Entropy Algorithm (MaxEnt) presents high performance for modeling presence-only data and is widely used to model species distribution (Bradie & Leung 2017). Here, we use this method to improve the knowledge on the geographical distribution of *Omalonyx* spp., assembling literature data with specimens either collected in our surveys, from malacological collections or donated by partner laboratories. These data on species presence and the associated bioclimatic variables were used to model species distribution, to investigate the main environmental factors related to the occurrence of *Omalonyx* species and to detect species with overlapping distribution areas. This work improves the knowledge on these slugs' distribution, providing information to propel advances in the morphological and molecular taxonomy of these neotropical succineids.

Material and Methods

Literature data on *Omalonyx* was reviewed (until December 2016) in order to find records of its occurrence across the Neotropical region (see the topic *literature review* in the results). Institutions harboring preserved specimens were identified in the literature review and by onsite and virtual searching in institutional collections (Table 1), and information regarding the lot number and collection site was organized in two tables (Table 2 and 3). Donated specimens were also included in these tables (Table 2 and 3).

To investigate in detail the distribution of these slugs in Brazil, malacological surveys comprising all geographical regions (North, Northeast, Southeast, South and Central West) were conducted between 2006 and 2016 (permission from environmental agency: ICMBio/SISBIO no. 12113-3). Specimens were manually collected from wet soil and vegetation in the vicinity of freshwater systems. Voucher specimens were deposited in the malacological collection of the Laboratório de Malacologia e Sistemática Molecular (LMSM), at Universidade Federal de Minas Gerais, Minas Gerais, Brazil.

All localities from the literature review, museums, collections, donations and our own malacological surveys were listed (Table 2 and 3) and plotted on a map (Figure 1) using the software ArcGIS 10.4 (ArcGIS Desktop 10.4 Geostatistical Analyst, Environmental Systems Research Institute ESRI, 2016). The geographic position of the localities and the information about South American hydrography and geography were cross-checked with Google Earth, Global Gazetteer, Hydro web (a hydrologic information system of Agência Nacional de Águas - ANA, the national water resources agency of the Environment Ministry, Brazil). Information regarding the hydrography of Austral South America was obtained from Bonetto (1994).

Morphological identification of specimens collected in our malacological surveys was based on characteristics of the reproductive system according to the following literature: *O. convexus* (Hylton-Scott & Lapuente 1968, Hylton-Scott 1971, Tillier 1981, Arruda & Thomé 2008b), *O. unguis* (Coscarelli & Vidigal 2011), *O. matheroni*, *O. pattersonae* and *O. geayi* (Tillier 1981). Specific identification provided in the papers (literature review) and on the labels (museum specimens) was also used in this work. Some of these records were not identified to species level and are designated *Omalonyx* sp. Until 1981, there were several species of *Omalonyx*, now recognized as synonyms. Throughout the text, species are designated according to Tillier (1981) and Arruda & Thomé (2008a, b), where synonyms for the six valid *Omalonyx* species can be found. However, when consulting the topic references, the reader will find the former designation.

Data of occurrence (Table 2 and 3) were used to model the species geographic distributions using one topographic variable (altitude) and the nineteen bioclimatic variables derived from the WorldClim database v. 1.4 as showed by Vogler et al. (2013) (Table 4). The classifications of climate were based on Koppen-Geiger climate classification types for South America (Peel et al. 2007). Maps for the distribution of *O. matheroni, O. pattersonae, O. geayi, O. convexus* and *O. unguis* were produced.

Table 1. Institutions harbouring specimens of *Omalonyx* found after literature review, visits and virtual search in collections databases.

Abbreviation	Museum or collection name
ANSP	Academy of Natural Sciences of Philadelphia, Pennsylvania, United States of America.
BMNH	British Museum of Natural History, London, United Kingdom.
CM	Carnegie Museum, Pittsburgh, Pennsylvania, United States of America.
CMIOC*	Coleção de Moluscos do Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.
FLMNH	Florida Museum of Natural, United States of America.
FMNH	Field Museum of Natural History, Chicago, Illinois, United States of America.
INPA*	Instituto Nacional de Pesquisas da Amazônia, Manaus, Amazonas, Brazil.
LMSM	Laboratório de Malacologia e Sistemática Molecular, Belo Horizonte, Minas Gerais, Brazil.
MACN	Museo Argentino de Ciencias Naturales, Bernadino Rivadavia, Buenos Aires, Argentina.
MCP	Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.
MCNZ	Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, United States of America.
MIRR*	Museu Integrado de Roraima, Boa Vista; Roraima, Brazil.
MLP*	Museo de La Plata, La Plata, Argentina.
MNHCL	Museo Nacional de Historia Natural del Chile, Chile.
MHNG	Muséum d'Histoire Naturelle, Genève, Switzerland.
MNKMO	Museo de Historia Natural Noel Kempff Mercado, Santa Cruz de la Sierra, Bolivia.
MNHN	Muséum national d'Histoire naturelle, Paris, France.
MNHNM	Museo Nacional de Historia Natural de Montevideo, Uruguay.
MNRJ*	Museu Nacional da Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil.
MPEG*	Museu Paraense Emílio Goeldi, Belém, Pará, Brazil.
MZUM	Museum of Zoology, University of Michigan, Ann Arbor, Michigan, United States of America.
MZUSP*	Museu de Zoologia da USP, São Paulo, São Paulo, Brazil.
RMNH	Rijksmuseum van Natuurlijke Histoire, Leiden, Netherlands
SMNH	Naturhistoriska Riksmuseet, Stockholm, Sweden.
UF	Florida Museum of Natural History (USA).
UFS	Universidade Federal de Sergipe, Aracajú, Sergipe, Brazil.
USDA	United States Department of Agriculture, Philadelphia, United States of America.
USMN	National Museum of Natural History, Smithsonian Institution, Washington, DC, United States of America.
ZMB	Museum für Naturkunde der Humboldt Universität, Berlin, Germany.
ZUECGAS	Museu de Zoologia da Universidade Estadual de Campinas, Campinas, São Paulo, Brazil.

Visited collections*

Table 2. Records of *Omalonyx* in Brazil based on literature data, institutional collections, and our own malacological surveys.

Species	Region	Locality	Record	[1]Source	[2] Hydrographic Region	Coordinates
O. matheroni	North	Rio Branco, Acre	LMSM 3346, 3378-83, 3438	Survey	5	10º 31'48"S, 68º 18'54"W
O. matheroni	North	Nações Unidas Ave. Macapá, Amapá	LMSM 3205-07, 3216-17, 3144, 3146	Survey	4	0º 28'19.2"N, 51º 39'0"W
O. matheroni	North	Amazon River, Santana, Amapá	LMSM 3199, 3200-03, 3232-33	Survey	4	0º 33'50.4"N, 51ºW 10'35.04"
O. matheroni	North	BR 156 Road, Calçoene, Amapá	LMSM 3215, 3220-21, 3145, 3147	Survey	4	2º 29'44.16"N, 50º 57'14.04W"
Omalonyx sp.	North	Manicore, Amazonas	INPA 445	Museum	4	6º 27'35.28"S, 61º 26'47.4W"
Omalonyx sp.	North	Novo Aripuanã, Amazonas	INPA769, 779, 781, 785, 801, 802, 808 (Pimpão 2007)	Literature	5	6º 49'30"S, 60º 28'37.92"W
Omalonyx sp.	North	Juruá River, Amazonas	MZSP 18444, 31962	Museum	5	2º 44'19.68"S, 66º 45'41.76"W
Omalonyx sp.	North	Barurua Island, Amazonas	MZSP 18445	Museum	5	2º 31'48"S, 67º 21'59.76"W
Omalonyx sp.	North	Amaña Lake, Japurá River Basin, Amazonas	MZSP 31874	Museum	5	2º 35'53.16"S, 64º 39'47.16"W
Omalonyx sp.	North	Boca do Acre, Amazonas	MZSP 31881-83	Museum	5	8º 45'37.44"S, 68º 53'52.8"W
Omalonyx sp.	North	Manaus, Amazonas	INPA 575, 598, 614, 620, 858, 1081; MZSP 31963	Museum	5	3º 6'6.84"S, 60º 15'0"W
O. pattersonae	North	Itacotiara, Manaus, Amazonas	ANSP 109524	Museum	5	_
Omalonyx sp.	North	Marchantaria Island, Amazonas	INPA 522	Museum	5	3º 14'23.28"S, 59º 56'15.36"W
	North					
O. geayi	North	Careiro Marchantaria Island, Amazonas	MCNZ 30548 (Arruda et al. 2016)	Literature	5	3º 15'14.11"S, 59º 58'19.09"W
Omalonyx sp.	North	Parana Uauaçu, Amazonas	INPA 532	Museum	5	4º 18'50.04"S, 62º 9'55.08"W

Table 2. Continued...

Species	Region	Locality	Record	¹Source	² Hydrographic Region	Coordinates
O.matheroni	North	Iranduba, Amazonas	LMSM 2270, 2276-77	Survey		3º 57'7.2"S, 60º 28'50.52"W
O.matheroni	North	Iranduba, Amazonas	INPA 551-2, 870	Museum	5	3º 57'7.2"S, 60º 28'50.52"W
O. geayi	North	Iranduba, Amazonas	INPA 1091 (Arruda et al. 2016)	Literature	5	3º 8'44.4768"S, 60º 15'26.6256"W
O. matheroni	North	Preto da Eva River, Amazonas	LMSM 2260, 2332-35	Survey	5	2º 31'47.64"S, 59º 38'17.52"W
O. matheroni	North	Preto da Eva River, Amazonas	INPA 1090 (Garcia et al. 2012)	Literature	5	2º 31'47.64"S, 59º 38'17.52"W
Omalonyx sp.	North	Preto da Eva River, Amazonas	INPA 1090 (Garcia et al. 2012)	Literature	5	2º 31'47.64"S, 59º 38'17.52"W
O. pattersonae	North	Preto da Eva River, Amazonas	LMSM 2260, 2332-35;	Survey	5	2º 31'47.64"S, 59º 38'17.52"W
O. pattersonae	North	Preto da Eva River, Amazonas	INPA 1090 (Garcia et al. 2012)	Literature	5	2º 31'47.64"S, 59º 38'17.52"W
Omalonyx sp.	North	Carauari, Amazonas	MCP 09184	Museum	5	5º 7'7.68"S, 67º 20'15.36"W
O. geayi	North	Carauari, Amazonas	INPA 1643; MCP 09183 (Arruda et al. 2016)	Literature	5	5º 7'7.68"S, 67º 20'15.36"W
O. matheroni	North		SMNH 1450 (Tillier 1981)	Literature		unespecified locality
O. matheroni	North	-	MHNG 53834.8 (Tillier 1981)	Literature		unespecified locality
Omalonyx sp.	North	Santarém, Pará	ZUECGAS 3313, 3215-3217; MZSP 31885	Museum	5	2ºS 45'52.56", 55ºW 56'16.8"
O. matheroni	North	Santarém, Pará	LMSM 2573-2579, 2583, 2646-49, 2663, 2670, 2761, 2765-67, 2789-94, 2835, 2849-76, 2910, 2930, 3234-36	Survey	5	2º 45'52.56"S, 55º 56'16.8"W
O. matheroni	North	Santarém, Pará	Lange de Morretes 1949; BMNH 1896.9.33 (Tillier 1981), Simone 2006	Literature	5	2º 45'52.56"S, 55º 56'16.8"W
O. matheroni	North	Alenquer, Pará	FMNH 29190 (Tillier 1981)	Literature	5	0º 32'10.68"S, 55º 41'56.4"W
Omalonyx sp.	North	Almerim, Pará	MZSP 31877	Museum	5	0º 17'56.4"N, 53º 53'37.68"W
Omalonyx sp.	North	Furo do Jurupari, Pará	MZSP 31879	Museum	5	2º 40'37.2"S, 53º 16'48"W
Omalonyx sp.	North	Lago Parú, Pará	MZSP 31959	Museum	5	1º 53'30.12"S, 55º 49'55.92"W
O. matheroni	North	Tucuruí, Pará	LMSM 2569, 2584-87, 2588, 2672, 2915-16	Survey	06/jul	3º 51'20.88"S, 49º 49'14.88"W
O. matheroni	North	Juruti, Pará	LMSM 3151-52, 3168	Survey	5	2º 37'21.72"S, 56º 13'16.32"W
O. matheroni	North	Belém, Pará	LMSM 3230-31	Survey	7	1º 14'26.52"S, 48º 27'33.84"W
O. matheroni	North	Cereja River, Bragança, Pará	LMSM 4037-4043	Survey	7	1º 20'49.2"S, 46º 45'42.84"W
O. pattersonae	North	Cereja River, Bragança, Pará	LMSM 4037-4043	Survey	7	1º 20'49.2"S, 46º 45'42.84"W
Omalonyx sp.	North	Curuá, Pará	ZUECGAS 3204-9, 3210-4, 3218	Museum	7	1º 50'45.24"S, 55º 6'47.52"W
O. matheroni	North	Ji-Paraná, Rondônia	LMSM3360, 3362, 3367	Survey	5	10º 27'43.92"S, 61º 45'25.56"W
O. matheroni	North	Madeira River, Porto Velho, Rondônia	LMSM 3347-48, 3358-59, 3364-65, 3372-76, 3403-04, 3440	Survey	5	8º 46'14.16"S, 63º 54'29.16"W
Omalonyx sp.	Northeast	Malvina District, Arari, Maranhão	MNRJ 6305; CMIOC 9519 (Cantanhede et al. 2014)	Museum	7	3º 27'30.96"S, 44º 46'30"W
Omalonyx sp.	Northeast	Amaral Village, Monção, Maranhão	CMIOC 9409 (Cantanhede et al. 2014)	Literature	7	3º 30'7.92"S, 45º 16'33.6"W
Omalonyx sp.	Northeast	Palmeirândia, Maranhão	Cantanhede et al. 2014	Literature	7	2º 41'34.08"S, 45º 15'0"W
Omalonyx sp.	Northeast	Pedro do Rosário, Maranhão	Cantanhede et al. 2014	Literature	7	2º 59'20.76"S, 45º 25'48"W
Omalonyx sp.	Northeast	Peri-Mirim, Maranhão	Cantanhede et al. 2014	Literature	7	2º 34'18.12"S, 44º 53'43.44"W
Omalonyx sp.	Northeast	Santa Terezinha District, Pinheiro, Maranhão	CMIOC 9452-53 (Cantanhede et al. 2014)	Literature	7	2º 30'55.08"S, 45º 48'7.2"W
Omalonyx sp.	Northeast	Santa Helena, Maranhão	Cantanhede et al. 2014	Literature	7	2º 25'33.96"S, 45º 22'59.52"W
Omalonyx sp.	Northeast	Porto Grande District, São Bento, Maranhão	CMIOC 9471 (Cantanhede et al. 2014)	Literature	7	2º 42'0"S, 44º 49'21.36"W
Omalonyx sp.	Northeast	São João Batista, Maranhão	Cantanhede et al. 2014	Literature	7	3º 27'36"S, 44º 45'32.04"W
Omalonyx sp.	Northeast	Vitória do Mearim, Maranhão	Cantanhede et al. 2014	Literature	7	3º 34'26.4"S, 44º 55'24.96"W
Omalonyx sp.	Northeast	Parnaíba, Piauí	LMSM 4558-64	Survey	7	2º 57'37.8"S, 41º 45'12.96"W
O. unguis	Northeast	Baturité, Ceará	MNRJ 12382	Museum	7	4º 22'49.44"S, 38º 51'10.08"W
O. matheroni	Northeast	Fortaleza, Ceará	LMSM 2027-29, 4579-4580	Survey	7	3º 47'10.32"S, 38º 31'38.64"W
Omalonyx sp.	Northeast	Fortaleza, Ceará	LMSM 2027-29, 4579-4580	Survey	7	3º 47'10.32"S, 38º 31'38.64"W
Omalonyx sp.	Northeast	Redenção, Ceará	CMIOC 5155	Museum	7	4º 14'57.48"S, 38º 45'56.52"W
Omalonyx sp.	Northeast	Sobral, Ceará	LMSM 4581-2	Survey	7	3º 42'54"S, 40º 20'58.92"W
O. matheroni	Northeast	Natal, Rio Grande do Norte	LMSM 2491, 2514, 2537, 2540-41, 3243	Survey	7	5º 48'11.88"S, 35º 13'44.4"W

Table 2. Continued...

Species	Region	Locality	Record	[1]Source	[2] Hydrographic Region	Coordinates
O. pattersonae	Northeast	Natal, Rio Grande do Norte	LMSM 2491, 2514, 2537, 2540-41, 3243	Survey	7	5º 48'11.88"S, 35º 13'44.4"W
O. matheroni	Northeast	Formosa Bay, Rio Grande do Norte	CMIOC 8569	Museum	7	6º 22'15.24"S, 35º 30'0"W
Omalonyx sp.	Northeast	João Pessoa, Paraíba	MZSP 41365	Museum	7	7º 9'59.04"S, 34º 52'10.92"W
O. matheroni	Northeast	Caruaru, Pernambuco	LMSM 2783, 2837, 2909	Survey	7	8º 10'48"S, 36º 9'54"W
O. matheroni	Northeast	Caruaru, Pernambuco	ANSPA18032- A18038 (Dutra-Clarke et al. 2001)	Literature	7	8º 10'48"S, 36º 9'54"W
O. matheroni	Northeast	Pernambuco	BMNH 1887.9.8.31 (Tillier 1981)	Literature	8	8º 28'56.64"S, 37º 46'21.72"W
Omalonyx sp.	Northeast	Tacaratu, Pernambuco	MZSP 31960	Museum	7	8º 57'24.48"S, 38º 45'54"W
Omalonyx sp.	Northeast	Dois Irmãos Zoo, Recife, Pernambuco	MZSP 31967, 47767-818, 48050	Museum	9	8º 6'0"S, 34º 56'51.36"W
Omalonyx sp.	Northeast	Maceió, Alagoas	MNRJ 32140	Museum	9	9º 31'12.36"S, 35º 42'37.08"W
O. geayi	Northeast	Satuba, Alagoas	ZMB 90832 (Arruda et. al. 2016)	Literature	7	9º 34'23.1924"S, 35º 49'31.8216"W
O. pattersonae	Northeast	Itabaiana, Sergipe	MCP 09276	Literature	9	10º 41'9.24"S, 37º 25'18.84"W
O. convexus	Northeast	Itabaiana, Sergipe	UFS 400, 403 (Jesus & Manso 2010)	Literature	9	10º 41'9.24"S, 37º 25'18.84"W
O. matheroni	Northeast	Tororó Dam, Salvador, Bahia	LMSM 3261-62	Survey	9	12º 59'42"S, 38º 30'22.32"W
Omalonyx sp.	Northeast	Tororó Dam, Salvador, Bahia	CMIOC 9865;MNRJ50523	Museum	9	12º 59'42"S, 38º 30'22.32"W
O. unguis	Northeast	Tororó Dam, Salvador, Bahia	Moricand 1836; Hidalgo 1870, 1872	Literature	9	12º 59'42"S, 38º 30'22.32"W
O. matheroni	Northeast	Vila Nova, Bahia	Lange de Morretes 1949	Literature	9	17º 30'14.04"S, 40º 30'28.8"W
Omalonyx sp.	Northeast	Uruçuca, Bahia	MZSP 18443	Museum	9	14º 30'58.32"S, 39º 13'35.76"W
Omalonyx sp.	Northeast	Campo Formoso, Bahia	MZSP 31880	Museum	8	10º 15'39.6"S, 40º 42'33.84"W
O. matheroni	Northeast	Prado, Bahia	LMSM 2914	Survey	9	17º 7'55.2"S, 39º 21'7.56"W
Omalonyx sp.	Northeast	Esplanada, Bahia	CMIOC 7973	Museum	9	11º 56'23.64"S, 37º 53'10.68"W
Omalonyx sp.	Northeast	Entre Rios, Bahia	CMIOC 8083	Museum	9	12º 43'26.4"S, 38º 25'22.8"W
Omalonyx sp.	Northeast	Wenceslau Guimarães, Bahia	CMIOC 8803	Museum	9	13º 37'49.44"S, 39º 37'36.48"W
O. matheroni	Southeast	Maracás, Bahia	MNRJ 14021, 32138	Museum	9	13º 29'50.64"S, 40º 33'11.16"W
Omalonyx sp.	Southeast	Maracás, Bahia	MNRJ 14021, 32138	Museum	9	13º 29'50.64"S, 40º 33'11.16"W
O. unguis	Southeast	Mocambinho, Jaíba, Minas Gerais	Oliveira & Almeida 2000. CMIOC 4307	Literature Museum	8	16º 6'53.64"S, 43º 58'28.92"W
O. unguis	Southeast	Mocambinho, Jaíba, Minas Gerais	CMIOC 4307	Museum	8	16º 6'53.64"S, 43º 58'28.92"W
O. matheroni	Southeast	Caratinga Biological Station, Caratinga, Minas Gerais	Arruda et al. 2006, Montresor et al. 2008	Literature	9	19º 43'21.36"S, 41º 48'21.96"W
O. matheroni	Southeast	Caratinga Biological Station, Caratinga, Minas Gerais	LMSM 711, 712, 714, 731-33, 744-45, 747-48, 763	Survey	9	19º 43'21.36"S, 41º 48'21.96"W
O. matheroni	Southeast	Caratinga Biological Station, Caratinga, Minas Gerais	MZSP 35387	Museum	9	19º 43'21.36"S, 41º 48'21.96"W
Omalonyx sp.	Southeast	Teófilo Otoni, Minas Gerais	MZSP 18442, 31886; MNRJ 18365	Museum	9	17º 42'55.08"S, 41º 23'24"W
O. matheroni	Southeast	Pampulha Lake, Belo Horizonte, Minas Gerais	LMSM 1378-87, 2383, 2516, 2525, 2527, 2529, 2536, 2787, 2918, 2929, 3153-55, 3162-63, 3166, 3186, 3208-10; MCP 09277-9,09192	Survey	8	19º 54'10.08"S, 43º 57'36.72"W
O. matheroni	Southeast	Rio Doce State Park, Marliéria, Minas Gerais	LMSM 1962-68, 2104-06, 2108; MNRJ 18365	Survey	9	19º 38'41.64"S, 42º 32'9.6"W
Omalonyx sp.	Southeast	Rio Doce State Park, Marliéria, Minas Gerais	MNRJ 18365	Museum	9	19º 38'41.64"S, 42º 32'9.6"W
O. matheroni	Southeast	Januária, Minas Gerais	LMSM 2526-27	Survey	8	15º 19'13.8"S, 44º 50'55.32"W
O. matheroni	Southeast	Nova Serrana, Minas Gerais	LMSM 713, 746	Survey	8	19º 51'8.64"S, 44º 58'27.84"W
O. matheroni	Southeast	Ipatinga, Minas Gerais	LMSM 3046	Survey	9	19º 26'20.76"S, 42º 36'14.4"W
O. matheroni	Southeast	Betim, Minas Gerais	LMSM 3079-81, 3097-99, 3101, 3105, 3147-48, 3164-65	Survey	8	19º 56'51"S, 44º 11'57.84"W
Omalonyx sp.	Southeast	Cachoeira Dourada, Minas Gerais	CMIOC 7098	Museum	10	18º 36'25.92"S, 49º 28'48.36"W

Table 2. Continued...

Species	Region	Locality	Record	[1]Source	[2] Hydrographic Region	Coordinates
Omalonyx sp.	Southeast	Ilha dos Frades District, Vitoria, Espírito Santo	MZSP 31965	Museum	9	20º 18'7.92"S, 40º 16'40.44"W
Omalonyx sp.	Southeast	Serra, Espírito Santo	LMSM 3027 (Donation from CMIOC)	Donation	9	20º 7'41.16"S, 40º 18'6.48"W
Omalonyx sp.	Southeast	Rio de Janeiro, Rio de Janeiro	MNRJ 32143; 9617	Museum	9	22º 55'24.24"S, 43º 26'51.36"W
O. unguis	Southeast	Rio de Janeiro, Rio de Janeiro	Lutz 1921	Literature	9	22º 55'24.24"S, 43º 26'51.36"W
O. unguis	Southeast	Rio de Janeiro, Rio de Janeiro	MNRJ 1916, 2199, 57865, 12378-79; 12381	Museum	9	22º 55'24.24"S, 43º 26'51.36"W
O. convexus	Southeast	Rio de Janeiro, Rio de Janeiro	MCZ unnumber (Parodiz 1963); MNHN, Gaudichaud coll. 1833 (Tillier 1981)	Literature	9	22º 55'24.24"S, 43º 26'51.36"W
O. matheroni	Southeast	Rio de Janeiro, Rio de Janeiro	LMSM 412-16, 3512-16 (Donation from CMIOC).	Donation	9	22º 55'24.24"S, 43º 26'51.36"W
O. unguis	Southeast	Araruama, Rio de Janeiro	MNRJ 12380	Museum	9	22º 45'16.92"S, 42º 17'36.24"W
Omalonyx sp.	Southeast	Parati, Rio de Janeiro	LMSM 419 (Donation from CMIOC)	Donation	9	23º 8'56.76"S, 44º 42'20.16"W
Omalonyx sp.	Southeast	Ilha Grande, Rio de Janeiro	LMSM 420-421 (Donation from CMIOC)	Donation	9	23º 9'7.56"S, 44º 13'44.04"W
Omalonyx sp.	Southeast	Macaé, Rio de Janeiro	LMSM 422-25, 431-32 (Donation from CMIOC). MNRJ30737	Donation	9	22º 17'42"S, 41º 58'29.64"W
Omalonyx sp.	Southeast	Raiz da Serra, Rio de Janeiro	LMSM 417, 426-28 (Donation from CMIOC)	Donation	9	22º 34'12"S, 43º 11'12.48"W
O. matheroni	Southeast	Duque de Caxias, Rio de Janeiro	LMSM 841, 857-58 (Donation from CMIOC)	Donation	9	22º 37'56.28"S, 43º 18'0"W
Omalonyx sp.	Southeast	Pendotiba, Niterói, Rio de Janeiro	CMIOC3877	Museum	9	22º 54'45"S, 43º 42'28.8W"
Omalonyx sp.	Southeast	São Fidélis, Rio de Janeiro	CMIOC 7515	Museum	9	21º 39'36"S, 41º 47'16.08"W
Omalonyx sp.	Southeast	Pinheiral, Rio de Janeiro	MCP 09187-8	Museum	9	22º 32'31.56"S, 43º 59'57.84"W
Omalonyx sp.	Southeast	Lagoa do Campelo, Campos, Rio de Janeiro	MNRJ 32139	Museum	9	21º 39'10.8"S, 41º 9'59.4"W
Omalonyx sp.	Southeast	Carmo, Rio de Janeiro	MNRJ1911	Museum	9	21º 54'7.56"S, 42º 34'8.4"W
O. convexus	Southeast	Campo dos Goytacazes, Rio de Janeiro	MNRJ 30735, 30785	Museum	9	21º 45'44.28"S, 41º 19'44.4"W
Omalonyx sp.	Southeast	Campos dos Goytacazes, Rio de Janeiro	MNRJ 30735, 30785	Museum	9	21º 45'44.28"S, 41º 19'44.4"W
Omalonyx sp.	Southeast	Santo Antônio de Pádua, Rio de Janeiro	MNRJ 32141	Museum	9	21º 33'24.84"S, 42º 11'33.72"W
Omalonyx sp.	Southeast	Itaguaí, Rio de Janeiro	MNRJ 32144	Museum	9	22º 50'29.4"S, 43º 49'12"W
Omalonyx sp.	Southeast	Cambuci, Rio de Janeiro	MNRJ 32148	Museum	9	21º 29'48.12"S, 41º 54'52.2"W
Omalonyx sp.	Southeast	Osasco, São Paulo	MZSP 17071	Museum	10	23º 31'44.04"S, 46º 47'20.76"W
Omalonyx sp.	Southeast	São José do Rio Preto, São Paulo	MZSP 18441, 31878; MNRJ 32137, 14024	Museum	10	20º 47'51"S, 49º 21'29.88"W
O. unguis	Southeast	São José do Rio Preto, São Paulo	MZSP 18441, 31878; MNRJ 32137, 14024	Museum	10	20º 47'51"S, 49º 21'29.88"W
Omalonyx sp.	Southeast	Caçapava, São Paulo	MZSP 31865	Museum	10	23º 6'12.6"S, 45º 42'48.24"W
Omalonyx sp.	Southeast	Barueri, São Paulo	MZSP 31875, 47749-51, 47819-50, 47879	Museum	10	23º 30'18.72"S, 46º 52'35.4"W
Omalonyx sp.	Southeast	Tremembé, São Paulo	MZSP 31876	Museum	9	22º 56'25.8"S, 45º 36'13.32"W
Omalonyx sp.	Southeast	Lins, São Paulo	MZSP 31966	Museum	10	21º 38'58.92"S, 49º 40'59.16"W
Omalonyx sp.	Southeast	Colômbia, São Paulo	MZSP 32664	Museum	10	20º 15'59.76"S, 48º 43'16.32"W
Omalonyx sp.	Southeast	Taubaté, São Paulo	MZSP 45129, 47718-26, 47738-47, 47858-67, 47907-10, 47939-82, 47995-8017, 48049, 48053	Museum	9	23º 52'48"S, 45º 30'7.92"W
Omalonyx sp.	Southeast	Paulínia, São Paulo	MZSP 47727-37, 47752-66	Museum	10	22º 44'51.72"S, 47º 8'41.64"W
Omalonyx sp.	Southeast	Americana, São Paulo	MZSP 47938	Museum	10	22º 43'23.52"S, 47º 17'20.04"W
Omalonyx sp.	Southeast	Santo André, São Paulo	MZSP 32774 (Eduardo et al. 2012)	Literature	10	23º 43'41.88"S, 46º 26'28.68"W
O. matheroni	Southeast	Santo André, São Paulo	MZSP 32774 (Eduardo et al. 2012)	Literature	10	23º 43'41.88"S, 46º 26'28.68"W
O. matheroni	Southeast	Ibitinga Powerplant, Ibitinga, São Paulo	MCP 09120 (Arruda et al. 2009)	Literature	10	21º 45'28.08"S, 48º 59'30.12"W
O. matheroni	Central West	Santa Rita do Novo Destino, Goiás	LMSM 3241-42	Donation	8	14º 52'35.4"S, 49º 33'46.8"W

Table 2. Continued...

Species	Region	Locality	Record	[1]Source	[2] Hydrographic Region	Coordinates
Omalonyx sp.	Central West	Passo da Lontra, Mato Grosso do Sul	MZSP 41206	Museum	10	19º 34’29.28”S, 57º 22’40.8”W
Omalonyx sp.	Central West	Três Lagoas, Mato Grosso do Sul	MZSP 47748	Museum	10	20º 26’19.68”S, 52º 12’41.04”W
O. unguis	Central West	Campo Grande, Mato Grosso do Sul	LMSM 2747	Survey	10	20º 54’47.16”S, 54º 14’58.92”W
O. unguis	Central West	Miranda, Mato Grosso do Sul	LMSM 2705-07, 2739-42, 2769, 2780-81, 2788, 2797-99, 2898-99, 2900-08, 2911-13, 2917	Survey	10	20º 7’12.36”S, 56º 35’52.8”W
O. unguis	Central West	Transpantaneira Road, Mato Grosso	MNRJ 9969	Museum	10	16º 22’30”S, 56º 40’12”W
O. unguis	Central West	Transpantaneira Road, Poconé, Mato Grosso	LMSM 2673; LMSM 429-430 (Donation from CMIOC).	Donation	10	16º 22’30”S, 56º 40’12”W
O. unguis	Central West	Cáceres, Mato Grosso	LMSM 3334	Survey	10	16º 31’26.4”S, 57º 50’16.8”W
O. unguis	Central West	Várzea Grande, Mato Grosso	LMSM 3344	Survey	10	15º 33’23.4”S, 56º 16’53.04”W
Omalonyx sp.	Central West	Barão de Melgaço, Mato Grosso	LMSM 433 (Donation from CMIOC). CMIOC 9565	Donation Museum	10	16º 49’14.52”S, 56º 19’19.2”W
Omalonyx sp.	Central West	Barão de Melgaço, Mato Grosso	CMIOC 9565	Museum	10	16º 49’14.52”S, 56º 19’19.2”W
Omalonyx sp.	Central West	Manso Lake, Mato Grosso	LMSM 784-86, 791 (Donation from CMIOC)	Donation	10	15º 26’20.4”S, 55º 11’9.96”W
Omalonyx sp.	Central West	Chapada dos Guimarães, Mato Grosso	LMSM 3032 (Donation from CMIOC)	Donation	10	15º 6’30.24”S, 55º 32’22.92”W
Omalonyx sp.	Central West	Nobres, Mato Grosso	LMSM 3028, 3030 (Donation from CMIOC)	Donation	10	14º 21’51.12”S, 55º 46’48”W
Omalonyx sp.	Central West	Santo Antônio do Leverger, Mato Grosso	LMSM 3029 (Donation from CMIOC)	Donation	10	16º 27’23.4”S, 55º 26’22.92”W
O. matheroni	Central West	Barra do Garças, Mato Grosso	MCP 09189	Museum	10	15º 21’34.56”S, 52º 29’50.28”W
O.matheroni	South	Paranaguá, Paraná	MZSP 18440 (Arruda et al. 2009)	Literature	12	25º 32’39.48”S, 48º 32’47.4”W
Omalonyx sp.	South	Paranaguá, Paraná	MZSP 18440 (Arruda et al. 2009)	Literature	12	25º 32’39.48”S, 48º 32’47.4”W
Omalonyx sp.	South	Sete Quedas, Guaíra, Paraná	MZSP 31884	Museum	12	24º 48’25.2”S, 54º 15’12.24”W
O. convexus	South	Curitiba, Paraná	LMSM 2667, 2975, 2990-91, 3003, 3008, 3011	Survey	12	25º 28’41.16”S, 49º 17’17.16”W
O. unguis	South	Foz do Iguaçu, Paraná	LMSM 3260	Survey	12	25º 28’15.24”S, 54º 28’56.28”W
O. unguis	South	Foz do Iguaçu, Paraná	MNRJ 32146, 32205	Museum	12	25º 28’15.24”S, 54º 28’56.28”W
O. unguis	South	Foz do Iguaçu, Paraná	MNRJ 32146, 32205	Museum	12	25º 28’15.24”S, 54º 28’56.28”W
O. convexus	South	Araranguá, Santa Catarina	LMSM 1522, 2988-89, 3001-02, 3005-07	Survey	12	28º 56’33.36”S, 49º 28’22.08”W
O. matheroni	South	Near to Tironi Park, Piçarras, Santa Catarina	LMSM 3942, 4024-4028	Survey	12	26º 45’56.88”S, 48º 42’38.88”W
O. convexus	South	São João do Sul, Santa Catarina	MCP 09271 (Agudo-Padron 2008)	Literature	12	29º 12’22.32”S, 49º 48’46.08”W
O. convexus	South	Camboriú, Santa Catarina	Agudo-Padron 2008	Literature	12	27º 42’43.2”S, 48º 42’32.4”W
O. convexus	South	Paulo Lopez, Santa Catarina	Agudo-Padron 2008	Literature	12	27º 57’53.28”S, 48º 45’39.6”W
O. convexus	South	Criciúma, Santa Catarina	Agudo-Padron 2008	Literature	12	28º 42’59.04”S, 49º 22’49.8”W
O. matheroni	South	Biguaçu River, Biguaçu, Santa Catarina	LMSM 3507, 3508	Survey	12	27º 28’27.84”S, 48º 40’9.84”W
O. convexus	South	Florianópolis, Santa Catarina	MCP 09190-1	Museum	12	27º 24’24.12”S, 48º 25’45.84”W
O. convexus	South	Palhoça, Santa Catarina	MCP 09227	Museum	12	27º 46’44.76”S, 48º 40’15.6”W
O. convexus	South	Porto Alegre, Rio Grande do Sul	Martens 1868, Heynemann 1868; Lange de Morretes 1949; Tillier 1981, Simone 2006; MCNZ: 8058, 1524, 31590, 4439, 5501, 35546 (Arruda & Thomé 2011)	Literature	12	31º 14’58.56”S, 51º 30’37.08”W
O. convexus	South	Porto Alegre, Rio Grande do Sul	MCP 02066, 08834, 08837-8, 08849, 08842-3, 08845, 09273-4	Museum	12	31º 14’58.56”S, 51º 30’37.08”W
O. convexus	South	Camaquã, Rio Grande do Sul	Parodiz 1963; MCP 8841 (Arruda & Thomé 2011)	Literature	12	30º 55’21.36”S, 51º 47’18.6”W

Table 2. Continued...

Species	Region	Locality	Record	[1]Source	[2] Hydrographic Region	Coordinates
Omalonyx sp.	South	Camaquã, Rio Grande do Sul	MCP 09272; MZSP 7539	Museum	12	30º 55'21.36"S, 51º 47'18.6"W
O. unguis	South	São Leopoldo, Rio Grande do Sul	MCP 10247, 10240-1, 10291; MZSP 31961,18439	Museum	12	29º 45'19.08"S, 51º 8'42.72"W
Omalonyx sp.	South	São Leopoldo, Rio Grande do Sul	MCP 10247, 10240-1, 10291; MZSP 31961,18439	Museum	12	29º 45'19.08"S, 51º 8'42.72"W
Omalonyx sp.	South	Pelotas, Rio Grande do Sul	MZSP 31964	Museum	12	31º 34'7.68"S, 52º 21'38.16"W
O. convexus	South	Rio Grande, Rio Grande do Sul	MCP 08836 (Arruda & Thomé 2011)	Literature	12	32º 13'10.92"S, 52º 24'15.48"W
O. convexus	South	Viamão, Rio Grande do Sul	LMSM 2650-51, 2666, 2668-69, 2704, 2759, 2762-63, 2768, 2895-97	Survey	12	30º 10'12"S, 50º 52'10.56"W
O. convexus	South	Viamão, Rio Grande do Sul	MCNZ 2506 (Arruda & Thomé, 2011)	Literature	12	30º 10'12"S, 50º 52'10.56"W
O. convexus	South	Arambaré, Rio Grande do Sul	LMSM 3009-10, 3021-23, 3149-50, 3167, 3175, 3187	Survey	12	30º 55'9.12"S, 51º 34'42.6"W
O. convexus	South	Cachoeira do Sul, Rio Grande do Sul	MCP 08840 (Arruda & Thomé 2011)	Literature	12	30º 12'22.32"S, 52º 59'11.04"W
O. convexus	South	Santa Maria, Rio Grande do Sul	MCP 08829-31 (Arruda & Thomé 2011)	Literature	12	29º 47'56.4"S, 53º 49'29.28"W
O. convexus	South	Novo Hamburgo, Rio Grande do Sul	MCP 08848 (Arruda & Thomé 2011)	Literature	12	29º 44'6.72"S, 51º 29'13.2"W
O. convexus	South	Mampituba Stream, Torres, Rio Grande do Sul	MCP 08832 (Arruda & Thomé 2011);	Literature	12	29º 35'29.76"S, 49º 57'36"W
O. convexus	South	Mampituba Stream, Torres, Rio Grande do Sul	LMSM 3932-3941	Survey		29º 35'29.76"S, 49º 57'36"W
O. convexus	South	Cachoeirinha, Rio Grande do Sul	MCP 09548 (Agudo Padron 2012); MCP 08839 (Arruda & Thomé 2011)	Literature	12	29º 55'15.96"S, 51º 56'56.4"W
O. convexus	South	Vacaria, Rio Grande do Sul	MCNZ 7559 (Arruda & Thomé 2011)	Literature	12	28º 22'14.88"S, 50º 55'24.96"W
O. convexus	South	Sapiranga, Rio Grande do Sul	MCNZ 2877 (Arruda & Thomé 2011)	Literature	12	29º 36'50.4"S, 50º 59'43.8"W
O. convexus	South	Guaíba, Rio Grande do Sul	MCNZ 6000 (Arruda & Thomé 2011)	Literature	12	30º 10'39.36"S, 51º 26'9.6"W
O. convexus	South	Triunfo, Rio Grande do Sul	MCNZ 8058 (Arruda & Thomé 2011)	Literature	12	29º 50'37.32"S, 51º 34'17.04"W
			MCP 06487	Museum		
O. convexus	South	Triunfo, Rio Grande do Sul	MCP 06487	Museum	12	29º 50'37.32"S, 51º 34'17.04"W
O. unguis	South	Imbé, Rio Grande do Sul	MCNZ 31969 (Arruda & Thomé 2011)	Literature	12	29º 55'50.16"S, 50º 7'55.2"W
O. convexus	South	Capão do Corvo, Canoas, Rio Grande do Sul	MCNZ 597 (Arruda & Thomé 2011)	Literature	12	29º 54'48.24"S, 51º 10'37.2"W
O. convexus	South	Taquara, Rio Grande do Sul	MCNZ 2610 (Arruda & Thomé 2011)	Literature	12	29º 40'15.6"S, 50º 45'47.52"W
O. convexus	South	Portão, Rio Grande do Sul	MCNZ 3264 (Arruda & Thomé 2011)	Literature	12	29º 42'36"S, 51º 14'56.76"W
O. convexus	South	Estrela, Rio Grande do Sul	MCP 08844 (Arruda & Thomé 2011)	Literature	12	29º 30'33.48"S, 51º 55'11.64"W
O. convexus	South	Eldorado do Sul, Rio Grande do Sul	MCP 08835	Museum	12	30º 46'58.8"S, 51º 29'54.24"W
Omalonyx sp.	South	São Borja, Rio Grande do Sul	MCP 09270; MCP 08828; MCP 08835	Museum	10	28º 44'46.68"S, 55º 47'50.28"W
O. convexus	South	Mampituba, Rio Grande do Sul	MCP 08833	Museum	12	29º 15'43.92"S, 50º 13'12"W
O. brasiliensis	South	Rio Grande do Sul	ZMB 45.913 (Arruda & Thomé 2008a).	Literature	10/dez	29º 36'18.24"S 53º 12'10.25"W unespecified locality
O. brasiliensis	South	Rio Grande do Sul	MNRJ 57927, 57955	Museum	10/dez	29º 36'18.24"S 53º 12'10.25"W unespecified locality

Note: **1**: Specimens from our malacological surveys in Brazil were deposited in LMSM and classified in the column source as "Survey"; Specimens that were donated by other researchers were deposited in LMSM and classified in the column source as "Donation"; **2**: Hydrographic region numbers are according to Figure 1 and to the metadata information system of the water resources national agency - ANA - of the Environment Ministry, Brazil and to Bonetto 1994).

Table 3. Records of *Omalonyx* in South America and Lesser Antilles based on literature data, institutional collections and our own malacological surveys.

Species	Country	Locality	[1]Record	[2]Source	[3]Hydrographic Region	Coordinates
O. unguis	Argentina	Rio Santiago, Buenos Aires	MACN 10218-1 (Hylton-Scott 1968); MACN 10208; 10268; MLP 31321 (Arruda & Thomé 2008b), Coscarelli & Vidigal 2011	Literature	10	34º 55’15.96”S, 57º 57’15.84”W
***Omalonyx* sp.**	Argentina	Punta Lara, Buenos Aires	MZSP 31866	Museum	10	34º 49’40.8”S, 57º 57’56.52”W
O. convexus	Argentina	Ezeiza, Buenos Aires	Camaggni coll. 1971 (Tillier 1981); MNHN unnumbered (Arruda & Thomé 2008b)	Literature	10	34º 51’13.68”S, 58º 31’22.44”W
O. convexus	Argentina	La Plata, Buenos Aires	LMSM 3273, 3237	Survey	10	34º 55’15.96”S, 57º 57’15.84”W
O. convexus	Argentina	Rio Santiago, Buenos Aires	MNHN, unnumbered; MACN 14472 (Arruda & Thomé 2008b)	Literature	10	34º 55’15.96”S, 57º 57’15.84”W
O. convexus	Argentina	Tucumán, Tucumán	Hylton-Scott 1968, 1971; SMNH 2507 (Tillier 1981); MNHN unnumbered (Arruda & Thomé 2008b)	Literature	11	26º 48’30.24”S, 65º 13’33.6”W
O. unguis	Argentina	Tucumán, Tucumán	MNRJ 4470	Museum	11	26º 48’30.24”S, 65º 13’33.6”W
O. convexus	Argentina	Rosario, Santa Fe	CM and MZUM unnumbered (Parodiz, 1963); MACN 26577 (Hylton-Scott & Lapuente 1968); USNM 124546 (Tillier 1981); ZMB28513 (Arruda & Thomé 2008b)	Literature	10	32º 56’40.2”S, 60º 39’10.8”W
O. convexus	Argentina	Rosario, Santa Fe	ANSP 64116	Museum	10	32º56’40.2”S, 60º 39’10.8”W
O. convexus	Argentina	Rio Negro, Chaco	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	27º 29’39.12”S, 58º 44’38.4”W
O. convexus	Argentina	Islands in Paraná River, near Santa Fe	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	31º 56’57.84”S, 60º 27’28.44”W
***Omalonyx* sp.**	Argentina	Lagunas Pampeanas, Buenos Aires	Tietze & Francesco 2012	Literature	10	_
O. unguis	Argentina	Locality between Santa Fe and Paraná, both in Argentina	Zilli et al. 2008	Literature	10	_
O. unguis	Argentina	Site within Chaco Province, on the west bank of the Paraná River at its confluence with Paraguay River	Franceschini et al. 2010	Literature	10	_
O. convexus	Argentina	Abra Vieja, Paraná River, Buenos Aires	CM and MACN unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	34º 34’43.32”S, 58º 48’15.12”W
O. convexus	Argentina	Perucho Verna Stream, Entre Rios	UF 159845 (Arruda & Thomé 2008b)	Literature	10	23º 34’25.32”S, 64º 8’31.2”W
O. convexus	Argentina	Vinalito, Jujuy	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	23º 34’25.32”S, 64º 8’31.2”W
O. convexus	Argentina	Santa Bárbara Ridge, Jujuy	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	23º 46’33.6”S, 64º 40’10.56”W
O. convexus	Argentina	Termas del Palmar, Salta	CM unnumbered (Parodiz 1963); MACN 27246 (Arruda & Thomé 2008b)	Literature	10	24º 14’54.96”S, 63º 27’12.96”W
O. convexus	Argentina	Orán, Salta	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	23º 8’9.96”S, 64º 19’19.92”W
O. unguis	Argentina	Resistencia, Chaco	MACN22931 (Arruda & Thomé 2008b)	Literature	10	27º 29’39.12”S, 58º 44’38.4”W
O. unguis	Argentina	Resistencia, Chaco	MZSP 14748	Museum	10	27º 29’39.12”S, 58º 44’38.4”W
O. convexus	Argentina	Guaycurú Stream, Chaco	CM unnumbered (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	26º 21’50.4”S, 60º 51’16.2”W
O. convexus	Argentina	Navarro, Buenos Aires	MACN 27241 (Hylton-Scott & Lapuente 1968)	Literature	10	34º 59’57.84”S, 59º 16’42.96”W

Table 3. Continued...

Species	Country	Locality	[1]Record	[2]Source	[3]Hydrographic Region	Coordinates
O. convexus	Argentina	Las Rosas, Buenos Aires	MACN 14860 (Hylton-Scott & Lapuente 1968), (Arruda & Thomé 2008b)	Literature	10	36º 26’56.4”S, 58º 50’27.96”W
O. convexus	Argentina	Chapadmalal, Buenos Aires	Hylton-Scott & Lapuente 1968; MACN 27242 (Arruda & Thomé 2008b)	Literature	11	38º 54’43.2”S, 58º 14’46.68”W
O. unguis	Argentina	Barca Grande, Buenos Aires	MACN 13996 (Hylton-Scott & Lapuente 1968, Arruda & Thomé 2008b); Coscarelli & Vidigal 2011	Literature	10	34º 14’54.24”S, 58º 44’45.6”W
O. unguis	Argentina	Guaycolec, Formosa	Hylton-Scott & Lapuente 1968, Coscarelli & Vidigal 2011	Literature	10	26º 11’49.2”S, 58º 10’32.88”W
O. unguis	Argentina	Formosa, Formosa	MLP 4567-1 (Arruda & Thomé 2008b), Coscarelli & Vidigal 2011	Literature	10	26º 11’49.2”S, 58º 10’32.88”W
O. unguis	Argentina	Manantiales, Corrientes	Hylton-Scott & Lapuente 1968, Coscarelli & Vidigal 2011	Literature	10	27º 55’19.2”S, 58º 59’42”W
O. unguis	Argentina	Oro River, Chaco	MACN unnumbered (Hylton-Scott & Lapuente 1968), Coscarelli & Vidigal 2011	Literature	10	26º 47’16.08”S, 59º 26’42”W
O. unguis	Argentina	Tragadero River, Locality between Barraqueras and Antequera, Chaco	Poi de Neiff et al. 1977	Literature	10	27º 29’39.12”S, 58º 44’38.4”W
O. unguis	Argentina	Catamarca, Catamarca	Cazzaniga 1985	Literature	11	28º 28’21”S, 65º 46’45.48”W
***Omalonyx* sp.**	Argentina	Ensenada, Buenos Aires	MLP 31321	Museum	10	34º 51’53.28”S, 57º 54’30.6”W
***Omalonyx* sp.**	Argentina	Atalaya, Buenos Aires	LMSM 23	Donation	10	35º 14’56.4”S, 57º 31’53.76”W
***Omalonyx* sp.**	Argentina	Esteros de Iberá, Corrientes	LMSM 16-22, 234-35	Donation	10	28º 20’24”S, 57º 22’56.64”W
O. convexus	Argentina	Ciudad Autónoma de Buenos Aires, Buenos Aires	LMSM 3239-40; ANSP 23465	Survey	10	34º 35’58.92”S, 58º 22’54.84”W
O. unguis	Argentina	Villafañe, Formosa	MLP 11878 (Arruda & Thomé 2008b)	Literature	10	26º 14’10.32”S, 59º 7’48”W
O. unguis	Argentina	Bahía Blanca, Buenos Aires	Cazzaniga 1985	Literature	11	38º 43’58.8”S, 62º 15’58.68”W
***Omalonyx* sp.**	Argentina	Misiones	MLP unnumbered (Gregoric et al. 2013)	Literature	10	27º 27’36”S, 54º 35’29.4”W
O. unguis	Argentina	Formosa	MLP 4567-1 (Arruda & Thomé 2008b)	Literature	10	24º 52’12”S, 60º 26’27.6”W
O. unguis	Argentina	Tigre, Buenos Aires	LMSM 734 (Arruda & Thomé 2008b)	Literature	10	34º 25’36.48”S, 58º 34’46.2”W
O. unguis	Argentina	Buenos Aires, Buenos Aires	ANSP 23465	Museum	10	34º 35’58.92”S, 58º 22’54.84”W
Omalonyx* sp.	Chile	Juan Fernandes	Odhner, 1922, Letelier et al. 2014	Literature	13	33º 46’23.52”S, 80º 46’33.6”W
Omalonyx* sp.	Chile	Valparaiso Region	ANSP 64117	Museum	13	32º 39’6.48”S, 71º 24’21.6”W
Omalonyx* sp.	Chile	Valparaiso Region Robinson Crusoe Island, Juan Fernandez Archipelago	ANSP 130450, A9768G	Museum	13	34º 10’12”S, 80º 49’59.88”W
***Omalonyx* sp.**	Colombia	–	FMNH unnumbered (Vera-Ardila 2008)	Literature	5	0º 14’8.88”S, 72º 30’0”W
O. matheroni	Ecuador	Limoncocha Lake	Hermann & Dundee 1967; FMNH 157321(Tillier 1981)	Literature	5	0º 23’57.12”S, 76º 36’33.12”W
O. geayi	Ecuador	Limonconcha, Sucumbios	FMNH 328261 (Arruda et. al 2016)	Literature	5	0º 24’41.91”S, 76º 37’31.36”W
O. matheroni	French Guiana	Kourou Bridge	Tillier coll. 4.78 (Tillier 1981); MNHN (Arruda & Thomé 2008a)	Literature	4	5º 9’39.24”N, 52º 38’57.48”W
O. geayi	French Guiana	Route Cayene-Kourou, Kaw Swamps	MNHN, Tillier coll. 29.4.1977 (Tillier 1980); MNH. (Tillier 1981), (Arruda et al. 2016)	Literature	4	4º 30’52.92”N, 52º 39’57.6”W

Table 3. Continued...

Species	Country	Locality	^{1}Record	^{2}Source	^{3}Hydrographic Region	Coordinates
O. geayi	French Guiana	Kaw Swamps	LMSM 3204, 3222-24, 3226	Survey	4	4º 30'52.92"N, 52º 39'57.6"W
O. matheroni	Guyana	Demerara	Gibbson 1879; BMNH 1930.5.14.6.11 (Tillier 1981)	Literature	4	6º 37'53.04"N, 58º 38'27.6"W
O. matheroni	Guyana	Manikol Swamps (upper Tuyuni)	BMNH 1936.12.2.27.29 (Tillier 1981)	Literature	4	5º 57'18"N, 57º 41'26.16"W
***Omalonyx* sp.**	Guyana	Georgetown	CMIOC 455	Museum	4	6º 47'52.8"N, 58º 9'19.08"W
O. matheroni	Lesser Antilles, Trinidad and Tobago,	Port Spain	ANSP A1173	Museum	_	10º 39'44.28"N, 61º 31'48"W
O. matheroni	Lesser Antilles, Guadeloupe	Pointe- à - Pitre	MNHN (Arruda & Thomé, 2008a)	Literature	_	16º 13'59.88"S, 61º 31'59.88"W
O. matheroni	Lesser Antilles, Guadeloupe	Pico Spring	LMSM 379-85, 2186-95 donated by Dr. J. P. Poitier collection	Donation	_	16º 13'59.88"S, 61º 31'59.88"W
O. matheroni	Lesser Antilles, Guadeloupe	Céligny and other localities	Lesson 1838, Tillier 1981 (Dr. J. P. Poitier collection)	Literature	_	16º 13'59.88"S, 61º 31'59.88"W
O. matheroni	Lesser Antilles, Guadeloupe	Vallet Pond	ANSP A22070	Museum	_	16º 13'59.88"S, 61º 31'59.88"W
O. matheroni	Lesser Antilles, Santa Lucia	-	Hermann & Dundee 1967	Literature	_	13º 54'7.2"N, 60º 58'42.96"W
O. pattersonae	Lesser Antilles, Antigua		Hermann & Dundee 1967; MZUM, (Patterson 1971); USNM 272284, 215047; BMNH 95.1.29.17 (Tillier 1981)	Literature	_	17º 42'14.4"N, 61º 48'25.2"W
O. unguis	Lesser Antilles, Antigua and Barbuda		ANSP 71975	Museum	_	17º 42'14.4"N, 61º 48'25.2"W
O. matheroni	Lesser Antilles, Trinidad and Tobago		USNM 162058	Museum	_	10º 26'44.52"N, 61º 16'17.04"W
O. matheroni	Lesser Antilles, Trinidad		BMNH 1950.618.407; LMNH (Tillier 1980, 1981)	Literature	_	10º 26'44.52"N, 61º 16'17.04"W
O. matheroni	Lesser Antilles, Trinidad and Tobago	Tunapuna-Piarco, Saint George East	USDA 100453 (Arruda et al. 2016)	Literature		10º 37'29.7336"N, 61º 13'6.5316"W
O. matheroni	Lesser Antilles, Monserrat	St. Peter	ANSP A23921	Museum	_	16º 44'18.24"N, 62º 12'31.68"W
***Omalonyx* sp.**	Lesser Antilles, Antigua and Barbuda	-	USNM 460663, 151347	Museum	_	17º 42'14.4"N, 61º 48'25.2"W
O. unguis	Paraguay	Paraná River near Corrientes and Moxos (Bolivia)	d'Orbigny 1835, 1837; Tillier (1981)	Literature	10	27º 57'57.6"S, 58º 14'35.52"W
O. unguis	Paraguay	Asunción	CM unnumber (Parodiz 1963); MACN 19968 (Arruda & Thomé 2008b)	Literature	10	25º 16'32.88"S, 57º 38'16.08"W
O. unguis	Paraguay	Asunción	LMSM 3436	Survey	10	25º 16'32.88"S, 57º 38'16.08"W
O. unguis	Paraguay	Pilar, Ñe	LMSM 3341	Survey	10	26º 51'11.88"S, 58º 17'44.88"W
O. convexus	Paraguay	San Bernardino	CM unumber (Parodiz 1963)	Literature	10	25º 16'46.92"S, 57º 17'7.08"W
O. unguis	Paraguay	Villa Rica	MCZ unumber (Parodiz 1963), (Arruda & Thomé 2008b)	Literature	10	25º 49'26.4"S, 56º 24'18"W
O. unguis	Paraguay	Puerto Guaraní	MACN 18181 (Hylton-Scott & Lapuente 1968), Coscarelli & Vidigal 2011	Literature	10	21º 17'20.4"S, 57º 55'26.4"W
***Omalonyx* sp.**	Paraguay	Riacho Nepengue, Concepción	INPA 485	Museum	10	22º 27'23.76"S, 57º 35'19.68"W
O. unguis	Paraguay	Paso de Patria, Ñe	LMSM 3434, 3350	Survey	10	27º 14'52.08"S, 58º 32'20.04"W
***Omalonyx* sp.**	Peru	Tarapoto, San Martín Morales, San Martín	MZSP 43266; USMN 612118 (Tillier 1981)	Literature	5	6º 11'6.36"S, 76º 28'44.4"W
O. matheroni	Peru	San Martín	MCP 08850	Museum	5	6º 25'30.72"S, 76º 49'51.24"W

Table 3. Continued...

Species	Country	Locality	^{1}Record	^{2}Source	^{3}Hydrographic Region	Coordinates
O. unguis	Peru	Lake Yarinacocha, Ucayali	Ramirez, 1991, Ramirez et al. 2003	Literature	5	8º 18’29.88”S, 74º 36’7.2”W
O. matheroni	Suriname	Zanderij	RMNH 15.6.67 (Tillier, 1981)	Literature	4	5º 27’21.24”N, 55º 12’39.6”W
O. matheroni	Suriname	Belwaarde	RMNH 2.9.69 (Tillier 1981)	Literature	4	5º 51’6.12”N, 55º 52’55.2”W
O. matheroni	Suriname	Paramaribo	RMNH 6.68 (Tillier 1981)	Literature	4	5º 51’7.2”N, 55º 12’13.32”W
O. geayi	Suriname	Paramaribo	FLMNH 463461 (Arruda et al. 2016)	Literature	4	5º 51’7.2”N, 55º 12’13.32”W
O. matheroni	Suriname	Brokobakka, Brokopondo	RMNH unnumbered (Tillier 1981)	Literature	4	3º 53’22.56”N, 55º 34’28.56”W
O. unguis	Uruguay	Fuente Salto, close to Salto city	Olazarri 1979	Literature	10	31º 23’31.2”S, 57º 49’55.2”W
Omalonyx **sp.**	Uruguay	Montevideo	MZSP 31873	Museum	10	34º 54’43.2”S, 56º 9’52.2”W
O. convexus	Uruguay	Montevideo	ANSP 70261	Museum	10	34º 54’43.2”S, 56º 9’52.2”W
O. unguis	Uruguay	Near from Carrasco Stream, Montevideo	Scarabino 2003	Literature	10	34º 54’43.2”S, 56º 9’52.2”W
O. convexus	Uruguay	Miguelete Stream, Prado, Montevideo	Scarabino 2003	Literature	10	34º 54’43.2”S, 56º 9’52.2”W
O. convexus	Uruguay	Canelones	MNHNM8691 (Arruda & Thomé 2008b)	Literature	10	34º 43’29.28”S, 55º 57’34.56”W
O. unguis	Uruguay	Colonia, Uruguai River in front of Punta Gorda	MNHNM 3368 (Arruda & Thomé 2008b)	Literature	10	33º 57’7.56”S, 58º 21’36”W
O. matheroni	Venezuela	Caracas	Martens 1873, Tillier 1981, Arruda et al. 2009	Literature	2	10º 28’43.68”N, 66º 54’12.24”W
O. matheroni	Venezuela	Laguna de Ramón Coronel, near Bejuma	Baker 1925, 1926; Martinez 1993	Literature	3	9º 50’47.4”N, 68º 12’52.92”W
O. pattersonae	Venezuela	Lake Valencia, Carabobo	Martinez 1993	Literature	2	10º 9’13.32”N, 67º 53’40.92”W
O. pattersonae	Venezuela	Zuata Dam, Aragua	Martinez 1993	Literature	2	10º 11’39.84”N, 67º 23’40.56”W
O. matheroni	Venezuela	Santa Elena de Uiarém, Bolívar	LMSM 2271-75, 2336-372353-54, 2356-57, 2372-73, 2377, 2378-79, 2522-24	Survey	5	4º 35’43.08”N, 61º 6’10.08”W
O. pattersonae	Venezuela	Lake Tacarigua	ANSP 161142	Museum	2	10º 48’10.8”N, 65º 48’21.24”W
O. pattersonae	Venezuela	-	ANSP 140961	Museum	-	unespecified locality
Omalonyx **sp.**	Venezuela	Amacuro Delta	MCP 09193	Museum	2	9º 29’31.2”N, 62º 31’22.8”W
O. unguis	Bolivia	Santa Cruz de la Sierra	MZUM unnumbered (Parodiz 1963)	Literature/	5	17º 7’59.88”S, 63º 55’12”W
O. pattersonae	Bolivia	Santa Cruz de la Sierra	MCP 09185	Museum	5	17º 7’59.88”S, 63º 55’12”W
O. geayi	Bolivia	Santa Cruz	MNKMO 7 (Arruda et al. 2016	Literature	10	19º 7’45.0912”S, 61º 41’57.804”W
Omalonyx **sp.**	Bolivia	-	ANSP A1826c	Museum	5	17º 7’59.88”S, 63º 55’12”W
O. unguis	Lesser Antilles, Guadeloupe		ANSP 23463	Museum	_	16º 15’47.52”N, 61º 33’10.8”W
O. matheroni	Lesser Antilles, Montserrat	Daly River, Saint Peter	ANSP A23919	Museum	_	16º 44’18.24”N, 62º 12’31.68”W
O. matheroni	Lesser Antilles, Montserrat		ANSP 144327	Museum	_	16º 44’13.92”N, 62º 11’33.36”W
O. matheroni	Lesser Antilles, Monserrat	Runaway Ghaut, Saint Peter	ANSP A23917-18, A23920, 466625, A23921	Museum	_	16º 45’27”N, 62º 13’12”W
O. matheroni	Lesser Antilles, Montserrat	Woollands, Saint Peter	ANSP A23921	Museum	_	16º 44’18.24”N, 62º 12’31.68”W

Note: **1**: Museum abbreviation is according to Table 1; **2**: Specimens from our malacological surveys were deposited in LMSM and they were classified in the column source as “Survey”; Specimens that were donated by other researchers were deposited in LMSM and they were classified in the column source as “Donation”. **3**: Hydrographic basin numbers are according to Figure 1 and to the metadata information system of the water resources national agency - ANA - of the Environment Ministry, Brazil and to Bonetto 1994); * Specimens from Chile that were previously identified as *O. gayana*, however, Tillier 1981 considered that this species belongs to the genus *Succinea.*

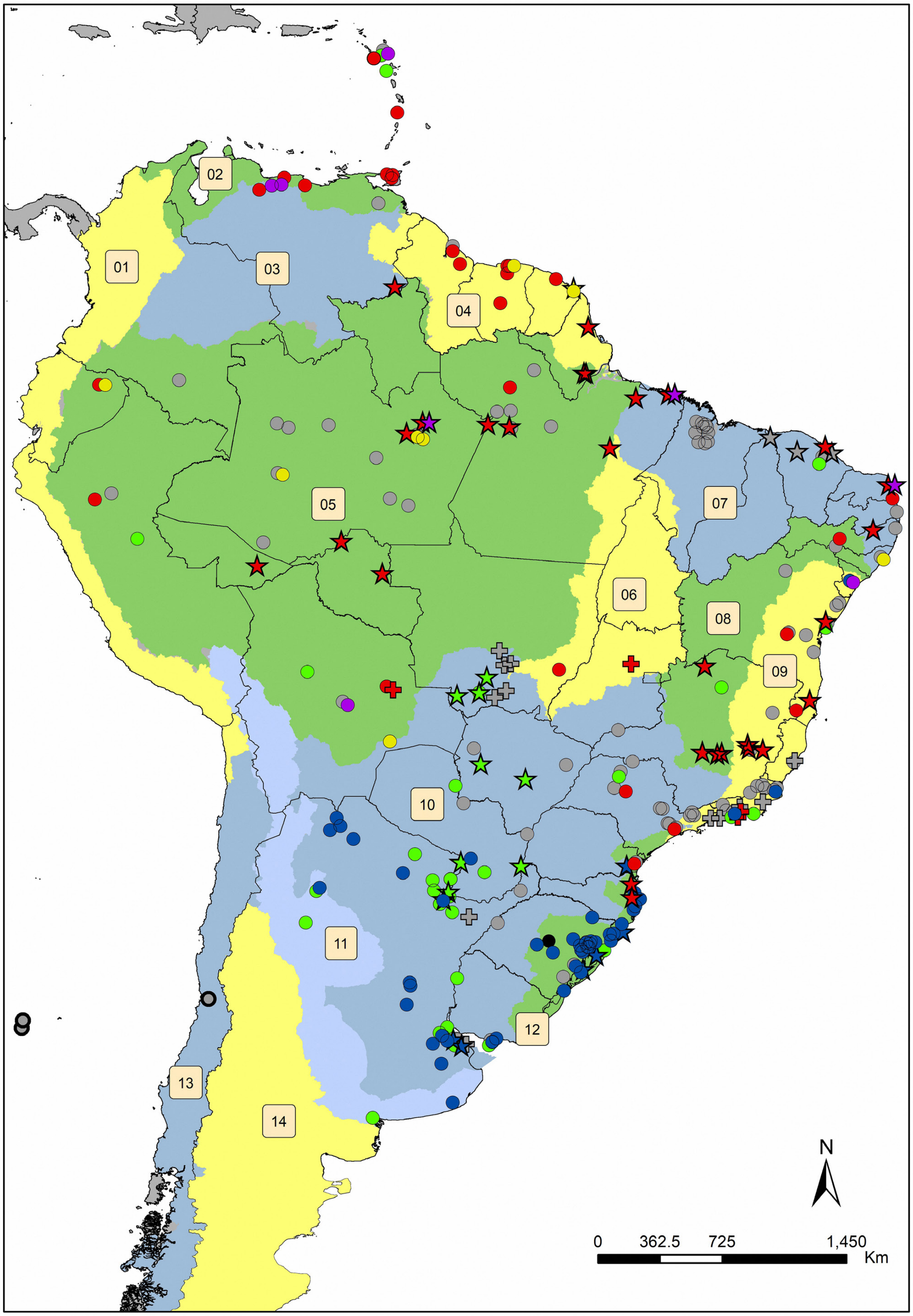

Figure 1. Updated distribution of the genus *Omalonyx* across Neotropical region. The coloured areas represent different hydrographic regions and their respective numbers. The solid black line indicates the borders among South American countries and within Brazilian administrative regions. The species records were represented by colours dots: red = *O. matheroni*; blue = *O. convexus*; green = *O. unguis*; yellow = *O. geayi*, purple = *O. pattersonae*, black = *O. brasiliensis*; gray = do not identified until species level, or *Omalonyx* sp. and gray circle with black border = Specimens from Chile that were previously identified as *O. gayana*. The stars represents the records made in our surveys, the circles represents literature or museums data and the cross symbol represents the samples that were donated by other researchers. Details about each sample are on tables 2 and 3. Hydrographic regions are indicated by numbers: 1) Pacific Coast; 2) Caribbean sea; 3) Orinoco River; 4) Amapá-Esequibo; 5) Amazon River; 6) Tocantins River; 7) North Atlantic; 8) São Francisco River; 9) Eastern Atlantic; 10) Del Plata; 11) Border strip of the Brasilica (North) and the Chilean-Patagonian (South West) Subregion; 12) Eastern; 13) Chilean-Patagonian Subregion of the Pacific Versant; 14) Chilean-Patagonian Subregion of the Atlantic Versant.

Table 4. Bioclimatic variables used in models development.

Variable	Description
Alt	Altitude
bio1	Annual mean temperature
bio2	Mean diurnal range (monthly mean, T° max-T° min)
bio3	Isothermality (bio2/bio7) x 100
bio4	Temperature seasonality (standard deviation x 100)
bio5	Maximum temperature of warmest month
bio6	Minimum temperature of coldest month
bio7	Temperature annual range (bio5-bio6)
bio8	Mean temperature of wettest quarter
bio9	Mean temperature of driest quarter
bio10	Mean temperature of the warmest quarter
bio11	Mean temperature of coldest quarter
bio12	Annual precipitation
bio13	Precipitation of wettest month
bio14	Precipitation of driest month
bio15	Precipitation seasonality (coefficient of variation)
bio16	Precipitation of wettest quarter
bio17	Precipitation of driest quarter
bio18	Precipitation of the warmest quarter
bio19	Precipitation of the coldest quarter

To minimize the spatial autocorrelation of the bioclimatic variables and to determine which variables contributed more to the development of the model, a principal component analysis (PCA) was carried out in the areas of *Omalonyx* species occurrence. The resulting rasters were used to generate a potential distribution model for South America. The input parameters followed the MaxEnt (version 3.3.3k) (Phillips et al. 2004) default options except for the threshold rule, which was set as "minimum training presence". The resulting models were processed and reclassified using ArcGIS. A binary map (absence-presence) for the potential distribution of a species was generated considering the average map that represents the induced and adjusted habitat of each species.

Results

1. Literature review

Inferences on the distribution of *Omalonyx* species have been made since its description, and it was assumed to be throughout South America east of the Andes (d'Orbigny 1837). This first inference was based on records from Bolivia and Paraguay (d'Orbigny 1835a, 1837). However, at that time (1837), the name *Omalonyx* was used as the subgenus *Succinea* (*Omalonyx*) *unguis*. Later, *Omalonyx* was used at the genus level (Herrmannsen 1849). In addition to the publications of d'Orbigny other species were described at that time as showed below. In the next century, Patterson (1971) extended the distribution of the genus to several West Indies islands and Central America. However, the basis for this conclusion was not clearly demonstrated. After that, Tillier (1981) demonstrated the occurrence of *Omalonyx* species in all South America east of the Andes and the Lesser Antilles. His conclusion was based on preserved specimens from malacological collections that did not include significant sampling material from Brazil, although there were already records on the presence of *Omalonyx* spp. in the country years before (e.g., Moricand 1836, Gibbons 1879). In fact, Tillier (1981) studied animals from only eight localities in Brazil, and four of these could not be clearly located and were excluded from his study. In this work, Tillier also discusses the origin and evolution of South American and Juan Fernandéz Islands (Chile) succineids. He suggests that the southeast region of Brazil is the center of *Omalonyx* radiation once three species can be found in this region (*O. unguis*, *O. matheroni* and *O. brasiliensis*). Details on the records of *Omalonyx* occurrence in the Neotropics are given below in the subsections "*Omalonyx* in Brazil" and "*Omalonyx* in other South American countries and Lesser Antilles".

1.1. Literature review: Omalonyx *in Brazil*

The first record of *Omalonyx* in Brazil was made in the 19th century in the Northeastern region (Moricand 1836) on a lake in Bahia State named "*la Digue*" or "*le Baril*". The specimens were identified as *O. unguis*. Hidalgo (1869) also identified as *O. unguis* specimens collected by Paz and Martinez in Bahia State. Two years later, the same author (Hidalgo 1872) stated that Paz and Martinez collected those specimens at "*Lago Dique*" (Dike Lake), Salvador, Bahia. We suppose that the specimens cited by Moricand (1836) and Hidalgo (1869, 1870) were collected in an urban lake known since the colonial period as "*Dique do Tororó*". Gibbons (1879) identified two different species of *Omalonyx* in Bahia State, *O. unguis* and *O. matheroni*. However, no other information about the locality was provided, and the morphology described in Gibbons (1879) is not compatible with *O. unguis*; thus these records were not included in Table 2. *Omalonyx convexus* was described by Martens (1968) as *Succinea convexa* from specimens collected in Porto Alegre, Rio Grande do Sul State, in southern Brazil (type locality), and it was later included in the genus *Omalonyx* (Arruda & Thomé 2008b). The map provided by Arruda and Thomé (2008b) shows overlapping distribution of *O. convexus* and *O. unguis* in the Argentinean region of the Paraná River basin (in Figure 1 the Paraná River basin is included in the Del Plata hydrographic region) and adjacent localities in Uruguay. *Omalonyx brasiliensis* was described by Simroth (1896) based on specimens collected by H. von Ihering in Rio Grande do Sul State.

In the 20th century, specimens of *Omalonyx* infected by *Leucochloridium*, an avian trematode, were found by Lutz (1921) in the surroundings of the institute where he used to work in Rio de Janeiro city, Rio de Janeiro State. The author also reported that this slug is abundant in the north of Brazil. This trematode was also found in birds from Mato Grosso and used in the experimental infection of *Omalonyx* (Travassos 1928). Both of these works have a parasitological focus, and the specific identification of the slug was not provided. Haas (1939) registered *O. unguis* for the State of Pernambuco but did not specify the locality. Parodiz (1957) recorded *O. unguis* in Brazil but did not include the locality. He also mentioned the occurrence of *O. unguis* in Porto Alegre (Rio Grande do Sul State) and observed its similarity to *O. convexus*. Based on specimens from the MCZ (lot number unavailable), Parodiz (1963) registered the occurrence of *Omalonyx* in Rio de Janeiro (*O. unguis*) and Camaquã, Rio Grande do Sul. The specimens from Camaquã were later identified as *O. convexus* (Arruda & Thomé 2008b). Lange de Morretes (1949) recorded *O. brasiliensis* and *O. unguis* in Vila Nova (Bahia State) and *O. matheroni* in Santarém (Pará State). In a taxonomical review using specimens from institutional collections, Tillier (1981) studied animals from eight localities in Brazil. Three of them were easily identified: Santarém and Alenquer in Pará State, and Rio de Janeiro in Rio de Janeiro State. The other four localities, due to insufficient labeling information, could not be precisely located. Two of them were only superficially specified: "Brazil" Pernambuco State and Praya (=Praia) Grande (Rio de Janeiro State). The other two localities could not be identified: Santa Amélia, Amazonas and "Makthlawara" (BMNH 1929.10.2.6.22). This author also mentioned the occurrence of *Omalonyx* in Porto Alegre, Rio Grande do Sul State, and cited Martens (1868) and Simroth (1896), who also recorded *O. brasiliensis* in this State (Tillier 1981). The distribution of *Omalonyx* in Brazil was revisited by two authors whose results were similar to those of Lange de Morretes (1949). First, Salgado & Coelho (2003) reported some species of the genus in a review about Brazilian land snails but did not cite any locality. After that, Simone (2006) cited the occurrence of *O. brasiliensis* in Rio Grande do Sul State and considered the occurrence area for the genus *Omalonyx* to be from Bolivia to Patagonia. Several other records were made in Brazil, including Minas Gerais (Oliveira & Almeida 2000, Arruda et al. 2006, Montresor et al. 2008) and São Paulo State (Arruda et al. 2009, Eduardo et al. 2012) in the southeast; Paraná (Arruda et al. 2009, Coscarelli & Vidigal 2011), Rio Grande do Sul (Arruda & Thomé 2008a, b, Arruda & Thomé 2011, Maltchik et al. 2010) and Santa Catarina State (Agudo-Padron 2008, 2012) in the south; Mato Grosso and Mato Grosso do Sul State (Coscarelli & Vidigal 2011) in the central west; Pernambuco (Dutra-Clarke et al. 2001), Sergipe (Jesus & Manso, 2010) and Maranhão State (Cantanhede et al. 2014) in the northeast; and Amazonas State in the North (Pimpão 2007, Garcia et al. 2012). Coscarelli & Vidigal (2011) investigated the distribution of *O. unguis* in Brazil and included new records for this species. Recently, new records for *O. geayi* extended the distribution range of this species in northern South America including Brazil (Arruda et al. 2016). The presence of *Omalonyx* was recorded in several Brazilian States representing all geographic regions (Table 2). All data and details (i.e., species and coordinates) on species distribution in Brazil were included in Table 2 and are shown in Figure 1.

1.2. Literature review: Omalonyx *in other South American countries and Lesser Antilles*

Several South American and Lesser Antilles countries are included in the distribution area of the genus *Omalonyx* (Figure 1, Table 3). The occurrence of *O. unguis* in Paraguay and Bolivia was reported by d'Orbigny (1835a, b, 1837). On this occasion, the author did a complete textual description of the external appearance of the animal and specified the collection sites: the flooded margins of Paraná River, near Corrientes, and the swamps of Moxos Province in Bolivia. Corrientes is a city on the riverbank of the Paraná River in Argentina, very close to the frontier with Paraguay, which is defined by the Paraná River itself. Later, Asunción, in Paraguay, was considered to be the type locality for *O. unguis* (Arruda & Thomé 2008b).

Omalonyx matheroni was described from individuals collected in Guadeloupe, Lesser Antilles (Potiez & Michaud 1835). Tillier (1981) studied specimens he collected or borrowed from institutional collections that were from several localities including the Lesser Antilles, Guiana, Suriname, French Guyana, Ecuador, Paraguay, Argentina and Peru (Table 3). *Omalonyx* occurrence in South American countries has been well documented by several authors: Argentina (Arruda & Thomé 2008a, b); Brazil (see above the topic geographic distribution of *Omalonyx* in Brazil); Colombia (Vera-Ardila 2008); Uruguay (Olazarri 1979, Scarabino 2003, Arruda & Thomé 2008b.); French Guiana (Tillier 1981, Arruda et al. 2016); Ecuador (Hermann & Dundee 1967, Tillier 1981, Arruda et al. 2016); Suriname (Tillier 1981, Arruda et al. 2016) Venezuela (Martens 1873, Baker 1925, 1926, Escarbassiere 1993); Guyana (Gibbons 1879, Tillier 1981); Peru (Tillier 1981, Ramirez, 1991; Ramirez et al. 2003) and Paraguay (Hylton-Scott & Lapuente 1968). Parodiz (1963) listed *Omalonyx* specimens from Paraguay, Argentina and Bolivia that were deposited in different institutional collections. Cazzaniga (1985), based on a bibliographic review, concluded that the known distribution area for *O. unguis* is southern Brazil, northern and eastern Argentina, Uruguay, Paraguay, and Bolivia and that the presence of *O. unguis* in the west of Argentina is known only in Tucumám (Hylton-Scott 1971) and Catamarca Provinces. The latter represents a new record made by Cazzaniga (1985) himself. Arruda & Thomé (2008b) showed that the distributions of *O. convexus* and *O. unguis* overlap in the Argentinean area of the Paraná River hydrographic region and adjacent localities in Uruguay. Gutiérrez Gregoric et al. (2013) recorded the occurrence of *Omalonyx* sp. in Misiones Province, Argentina using data from the La Plata Museum Mollusk Collection, literature and his own field work. For further literature data about *Omalonyx* occurrence in Argentina see Table 3. There are some records of *Omalonyx* in Chile (Odhner 1922, Letellier et al. 2003, 2014), and some of them are recent. However, in 1981, Tillier studied specimens from Chile that were identified as *O. gayana* and stated that, despite its limaciform shape, the species belongs to the genus *Succinea*. The recent records from Chile (Letellier et al. 2003, 2014) do not include a description of the specimens nor any discussion on this taxonomic issue related to Chilean succineids. Thus, we suppose that the specimens are in fact *Succinea gayana*.

The distribution of the genus in the Lesser Antilles is also well documented. *Succinea haliotidea* Mittre 1841 was found in Martinique by Mittre (Tillier 1981). However, Tillier (1981) did not include this species in the genus *Omalonyx*, and indicates that they do not even belong to the family Succineidae once neither he nor Fischer found any *Omalonyx* in Martinique. Therefore, we did not include Martinique within the occurrence area of *Omalonyx*. Hermann & Dundee (1967) studied *Omalonyx* from Sta. Lucia and Antigua, both in the Lesser Antilles. Other authors have also worked in this region. Patterson (1971) and Tillier (1981) studied specimens from Antigua, and Robinson et al. (2009) recorded the occurrence of *Omalonyx* in several localities in Dominica. Arruda et al. (2016) showed new records of *O. geayi* and extended the range of distribution of this species in South America to include Suriname, Ecuador, and Bolivia. All references and localities are in Table 3.

2. Records from institutional collections

Based on data of Brazilian institutional collections obtained through literature, museum databases or onsite research, we verified 21 records for the genus in the North region of the country, 28 in the Northeast, four in the Central West, 33 in the Southeast and 31 in the South (Table 2). Specimens were found in eight Brazilian institutional collections (INPA, MCP, MCNZ, MZSP, MNRJ, UFS, ZUECA and CMIOC). Among the institutional collections that were visited, two of them did not present specimens belonging to the genus (MIRR and MPEG) in (Table 1). Brazilian specimens of *Omalonyx* were also registered in six foreign institutional collections (ANSP, FMNH, BMNH, ZMB, MNHN, and MCZ). We also investigated the presence of *Omalonyx* in other South American countries and Lesser Antilles using engines for virtual searching in databases from Brazilian (CMIOC, MCP, MZSP, MNRJ) and foreign institutional collections (ANSP, BMNH, USNM, CM, MACN, MLP) (Table 3).

3. Malacological surveys in Brazil

Specimens of *Omalonyx* were sampled across Brazilian hydrographic regions, and the sites were classified above according to the presence or absence of *Omalonyx* spp. (Table 2 and 5).

3.1. Surveyed sites with Omalonyx *spp. presence*

Specimens were collected in all Brazilian geographic regions, and we found *Omalonyx* in three States of the South region (Rio Grande do Sul, Santa Catarina, Paraná) where six localities were sampled, one State in the Southeast region (Minas Gerais) where seven localities were sampled, two States in the Central-west region (Mato Grosso, Mato Grosso do Sul) where four localities were sampled, five States in the Northeast region (Piauí, Ceará, Rio Grande do Norte, Pernambuco and Bahia) where nine localities were sampled, and five States (Acre, Amazonas, Pará, Rondônia and Amapá) in the North region where 15 localities were sampled. Among these localities, we made new records for four Brazilian States: Acre, Rondônia, Amapá and Piauí. All coordinates for 41 surveyed sites are listed in Table 2 and indicated in the map (Figure 1).

Table 5. Surveyed sites within the Brazilian administrative regions where *Omalonyx* spp. was absent.

Administrative regions	Locality	Coordinates
Distrito Federal	Brasília	15° 51’36”S, 47° 52’20” W
		15° 50’21”S, 47° 54’26”W
		15° 50’41”S, 47° 54’59”W
		15° 44’26”S, 47° 52′52”W
	Brazlândia	15° 42’49”S, 48° 12’14”W
Tocantins	Palmas	10° 12’34”S, 48° 19’21”W
		10° 13’18”S, 48° 21’51”W
		10° 07’49”S, 48° 18’25”W
	Porto Nacional	10° 41’54”S, 48° 24’39”W
		10° 44’39”S, 48° 26’06”W
		10° 46’04”S, 48° 24’50”W
	Lagoa da Confusão	10° 47’54”S, 49° 37’21”W
		10° 43’50”S, 49° 32’27”W
Roraima	Boa Vista	02° 48’30”N, 60° 44’27”W
	Anauá Park	02° 49’52”N, 60° 40’47”W
	Caracaraí	01° 25’16”N, 60° 59’17”W
	Estação Ecológica de Viruá	01° 29’24”N, 61° 00’07”W
	Maloca da Raposa	03° 50’25”N, 59° 58’24”W
	Mucajaí	02° 41’05”N, 61° 12’56”W
	Villa Villena	02° 12’30”N, 60° 05’06”W
	Igarapé do Fogo	02° 18’53”N, 60° 04’56”W
	Taboca	02° 27’25”N, 60° 12’32”W
	Açude do Barroso	02° 30’38”N, 60° 17’54”W
	Tepequém	03° 41’31”N, 61° 42’41”W
	Alto Alegre	02° 53’54”N, 61° 29’30”W
	Caracaranã Lake	03° 46’21”N, 59° 50’02”W
	Estação Ecológica de Maracá	03° 22’51”N, 61° 26’13”W
		03° 22’13”N, 61° 26’13”W
		03° 21’46”N, 61° 25’59”W
	Serra do Murupu	03° 08’55”N, 60° 40’54”W
	Localities known only from the coordinates	04° 11’46”N, 60° 47’56”W
		03° 51’42”N, 60° 12’58”W
		02° 55’37”N, 60° 42’57”W
		03° 05’18”N, 60° 52’52”W
		02° 14’38”N, 60° 06’13”W
		02° 16’58”N, 60° 05’16”W

3.2. Surveyed sites with Omalonyx *spp.* absence

Despite the surveying effort, there were no records of *Omalonyx* spp. in three States: Distrito Federal (Central-west region), Tocantins and Roraima (North region). The coordinates are shown in Table 5.

4. Modeling of species distributions

The potential distribution of each species is indicated in the maps (Figure 2) where different colors and color densities are related to different probabilities of occurrence of suitable conditions for their presence. The environmental model applied here agrees with the data on species distribution (Figure 1) and supports geographic constraints on the species. Some species presents overlapping ranges of distribution. This is the case for *O. matheroni, O. pattersonae* and *O. geayi*, which tend to occur in tropical areas within the northern half of South America (Figure 2A, B and C) and for *O. convexus* and *O. unguis*, which tend to occur in temperate areas in the southern half of South America (Figure 2 D and E). *Omalonyx matheroni, O. pattersonae* and *O. geayi* present moderate probability of occurrence in a vast area within northern South America, merged with intermittent areas of high probability of occurrence (Figure 2A, B and C). *Omalonyx convexus* and *O. unguis* present a smaller area of occurrence; however, there are many regions of high probability of occurrence.

There was a high probability of occurrence of *O. matheroni* in the Lesser Antilles, in the central region of South America (hydrographic region 5 and 10), in the Atlantic coast (hydrographic region nine), and in the south of South America (hydrographic region 13) (Figure 2A). These areas are predominantly tropical; however, there are also some temperate areas.

Omalonyx pattersonae showed a high probability of occurrence in tropical and arid regions (Figure 2B) in hydrographic region one, two and three (at isolated points in arid regions within hydrographic regions two and three) and in hydrographic regions six, seven and eight (in tropical and arid regions). Moderate probabilities of occurrence of *O. pattersonae* were observed in hydrographic regions nine and five (tropical region), where discontinuous areas of high probability of occurrence were observed in the Amazonian region (tropical and arid region) (Figure 2B).

Omalonyx geayi is a predominantly tropical species that can also occur in arid areas with high probability of occurrence within hydrographic regions one, two, three, four and five (Figure 2C). In hydrographic region five there are several small areas of high probability of occurrence merged in a vast area of moderate probability. There are areas of moderate probability of occurrence of *O. geayi* in hydrographic regions six, seven and 13.

Temperate areas along the southeast of South America present the highest probability of *O. convexus* occurrence. Areas with high probability of occurrence are within hydrographic regions 10, 12 and 13 (Figure 2D). There is moderate probability of occurrence in a vast area of hydrographic region 10 and in small areas within hydrographic regions 11 and 13.

Temperate areas in the southeast of South America that presented high probability of *O. unguis* occurrence includes hydrographic regions 10, 11, 12 and 13 (Figure 2E). Some areas within tropical (hydrographic regions five, nine and 10), arid (hydrographic region 11) and temperate areas (hydrographic region 14 and Malvinas Islands) were considered to have moderate probability of occurrence.

The PCA analysis resulted in three layers with highest eigenvalues that better explain the relationship between the variables in the selected study area. The most representative variables within these PCA-analysis axes were BIO4 (Temperature seasonality), BIO12 (Annual precipitation), and Altitude. As a consequence, the modeling of the species of *Omalonyx* was strongly influenced by these three variables, and their potential distribution was mainly determined by them. There is only one record of *O. brasiliensis*, thus, it was not possible to model this species' distribution due to technical limitations.

Discussion

This work addresses *Omalonyx* species distribution. The genus has a widespread Neotropical distribution, covering most South American hydrographic regions (except hydrographic regions one and 14) and the Lesser Antilles. The species occurrence map and the model of species distribution showed that three species are related to the north of South America (*O. matheroni*, *O. pattersonae*, *O. geayi*) and Lesser Antilles, and three species are related to the south of South America (*O. unguis*, *O. convexus, O. brasiliensis*).

Omalonyx matheroni is the most widespread species, and its distribution is more concentrated in northern South America (North and Northeast of Brazil, Guyana, French Guiana and Suriname) and in the Lesser Antilles. *Omalonyx matheroni* occurs in all Brazilian hydrographic regions. The most southern record for this species is Santa Catarina State in the south of Brazil (12, Figure 1).

Among northern species, *O. matheroni* is the most widespread. On the other hand, the records for *O. geayi* and *O. pattersonae* are discontinuous.

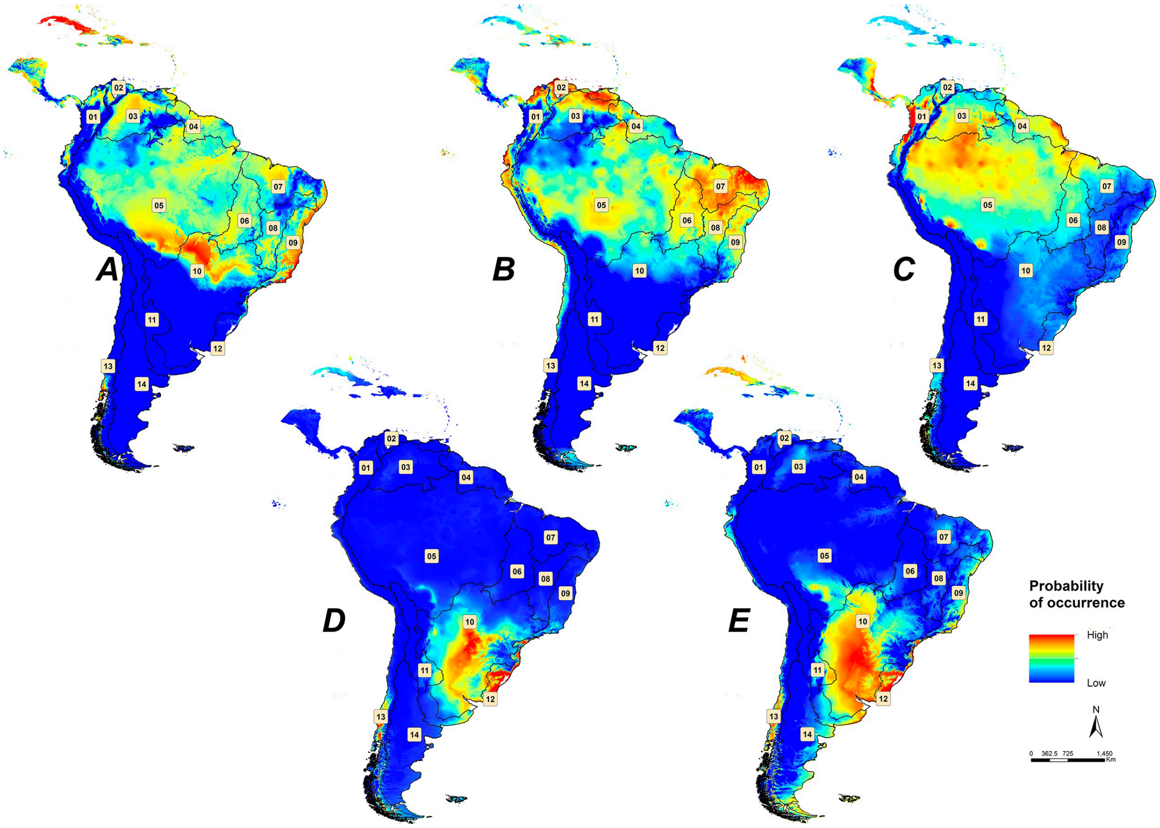

Figure 2 Potential distribution of *Omalonyx* species modeled with Bioclim analyses using all 19 bioclimatic variables, altitude and the full dataset showed on tables 2 and 3. (A) *Omalonyx matheroni*; (B) *Omalonyx pattersonae*, (C) *Omalonyx geayi*, (D) *Omalonyx convexus*, (E) *Omalonyx unguis*. The numbers represents the mainwatersheds within South America. The hydrographic regions are indicated by numbers: 1) Pacific Coast; 2) Caribbean sea; 3) Orinoco River; 4) Amapá-Esequibo; 5) Amazon River; 6) Tocantins River; 7) North Atlantic; 8) São Francisco River; 9) Eastern Atlantic; 10) Del Plata; 11) Border strip of the Brasilica (North) and the Chilean-Patagonian (South West) Subregion; 12) Eastern; 13) Chilean-Patagonian Subregion of the Pacific Versant; 14) Chilean-Patagonian Subregion of the Atlantic Versant.

Despite a huge area with high or intermediate probability of occurrence (Figure 2), the records for *O. pattersonae* and *O. geayi* are sparser (Figure 1). *Omalonyx geayi* was known to occur only in Kaw Swamp, French Guiana, the type locality (Tillier 1981). However, new records published recently expanded its distribution to Ecuador, Bolivia, Suriname and some localities in Brazil (Arruda et al. 2016). In this work, we recorded *O. pattersonae* for the first time in three hydrographic regions in North and Northeast of Brazil. There is one state in Brazil (Amazon State) where all three of these species occur at sites that are very close.

The other three species (*O. unguis*, *O. convexus, O. brasiliensis*) are concentrated in the south of South America. Among these species *O. unguis* reaches the most northern positions. This species was even found outside its distribution range (Lesser Antilles, Bolivia, Peru and in Ceará State, in the Northeast of Brazil). *Omalonyx convexus* records are very concentrated (Figure 1) and are restricted to sites where high or intermediate probability of occurrence was predicted (Figure 2B). However, there are some exceptions, and the species was also recorded in the north shore of Venezuela and in the Northeast of Brazil (Sergipe State). Until now, *O. brasiliensis* was known only from its type locality in Rio Grande do Sul State, Brazil. Despite all the surveying efforts already concentrated in Rio Grande do Sul State (Figure 1), *O. brasiliensis* was never found again since its description and the species remain known only for the type specimens from an unspecified site that could be related to hydrographic region 10 or 12 (Figure 1). Thus, *O. brasiliensis* remains to be rediscovered. This is a large State where *O. convexus* is widespread (Table 2, Figure 1); thus, both species may occur in sympatry.

There are several records from literature review, museums and collections that were not identified to species level and are designated *Omalonyx* sp. They were not used to model species distribution. These records are widespread throughout South American hydrographic regions (e.g., five, seven, nine and 10) and should also be carefully analyzed in future studies. On the other hand, some records identified to species level seem to be inconsistent (e.g., *O. convexus* was recorded for the Northeast of Brazil) and should be revised. All the records situated outside the area predicted for the species distribution should be carefully investigated. We suppose that the lack of taxonomists dealing with this group is the main cause for the great amount of records identified to genus level. On the other hand, the lack of training and the subtle morphological differences among species must be a cause of misidentification. These problems contribute to gaps in the knowledge regarding *Omalonyx* distribution (Tillier 1981, Coscarelli & Vidigal 2011).

Brazil is the best-sampled region in South America. Here, based on our malacological surveys, new records were made in Acre, Rondônia, Piaui, and Amapa. However, despite the absence of records even after intensive surveys in some administrative unities (Roraima, Tocantins and Distrito Federal) (Table 5), the genus was registered in all Brazilian hydrographic regions. There were no records for the central area of Tocantins River hydrographic region (surveyed sites: Palmas and other surrounding areas such as Lagoa da Confusão and Porto Nacional). However, the genus was present in the upper part of the hydrographic region (comprising Goiás and Pará States; Figure 1 and Table 2). Distrito Federal is an administrative unity that encompasses a small area when compared to the Brazilian States, and within this small area, there are the frontiers of three hydrographic regions: Tocantins River, São Francisco River and Del Plata. Despite its proximity to Goiás State where we found *Omalonyx* sp., there were no records in Distrito Federal (surveyed sites: the margins of Paranoá Lake, in Brasília, and in Brazlândia, all of them in the upper Del Plata hydrographic region where the genus is widely distributed; Figure 1). In Roraima State, northern Brazil, despite the surveying efforts (23 records of absence) no record was made. Roraima is within the Amazon River hydrographic region in which *Omalonyx* is widely distributed (Figure 2). However, all these negative localities are in the northeastern quadrant of Roraima, which is characterized by unusual vegetation, a seasonally flooded savannah, locally called "Lavrado" (Barbosa et al. 2007), which may have some ecological features that prevent *Omalonyx* spp. colonization. Future sampling efforts should focus on areas within these States that were not investigated until now. Considering that *Omalonyx* spp. occur in the hydrographic regions that cover these States, the absence of records is probably due to insufficient sampling effort.

In South America *Omalonyx* species were found in all hydrographic regions (except hydrographic regions one and 14) and countries. However, there were only isolated records in Colombia, Ecuador and Chile, indicating the need for more sampling effort in these areas (Figure 1). However, the occurrence of this genus in Chile must be further investigated due to different point of views regarding the identification of the Chilean slug-like succineids (Tillier 1981). The record from Chile (Letellier et al. 2014) should be carefully investigated since Tillier (1981) considered that the species recorded in Chile, *O. gayana*, belongs to the genus *Succinea.* In fact, Chile is separated from the other South American countries by the Andes mountain range, which is the major water divider in South America. d'Orbigny (1837) stated that the genus occurs only on the east side of the ridge, and until now, there is no evidence suggesting otherwise. Our results agree with those from d'Orbigny (1837) and Tillier (1981). Bioclimatic analysis proved to be a valuable tool for gaining insight into the geographical distribution of a species, especially when few records are available (i.e., *O. geayi* and *O. pattersonae*). Predictions showed that *O. matheroni* and *O. geayi* are tropical species. *Omalonyx pattersonae* usually occurs in arid regions; however, its distribution moderately extends over tropical areas. However, the prediction of *O. geayi* to hydrographic region 13 (temperate and very cold area) could be related to low numbers of records. In fact, bias can occur if species are insufficiently sampled. Thus, climatic envelopes may generate incomplete results, and the accuracy of predicted distributions will be decreased (Beaumont et al. 2005).

The occurrence records and the predictions both indicated that *O. convexus* and *O. unguis* have largely overlapping distributions in temperate areas within southern South America. These results are in accordance with those from Arruda & Thomé (2008b) and Coscarelli & Vidigal (2011). However, the distribution of *O. convexus* is constrained to temperate regions while *O. unguis* is well represented in tropical, temperate and arid regions indicating that the latter presents a broader tolerance range for environmental variables and is adapted to different bioclimatic conditions.

Considering the extension and the great diversity of habitats in the neotropical region and the wide occurrence area of *Omalonyx* species, bioclimatic models can be used to extrapolate information on species distribution if the high cost of field surveys in such a vast area is unfeasible. Information on the distribution of *Omalonyx* species will help to improve the taxonomic and biogeographic studies on these slug-like gastropods.

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Author Contributions

Ms. Daniel Coscarelli: contributed to the conception and design of the study. He did most of the malacological surveys, dissections (morphological identification) and visits to museums and collections. Moreover he wrote the first version of the manuscript and after some changes in the manuscript structure, he critically reviewed the paper.

Dra. Lângia C. Montresor: contributed to this manuscript since the beginning of the project. She reviewed the project that was submitted in order to get grants. She visited malacologycal collections in Rio de Janeiro State and also provided some field specimens. She is working in the manuscript since the first versions, writing and critically revising the text and improving the quality of the analysis.

Dr. Alan de Melo: has a vast experience with invertebrates and scientific writing and improved the quality of the manuscript. The contributed to data analysis and interpretation and critically revised the manuscript.

Philipp Russo: used our distribution data and WorldClim database to build the models of species distribution using the Maximum Entropy Algorithm (MaxEnt).

Teofânia Vidigal: contributed to the conception and design of the study. She wrote the project and got the grants that gave origin to this paper. She supervised and coordinated the project. She also provided field specimens, dissected (morphological identification), analyzed data and worked in the manuscript since the first until the last version, critically revising the manuscript.

Conflicts of interest

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

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Jaguarundi (*Puma yagouaroundi*) predation by puma (*Puma concolor*) in the Brazilian Atlantic Forest

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Abstract: The jaguarundi (*Puma yagouaroundi*) is the second most widespread carnivore of the Americas, and considered a threatened species in Brazil. However, most of its ecology is unknown, and few information is available about species that may act as its predator. In this study we present evidence of the predation of a jaguarundi by puma (*Puma concolor*) in a protected area (Carlos Botelho State Park) in the state of São Paulo, southeastern Brazil. We collected fecal samples in trails and dirt roads in the study area, and by using hair cuticle imprints and medullar patterns, we identified a puma scat containing hair and claws of a jaguarundi. Pumas usually consume carnivores, but the presence of felids is uncommon, especially in Brazil. This could be considered an antagonist interaction, but pumas and jaguarundis do not compete for territory or prey in large protected areas, since pumas have a high intake of larger prey (> 1 kg). Thus, we conclude that this an episode of intraguild predation for feeding purposes, not a kill in response to competition for prey or territory. This is an important record that helps to understand a bit more of the complex trophic relationships in tropical forests.

Keywords**:** *Intraguild predation; Trophic interactions; Diet; Felids; Feces*

Predação do gato-mourisco (*Puma yagouaroundi*) por onça-parda (*Puma concolor*) na Mata Atlântica

Resumo: O gato-mourisco (*Puma yagouaroundi*) é o segundo carnívoro com maior distribuição nas Américas, e considerado vulnerável a extinção no Brasil. No entanto, grande parte de sua ecologia é desconhecida, e há pouca informação disponível sobre espécies que poderiam atuar como seu predador. Nesse estudo, apresentamos evidências da predação do gato-mourisco por onça-parda (*Puma concolor*) em uma unidade de conservação (Parque Estadual Carlos Botelho) no estado de São Paulo. Coletamos amostras fecais de onça-parda em trilhas e estradas de terra na área estudada, e por meio da análise de microestrutura dos pelos, identificamos pelos e garras de um gato-mourisco em uma das amostras fecais. As onças-pardas normalmente consomem carnívoros, mas a predação de outros felinos é incomum, especialmente no Brasil. Essa poderia ser considerada uma interação antagonista, mas a onça-parda e o gato-mourisco não competem por território ou presas em grandes áreas protegidas, uma vez que as onças-pardas consomem preferencialmente presas maiores (> 1 kg). Portanto, concluímos que esse é um episódio de predação intraguilda com propósito alimentar da onça-parda, e não uma predação por conta de competição por território ou presas. Esse registro é importante e ajuda a compreender um pouco mais sobre as complexas relações tróficas em florestas tropicais.

Palavras-chave**:** *Predação intraguilda; Relações tróficas; Dieta; Felídeos; Fezes*

Introduction

The jaguarundi, *Puma yagouaroundi* (É. Geoffroy Saint-Hilare, 1803), occurs in the Latin America, from the borders of USA/Mexico to Argentina (Caso et al. 2015), but is possibly extinct in the USA. In Brazil, where the species is most widely distributed, it was included in the latest version of the national red list, and now is considered 'vulnerable' to extinction (Portaria 444 2014). Besides to the puma, *Puma concolor* (Linnaeus, 1771), the jaguarundi is the second most widespread felid of the Americas (Giordano 2016), and probably occurs at low densities (Caso 2013, Caso et al. 2015). It is most diurnal (Caso 2013, Giordano 2016), and can inhabit from more pristine areas – from pine-oak forests to more tropical humid ones – to highly modified landscapes (Giordano 2016, Magioli et al. 2016).

As a generalist mesocarnivore (Oliveira & Cassaro 1996), the jaguarundi consumes basically small vertebrates such as mammals (rodents and marsupials), its main prey, and significative percentages of birds, reptiles and arthropods (Bianchi et al. 2011, Silva-Pereira et al. 2011, Giordano 2016). Yet, most of the jaguarundi ecology is poorly known (Giordano 2016), and few information is available about species that may act as its predator. Oliveira & Pereira (2014) present evidence that intraguild and interspecific predation between felids in South America is an important interaction for structuring carnivoran communities. Apex predators such as puma and jaguars *Panthera onca* (Linnaeus, 1758), may have an important impact over mesocarnivore populations, since they consume some of them over time (Crawshaw & Quigley 2002, Vidolin 2004, Pereira et al. 2010). Thus, information about these interactions is important as supporting evidence.

Here we present evidence of the predation of jaguarundi by puma, in a protected area in the Brazilian Atlantic Forest from fecal samples collection and identification.

Material and Methods

We carried out our research in Carlos Botelho State Park (PECB; 24°00'–24°15'S, 47°45'–48°10'W), a large protected area (37,000 ha) in São Paulo State, Brazil (Figure 1), inserted in the largest forest continuum of the Atlantic Forest (~1,000,000 ha), between Serra do Mar and Corredor Ecológico de Paranapiacaba. PECB has high richness and diversity of mammal species (Brocardo et al. 2012), being one the most preserved Atlantic Forest remnants, including some threatened species such as *Brachyteles arachnoides*, *Tayassu pecari*, *Panthera onca* and *Speothos venaticus*, and an important source area of mammals' ecological functions (Magioli et al. 2015).

We collected puma fecal samples in trails and dirt roads in PECB (Figure 1), between October 2014 and July 2016, comprising four sampling campaigns lasting five consecutive days. The samples were stored in plastic bags labeled with data and the trail or dirt road it was collected. The total sampling effort was of 280 km traversed, and we collected 19 fecal samples characteristics from large felids. We screened the fecal samples adapting the method proposed by Korschgen (1980), which consisted in fragmenting and soaking the feces in water with detergent and alcohol for at least a day, subsequently washing them in running water with a 1 × 1 mm mesh sieve. The resulting material was dried in an oven at 50 °C, and then screened by separating food items (e.g., hair, bones, claws, feathers), which were placed in plastic bags for later identification of prey and predator guard-hairs.

We identified the species (predator and prey) using cuticle imprints and medullar patterns of the hair samples, adapting the method proposed by Quadros (2002). First, we cleaned the guard-hair with 70% alcohol and dried it with absorbent paper. Then, we deposited the hair over a slide containing a thin layer of partially dried transparent nail polish and covered it with another slide. The set of slides was pressed in a manual vise and left to rest for ~30 min. Finally, the hair was carefully removed from the slide, and its imprint was observed and photographed under a microscope at ×400 magnification.

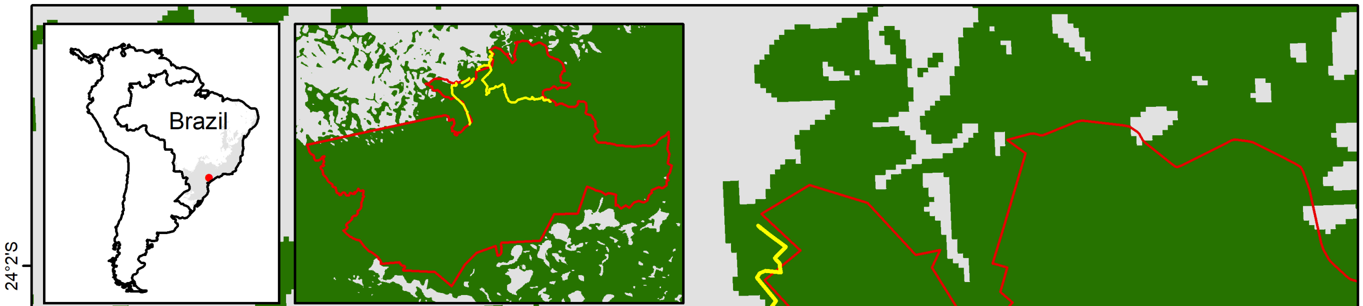

Figure 1. Location of the Carlos Botelho State Park (PECB), São Paulo, Brazil, in the Atlantic Forest biome, highlighting trails and dirt roads used for sampling, and the site where the fecal sample containing evidence of *Puma yagouaroundi* predation was collected.

To analyze the medullar patterns, we deposited the guard-hair over a slide containing water and covered it with a cover glass. Then, we observed and photographed the medullar pattern in a microscope at ×400 magnification. To identify the hair cuticle imprints and medullar patterns, we compared our records with photos from Quadros (2002), Miranda et al. (2014) and Amaro (2016). Some samples were previously identified by the presence of tracks close to the feces, or those that were placed at scratches made by pumas.

Fecal samples collection was authorized by IBAMA, through SISBIO permission n. 43680-3, and access to the study area was authorized by COTEC through permission n. 260108 – 003.547/2014.

Results and Discussion

From 19 puma fecal samples collected, one presented the jaguarundi as the prey (24°03'22"S, 47°58'28"W) (Figure 1). The sample was composed of a huge amount of grey hair, three claws, and few feathers and bones from a bird, a sample very different from any other collected in PECB. Both jaguarundi and puma guard-hairs were identified by cuticle imprints and medullar patterns, besides to the claws of the jaguarundi, which helped to corroborate the identification (Figure 2). We could not tell if the bird items present were eaten by the puma or the jaguarundi.

To our knowledge, the predation of jaguarundis was mentioned in literature only three times. Crawshaw (1995) in Iguaçu National Park, between Brazil and Argentina, and Martins et al. (2008) in Núcleo Juréia-Itatins, an administrative division of the Serra do Mar State Park, São Paulo, Brazil, reported the predation of jaguarundis by pumas. These areas are within the Atlantic Forest, but the area studied by Martins et al. (2008) is closer to PECB (~39 km in a straight line), located also in the forest continuum of Serra do Mar and Corredor Ecológico de Paranapiacaba. In Central Mexico, close to the extreme part of jaguarundi current distribution, Monroy-Vilchis et al. (2011) reported the predation of an adult male individual by a boa snake, *Boa constrictor* Linnaeus, 1758 (Squamata, Boidae), but the snake was killed by people before eating the whole individual.

Carnivores are a common prey for big cats such as the puma. In South America, there are several records of carnivore species that had been consumed by pumas (Oliveira & Pereira 2014). However, the predation of wild cats is uncommon, especially in Brazil. In the south portion of the

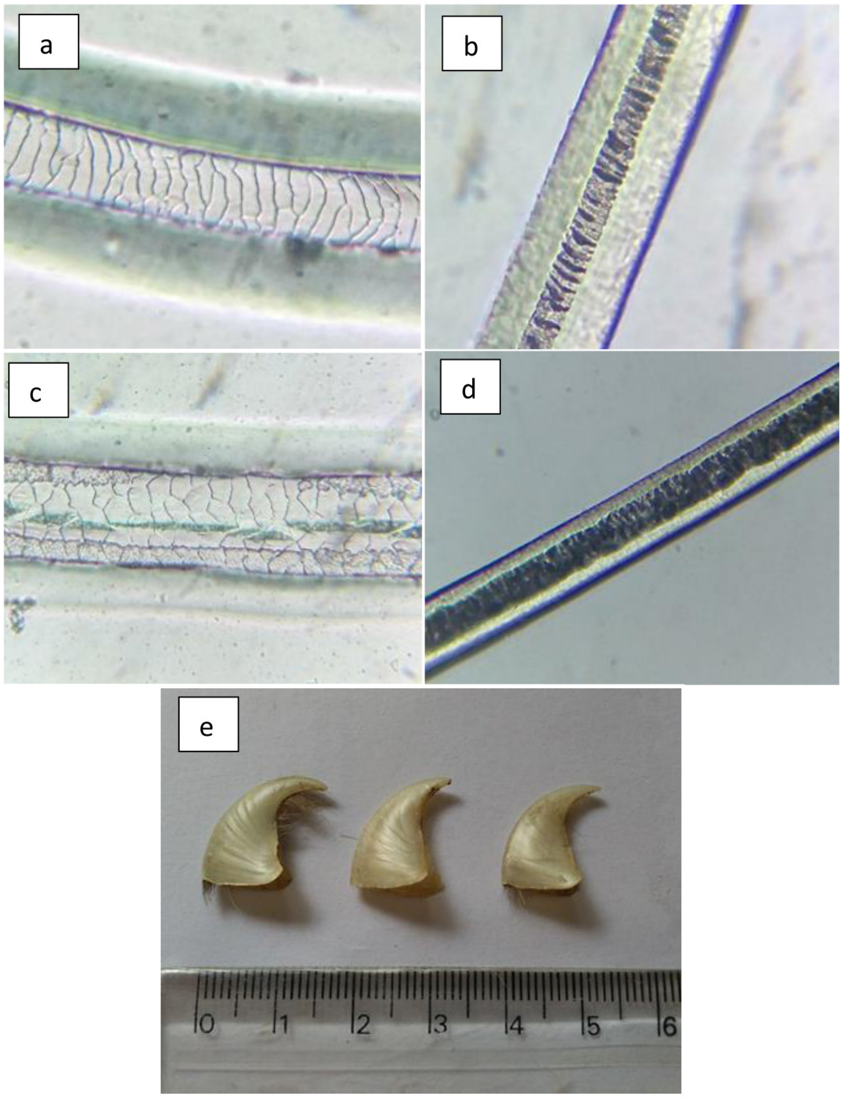

Figure 2. Hair cuticle imprints and medullar patterns of *Puma concolor* (a, b) and *Puma yagouaroundi* (c, d), and claws from *P. yagouaroundi* (e) identified from fecal samples in Carlos Botelho State Park (PECB), São Paulo, Brazil, an Atlantic Forest remnant.

Brazilian Atlantic Forest, Vidolin (2004) recorded the presence of *Leopardus pardalis* in two puma feces at the Reserva Natural Salto Morato, state of Paraná. In other countries of South America, it has been cited some other felids such as *Leopardus* sp., *Leopardus geofroyi* and *Leopardus colocolo* as part of puma's diet (Oliveira & Pereira 2014). On the other hand, the predation of bobcats *Lynx rufus* by pumas in the USA, seems to be a common record, since these species have a great niche overlap (Haas 2009).

The jaguarundi predation could be considered an antagonist interaction – competition for prey and/or territory. However, in large preserved areas of the Atlantic Forest, the feeding habits of pumas and jaguarundis are very different. Pumas feed mainly on medium- and large-sized prey (> 1 kg) (Brito 2000, Azevedo 2008, Martins et al. 2008), while jaguarundis consumes preferentially small vertebrates (< 1 kg) (Bianchi et al. 2011, Silva-Pereira et al. 2011). In modified areas, this antagonist interaction could have a higher possibility of happening, since pumas include a large percentage of small vertebrates in their diet (Rocha et al. 2010, Magioli et al. 2014). Then, it will be more reasonable to think that the pumas would be competing with other small felids for territory and/or prey.

Thus, we conclude that this is an episode of intraguild predation for feeding purposes, not a kill in response to competition for prey or territory. PECB has a high diversity of mammals, presenting several species that are included in pumas' diet, especially the medium- and large-sized mammals. Despite that, pumas are very plastic regarding their habitat use (Magioli et al. 2014) and considered generalist predators, which puts the jaguarundi as an occasional prey. This is an important record that helps to understand a bit more of the complex trophic relationships in tropical forests.

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Author contributions

Marcelo Magioli: collected and analyzed the data, and wrote the manuscript. Katia Maria Paschoaletto Micchi de Barros Ferraz: wrote the manuscript.

Conflicts of interest

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

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Article

Endozoochory by *Didelphis albiventris* Lund, 1840 (Mammalia, Didelphimorphia) in a Semideciduous Seasonal Forest remnant in the South of Brazil

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Abstract: Seed dispersal is a process that is fundamental to maintenance of forest ecosystems, enabling plants to successfully germinate in sites that are favorable to their growth, minimizing risks of competition, the action of pathogens and predation by herbivores. Intraspecific and seasonal variations in fruit consumption by *Didelphis albiventris,* and its contribution to dispersal and germination of endozoochorous seeds were analyzed in a Semideciduous seasonal forest. The study was conducted at Morro do Coco, which is a hilly area in the municipal district of Viamão, RS, Brazil, bordering the shore of Guaíba lake (30º16'15"S, 51º02'54"W), between June 2013 and May 2014, with a total sampling effort of 2992 trap-nights. A total of 18 individuals were captured and 24 fecal samples were collected. Fruits were identified in 96% of the samples, corresponding to 18 plant species, belonging to 10 families. The most common species were *Ficus cestrifolia* and *Syagrus romanzoffiana*, which occurred in 66% of the samples, followed by *Banara parviflora* and *Cecropia pachystachya*, both with a 25% rate of occurrence. There were no differences between the richness of fruit consumed by males and females (t = 0.083; DF = 32; p = 0.934) and there were no seasonal variations (H = 3.165; p = 0.367). The greatest breadth of dietary niche occurred during the summer, when twice as many fruit species were recorded in the diet than during the autumn, which was the season with the smallest breadth. Both germination percentage and germination velocity of *Ficus cestrifolia* and *Psidium* sp. seeds increased after passage through the animals' digestive tracts (percentage germination increased more than 40% and velocity was up to 7 times highest). *Didelphis albiventris* can be considered a frugivorous-omnivorous species, since fruit are an important item of its diet, and it contributes to dispersal of a large quantity of small endozoochorous seeds, increasing both germination percentage and germination velocity of some species.

Keywords: *white-eared opossum, germination inductor, marsupial, Atlantic Rain Forest, zoochory.*

Endozoocoria por *Didelphis albiventris* Lund, 1840 (Mammalia, Didelphimorphia) em remanescente de Floresta Estacional Semidecidual no sul do Brasil

Resumo: A dispersão de sementes é um processo fundamental à manutenção de ecossistemas florestais, favorecendo o sucesso germinativo de plantas em locais adequados ao seu crescimento, minimizando a competição, a ação de patógenos e a predação por herbívoros. Variações intra-específicas e sazonais no consumo de frutos por *Didelphis albiventris,* bem como sua contribuição à dispersão e germinação de sementes endozoocóricas foram avaliados em Floresta Estacional Semidecidual. O estudo foi realizado no Morro do Coco, localizado no município de Viamão, RS às margens do Lago Guaíba (30º16'15"S, 51º02'54"W), entre junho de 2013 a maio de 2014, resultando em um esforço amostral de 2992 armadilhas-noite. Foram capturados 18 indivíduos e coletadas 24 amostras fecais. Frutos estiveram presentes em 96% das amostras e corresponderam a 18 espécies vegetais, pertencentes a 10 famílias. As espécies mais frequentes foram *Ficus cestrifolia* e *Syagrus romanzoffiana*, que ocorreram em 66% das amostras, seguidas de *Banara parviflora* e *Cecropia pachystachya*, ambas com 25% de ocorrência. Machos e fêmeas não diferiram quanto à riqueza de frutos consumidos (t = 0.083; DF = 32; p = 0.934), o que também não variou sazonalmente (H = 3.165; p = 0.367). A maior amplitude de nicho trófico ocorreu no verão, registrando-se duas vezes mais frutos na dieta do que no outono, estação com a menor amplitude. Tanto a porcentagem quanto a velocidade de germinação das sementes de *Ficus cestrifolia* e *Psidium* sp. aumentaram após a passagem pelo trato digestório do animal (a porcentagem de germinação aumentou mais de 40% e a velocidade foi até 7 vezes maior). *Didelphis albiventris* pode ser considerada uma espécie frugívora-onívora, uma vez que frutos representam um item importante na dieta, e contribui para a dispersão de grande quantidade de sementes endozoocóricas pequenas, aumentando tanto o percentual quanto a velocidade de germinação de algumas espécies.

Palavras-chave: *gambá-de-orelha-branca, indutor de sementes, marsupial, Mata Atlântica, zoocoria.*

Introduction

Seed dispersal is a process that is fundamental for maintenance of plant species in forest systems, enabling successful germination in sites that are favorable to their growth, minimizing risks of competition, the action of pathogens and predation by insects and other herbivores (Howe & Smallwood 1982, Jordano et al. 2006). It has been estimated that around 50 to 90% of the trees in tropical forests produce fruit that are adapted to dispersal by animals and in these environments the diversity of frugivores is relatively high, primarily comprising reptiles, fish, mammals and birds (Howe & Smallwood 1982, Fleming 1987).

Many authors (e.g. Bocchese et al. 2008, Camargo et al. 2011, Oliveira & Leme 2013) consider that endozoochory is the most important of all of the various different animal-plant dispersal mechanisms. However, this interaction process can have varying results in terms of induction of germination because animals species' habits and behavior differ. Many plants also have features that make germination less likely after consumption by an animal. For example, small seeds can be damaged by gastric juices. As a result, several species may not be considered potential dispersers (Oliveira & Leme 2013).

Among neotropical mammals, marsupials are part of a rich fauna of small mammals and the majority of species are considered omnivorous, although some are more frugivorous. The degree to which didelphids specialize in frugivory is variable, and depends on factors such as the plant's capacity to attract consumers by smell, color and shape of fruit, the position of fruit on the trunk and their availability in the environment (Howe & Smallwood 1982, Cáceres et al. 2009). Marsupials do not fit into a defined dispersal syndrome, but are attracted by fruit that is also dispersed by birds or bats, and for this reason their frugivory is considered eclectic (Cáceres & Lessa 2012). Notwithstanding the fact that they don't have a defined dispersal syndrome, some species are considered more frugivorous than others, such as those that belong to the *Caluromys* genus, with a diet that includes around 45 species of fruit, in addition to nectar and sap (Cáceres 2006).

Didelphis albiventris Lund, 1840 is a generalist and omnivorous marsupial species that eats invertebrates, fruit and small vertebrates and occasionally carrion and parts of plants (Santori & Moraes 2006). Its behavior is also considered opportunist, because it feeds on both vegetable and animal material, depending on the availability in its environment or seasonal variations in the supply of fruit (Lessa & Costa 2010). Cantor et al. (2010) reported that *D. albiventris* ate 37 plant species in a disturbed area in the state of São Paulo, Brazil, including three genera that are considered pioneers in succession of tropical forests (*Piper*, *Cecropia* and *Solanum*), demonstrating the potential importance of these didelphids to recovery of degraded areas. Furthermore, the period of time that a seed may remain within the animal's intestine can be as long as 24 hours, which means it is possible that the seed may be deposited further from the seeding plant, compared to dispersal by other small mammals (Cáceres 2006).

Knowledge about the distinct dietary habits of didelphids is fundamental to understanding the ecological functions that they perform in their respective habitats, as predators of small vertebrates and invertebrates, and as seed dispersers. Although the number of studies investigating dietary habits has increased over recent years, primarily focusing on *Didelphis*, *Micoureus* and *Philander*, further studies of this type are considered indispensable to acquisition of knowledge about relationships between niches and the distribution of resources in the environment (Cáceres 2006, Cantor et al. 2010, Lessa & Cost, 2010; Lessa & Geise 2010). In particular, little is known and has been studied about the contribution made by *D. albiventris* to dispersal of seeds and induction of germination. The few studies that do exist were conducted in Brazil, in the Cerrado, Southeast and South, investigating this marsupial's potential to contribute to regeneration of forest fragments, and the results suggest that it can be an effective disperser, since seeds remain intact even after defecation and it is tolerant of altered environments and of urbanization (Cáceres 2002, Cantor et al. 2010, Oliveira & Leme 2013). In view of this knowledge gap and the fact that this is a common and widely-distributed species, including in degraded habitats, this study was conducted in a remnant of the Atlantic Rain Forest Domain in South Brazil to investigate: 1. intra-species and seasonal variations in fruit consumption by *D. albiventris* and its contribution to endozoochorous seed dispersal, and 2. the viability and speed of germination of seeds after passage through the animal's digestive tract.

Materials and Methods

1. Study area

The study was conducted on the Morro do Coco, which is a hill in the municipal district of Viamão, RS, Brazil, bordering the Guaíba lake (30º16'15"S, 51º02'54"W) (Figure 1). This is an area of Semideciduous seasonal forest of about 140 ha that is part of the Atlantic Rain Forest domain. It has characteristics that are still close to the original characteristics and is made up of mosaics of more preserved areas, in advanced stages of succession, and degraded areas, in initial stages (Backes 2000). The region has a type Cfa climate, according to the Koeppen classification, i.e., subtropical with a cool winter and hot summer, mean temperature greater than 22 ºC in the hottest month, and rainfall well-distributed throughout the year (Livi 1998, Backes 2000).

The entire hill is covered with arboreal vegetation, with a top stratum that can reach 35 m in height, in which species such as *Syagrus romanzoffiana* (Cham.) Glassmsn (Arecaceae) and *Ficus cestrifolia* Schott ex Spreng. (Moraceae) (Knob 1978) predominate. Backes (1999) recorded 44 arboreal species in the upper stratum, predominantly members of the families Euphorbiaceae, Lauraceae, Moraceae, Mimosaceae and Myrtaceae. In the middle and lower strata, the same study recorded 28 and 19 species respectively, predominantly members of the Myrtaceae family in both strata. The formation also includes many epiphytes, primarily of the Bromeliaceae (14 species) and Orchidaceae (22 species) families, which are more abundant at the margins of Guaíba lake, where the humidity of the air is higher (Backes 1999).

2. Capture methods

Sampling was conducted between June 2013 and May 2014, over 11 expeditions, each lasting four consecutive nights, resulting in a total sampling effort of 2992 trap-nights, according to the Stallings method (1989). In order to minimize the effects of moonlight intensity, dates when the moon was new or in the third quarter were preferred (Auricchio 2002). All four seasons of the year were sampled (winter, from June to August 2013; spring, from September to November 2013; summer, from December 2013 to February 2014; and autumn, from March to May 2014), with the objective of encompassing seasonal variations in supply of fruit and its influence on the diet.

Sixty-five "Tomahawk" type traps were used for captures, 50 with dimensions of 40 cm x 20 cm x 20 cm and 15 with dimensions of 45 cm x 25 cm x 21 cm. The traps were arranged in five parallel lines of 10 capture stations each (spaced equidistantly at 20 m intervals), forming a 100 m x 200 m grid. Three stations in each line (one, five and nine) had both a ground trap and an understorey trap (at 1.60 m from the ground, fixed to a wooden platform). Another three traps were set outside of the grid described above, close to the margins of the Guaíba lake, taking the total number of traps to 68. Trap bottoms were lined with plastic sheeting to facilitate collection of feces and avoid loss of material. Bait was put inside the traps, comprising a mixture of sardines, peanut paste, vanilla essence and banana.

Each individual captured for the first time was identified with a numbered tag (SISBIO Permanent License number 11066-1, granted on 17th November 2010). The following details and biometric data were recorded for each individual: species, sex, weight (g), head and body length (mm), tail length (mm) and hind feet length (mm), and reproductive status for females. Animals that were captured more than once in the same expedition were released without collecting biometric data again after the first capture, to avoid pseudo-replicas. Only animals recaptured during different expeditions were included in the analyses more than once.

3 Collection of fecal content

All fecal collections were conducted manually, from inside the cages and during handling of animals and samples were identified and stored individually. Each sample comprised all stools collected from a single individual. At the Unilasalle Biodiversity Conservation and Management Laboratory, feces were washed under running water, using filter paper bags, and dried at room temperature. Once fully dry, fecal content was sorted and seeds were identified using a stereoscopic microscope and the relevant literature (Lorenzi 2003, 2008, 2009), and compared with seeds from fruit gathered in the study area and with a reference collection maintained by the MCN La Salle museum.

4 Tests of seed germination

Experiments to test seed germination were conducted at the UNIVATES Plant Propagation Laboratory in the town of Lajeado, Rio Grande do Sul. Tests were conducted with the seeds of seven plant species: *Ficus cestrifolia* Schott ex Spreng., *Psidium* spp., *Banara parviflora* (A.Gray) Benth, *Cecropia pachystachya* Trécul, *Passiflora amethystina* J.C. Mikan and one species of Solanaceae. Control groups were also tested, comprising seeds from fruit collected in the study area. The test for *Psidium* sp. used *P. guajava* L. and *P. cattleianum* Sabine as controls, since both occur in the study area. The germination test for *P. amethystina* and the unidentified Solanaceae species were only conducted with seeds from the fecal samples, because no fruit for control groups could be found in the study area.

The seeds were sorted, de-infested and immersed for 20 minutes in commercial hypochlorite at 70% and then in 70% ethanol for 60 seconds, before once more being washed three times using autoclaved water (Maguiri 1962). After disinfection in a laminar flow chamber, the seeds were inoculated onto transparent (glass) Petri dishes containing two sheets of Germitest paper moistened with distilled water. This process was performed with two groups of seeds: control groups (seeds extracted from fruit collected in the study area) and test groups (seeds obtained from fecal samples), as shown in Table 1. Wherever possible, repetitions with 20 seeds were conducted of each treatment (control and test), subject to sufficient numbers of seeds being found in the fecal samples. Dishes were kept in propagators with fluorescent lighting at a photoperiod of 16 h light per day and a temperature of 25 ºC (Maguiri 1962, Camargo et al. 2011).

Table 1. Number of seeds per plant species and per treatment used to test seed germination in experiments with marsupial *Didelphis albiventris* from a remnant of Semideciduous seasonal forest in South Brazil, RS.

Plant species	**Group of seeds**	
	Control	**Test**
Banara parviflora (A.Gray) Benth	133	11
Cereus hildmannianus K.Schum.	8	2
Ficus cestrifolia Schott ex Spreng.	280	134
Psidium sp.	*70	20
Passiflora amethystina J.C.Mikan	-	11
Solanaceae	-	6

* *P. guajava* Linnaeus, Carl von, *P. cattleianum* Sabine

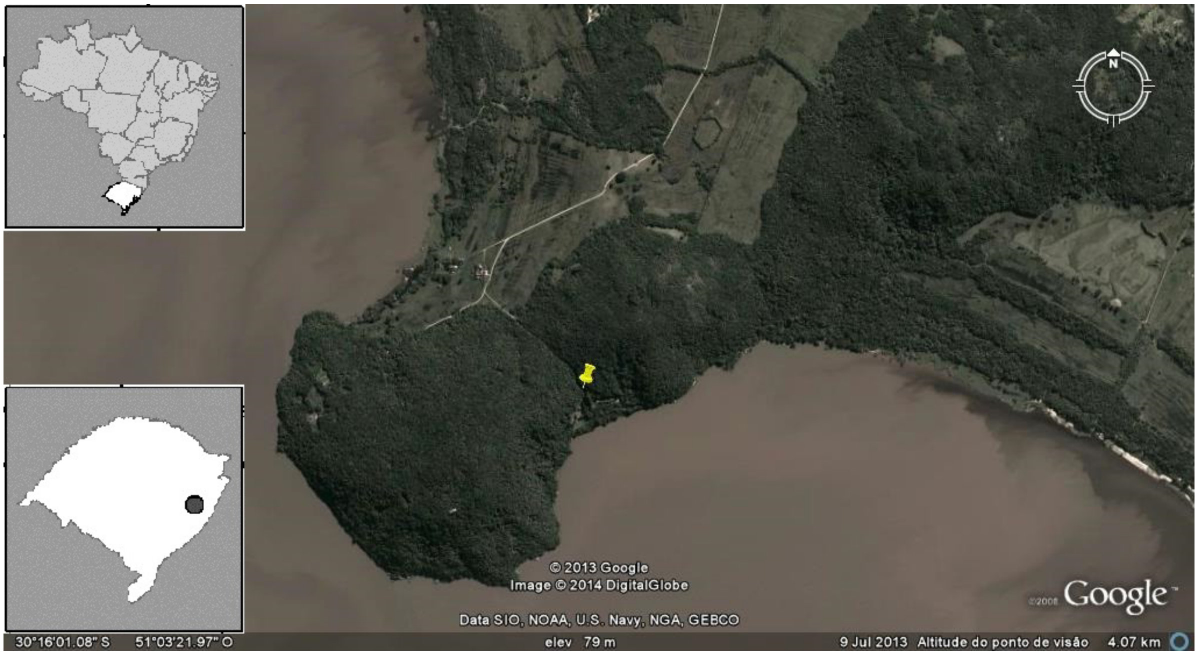

Figure 1. Location of the study area, a hill named Morro do Coco, within the municipal district of Viamão, RS, Brazil (Image from Google Earth, 2014).

The experiment was monitored every 2 days for up to 70 days after inoculation. Sprouting of the primordial radicle was considered evidence of germination.

5 Data analysis

The representativeness of vegetable items identified in the diet was estimated on the basis of the Frequency of Occurrence (FO) of these items in samples, where: FO = number of samples containing the item / total number of samples x 100. Student's *t* test with Welch's correction (for samples with unequal variances) was used to evaluate the significance of differences between sexes in richness of fruit and number of seeds in the fecal samples.

The diversity of fruits in the diet of males and females and per season was measured in terms of breadth of dietary niches, calculated using Levin's Index (B_A), which, according to Krebs (1999), attributes greater weight to fruits that are more abundant in the diet. The Kruskal-Wallis test (H) was used to test whether animals had a preference for any of the fruits in their diet, comparing all of the plant species found in the samples. Seasonal variations in diet were also investigated using the same test. Differences between the numbers of seeds of different species found in fecal samples were tested using ANOVA (F). Dunn's post hoc test for multiple comparisons was used to identify which samples differed from each other.

Analyses of germination were conducted separately for each species, as per Borghetti & Ferreira (2004), by calculating the percentage of germinated seeds (PG) in relation to the number of seeds under experimental conditions, using the formula: $PG = (\sum n_i . N^{-1}) . 100$, where n represents the number of plantlets germinated and N the total number of seeds used in the experiment. The germination velocity index (GVI), in which the number of seeds or plantlets is observed and counted regularly (every 2 days in this study), was obtained as per Borghetti & Ferreira (2004), using the formula: $GVI = G_1/N_1 + G_2/N_2 + ... + G_n/N_n$, where G represents the number of seeds germinated each time they were counted and N is the number of days since seeds were sown. The significance of differences observed between two groups of seeds (control and test) was assessed using Student's *t* test with Welch's correction, Fisher's exact test or the G test (goodness-of-fit), when the number of seeds inoculated was low (six seeds). Each plant was also assessed by calculating odds ratios for the probability of germination after passage through the intestinal tract, in comparison to the control group.

Statistical analyses were conducted using the programs PAST 3.11 and Biostat 5.3 (Ayres et al. 2007). Differences were considered significant when $p \leq 0.05$.

Results and Discussion

During the study, 18 *D. albiventris* individuals (seven females and 11 males) were captured a total of 26 times, producing 24 fecal samples. All individuals were captured on the ground and no animals were captured on the platforms in the understorey.

Among the dietary items found in the fecal samples, fruits were identified in 96% of the samples and corresponded to 18 plant species, belonging to 10 families (Table 2). The most common species were *Ficus cestrifolia* and *Syagrus romanzoffiana*, which occurred in 66% of the samples, followed by *Banara parviflora* and *Cecropia pachystachya*, both with a 25% rate of occurrence.

Cáceres et al. (2009) conducted a 6-month study in Atlantic Rain Forest in South Brazil, using 127 traps and captured 19 *Didelphis aurita* Wiend-Neuwied, 1826, collecting 63 fecal samples and recording consumption of eight plant species. Silva et al. (2014) analyzed 40 fecal samples from *D. albiventris* in two areas in South Brazil and recorded consumption of the fruit of six plant species (accounting for 77.5% of samples), of which *S. romanzoffiana*, *F. cestrifolia* and *Coussapoa microcarpa* (Schott) Rizzini were the most frequent. The *Ficus* genus is considered one of the most important for frugivores in tropical forests, providing a food resource during seasons in which fruit is scarce in the environment (Sugai & Cara 2009). Many frugivorous mammals are attracted by the yellow-green fruit of *F. cestrifolia*, which only become purple during the final phase of maturity (Carauta & Dias 2002).

The high rate of occurrence of the seeds of *F. cestrifolia* in the fecal samples may be related to the species' asynchronous fruit bearing, since its fruit is available in all seasons of the year, which is the reason why members of *Ficus* are considered key species in tropical forests (Carauta & Days 2002, Sugai & Cara 2009). Another relevant factor is the fact that *F. cestrifolia* and *F. luschnathiana* (Miq.) Miq. are common species that are well-distributed in the study area, making their fruits accessible to many frugivores. Although in this study occurrence of *F. luschnathiana* was not identified in the samples analyzed, Silva et al. (2014) have observed seeds of this species in the feces of *D. albiventris* in the same study area.

Syagrus romanzoffiana is also considered a key species in tropical forests, because its fruits are fleshy and rich in nutrients and it provides an

Table 2. List of plant species with life forms (according to Lorenzi 2003, 2008, 2009) and occurrence in feces of marsupial *Didelphis albiventris* collected in a remnant of Semideciduous seasonal forest in Rio Grande do Sul, Brazil, according to APG III (2009).

Family	Taxon	Common name	Life form	Frequency of occurrence (%)
Anacardiaceae	*Lithraea brasiliensis* Marchand	Aroeira-brava	tree	8
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassm.	Gerivá	tree	66
Cactaceae	*Cereus hildmannianus* K.Schum.	Tuna	tree	8
Moraceae	*Ficus cestrifolia* Schott ex Spreng.	Figueira	tree	66
Myrtaceae	*Campomanesia xanthocarpa* (Mart.)	Guabiroba	tree	4
	Psidium sp. *	Araçá/goiaba	tree	4
Passifloraceae	*Passiflora amethystina* J.C.Mikan	Maracujá-azul	liana	4
Primulaceae	*Myrsine* sp.	Capororoca	tree	4
Salicaceae	*Banara parviflora* (A.Gray) Benth	Farinha-seca	tree	25
Solanaceae	Solanaceae	-	-	4
Urticaceae	*Cecropia pachystachya* Trécul	Embaúba	tree	25
	Coussapoa microcarpa (Schott) Rizzini	Mata-pau	tree	8
Not identified sp.1				17
Not identified 5 spp.				**4

* *P. guajava* Linnaeus, Carl von, *P. cattleianum* Sabine; ** each one

energy source for many tropical frugivores. Additionally, this palm bears fruit throughout the year, with greater intensity from March to August, providing fauna with food in months of seasonal scarcity (Lorenzi 1992, Costantin et al. 2013). Messias & Alves (2009) identified 10 species of mammals feeding on *S. romanzoffiana* in an area of Atlantic Rain Forest in the state of São Paulo, Brazil, one of which was *Didelphis* sp. Larger marsupials, such as *D. albiventris*, are considered occasional dispersers of large seeds, in common with the majority of didelphids, because they only eat the flesh of the fruit, leaving the seeds in the place where they eat (Cáceres 2006). This is the case of *S. romanzoffiana*, according to recent studies (Cáceres et al. 2009, Cantor et al. 2010, Silva et al. 2014). Roman et al. (2010) studied removal of *S. romanzoffiana* fruit, observing that *D. albiventris* removed 13% of the fruit, left 39% intact and masticated 63%, leaving the seeds at the site in a synzoochorous manner. Therefore, because this species does not disperse seeds over long distances, it is not a good disperser, since for palms to germinate successfully the seeds must be a minimum of 4 m from the mother plant.

The size of the most frequent seeds in the samples varied from 0.3 to 3.7 mm (90%), and seeds larger than 3.7 mm were infrequent (10%). Fecal samples differed significantly in terms of the quantity of seeds (F = 31.134; Degrees of freedom [DF] = 17; p = 0.019), and *F. cestrifolia* was the species with the largest number of seeds in the samples. The quantity of seeds found in the samples varied from one to 1480 seeds (Mean = 150.125; SD = 311.15) in a single sample, but the majority of samples contained large numbers of seeds. As such, small seeds predominated in the samples, and the seeds of *F. cestrifolia* and *B. parviflora* were the smallest seeds found. Cantor et al. (2010) also found large numbers of seeds in samples from *D. albiventris,* ranging from one to 566 seeds per sample. Cáceres (2006) suggests that animals' body sizes can have an influence on the quantity of seeds dispersed and that small seeds (< 5 mm) may be dispersed by both small and large marsupials. In view of this, the observation of a single *S. romanzoffiana* seed (around 17 mm in size) in a fecal sample from a reproductive female with young is considered atypical, since it was the only such record. This does, however, provide evidence that an animal with the body size of *D. albiventris* can occasionally disperse the fruit of palms by endozoochory. Cantor et al. (2010) found a larger amplitude of seed sizes in stools, ranging from 0.5 to 13.3 mm, but still with a higher frequency of small seeds, and also observed that larger seeds were damaged. Lessa et al. (2013) reported similar results, finding small seeds in 68% of samples from seven species of marsupials, including *D. albiventris*. Seeds larger than 6 mm were damaged.

The richness of plant species found in samples from males and females did not differ significantly (t = 0.083; DF = 32; p = 0.934), and there was no significant seasonal variation in fruit richness either (H = 3.165; p = 0.367), as illustrated in Figure 2. However, the greatest dietary niche breadth was observed in the summer (B_A= 0.3176), when twice as many fruits were recorded in the diet than in the autumn, which was the season with the narrowest dietary niche breadth (B_A= 0.1513). This difference may be related to availability of fruit in the environment, since 47 arboreal individuals bearing fruit were recorded in the summer and just 16 individuals in the autumn in the same sampling grid (Luana S. Guimarães, personal communication, 2014, data not yet published). Cantor et al. (2010) also failed to detect seasonal differences in fruit richness for *D. albiventris* and attributed this result to continuous fruit bearing by zoochorous plants in the area studied.

Males had a dietary niche breadth (B_A= 0.3771) that was 1.3 times larger than that of females (B_A= 0.2834). These results are similar to findings reported by Silva et al. (2014) for the same study area. *Didelphis aurita* also exhibited a similar pattern in fragments of Atlantic Rain Forest (Carvalho et al. 2005, Cáceres et al. 2009). Both the increase in size of males' living areas during the reproductive period and the report that young males are more nomadic and don't have a fixed living area (Cáceres & Monteiro-Filho 2006, Graipel & Filho 2006) may explain why it was observed that males' trophic niches were wider in this study.

The quantity of seeds was also not significantly different between the sexes (t = 1.547; DF = 11; p = 0.150), confirming that the frugivorous-omnivorous diet is independent of sex. So, although males have larger living areas and young males are more nomadic and arboreal (Cáceres & Monteiro-Filho 2006, Graipel & Filho 2006, Vieira 2006), it seems that this ecological differences between the sexes don't influence the consumption of fruits. Cáceres et al. (2009) analyzed fecal samples from *D. aurita* in South Brazil and also failed to detect differences between sexes in terms of the quantity of seeds, which confirms the results obtained in this study.

In relation to the life forms of the plant species that were found in the samples, 91% are tree forms and just one is a liana. All of the species recorded are native, with the exception of the *Psidium* sp., which may have been *P. guajava*, which occurs in the study area as well as other exotic species of plants.

The germination tests of *Ficus cestrifolia* seeds demonstrated that differences observed were not the result of chance. For the first repetition, there was a significant difference in percentage germination (t = 3.793; DF = 5; p = 0.013) between the control group (PG = 6.6%) and

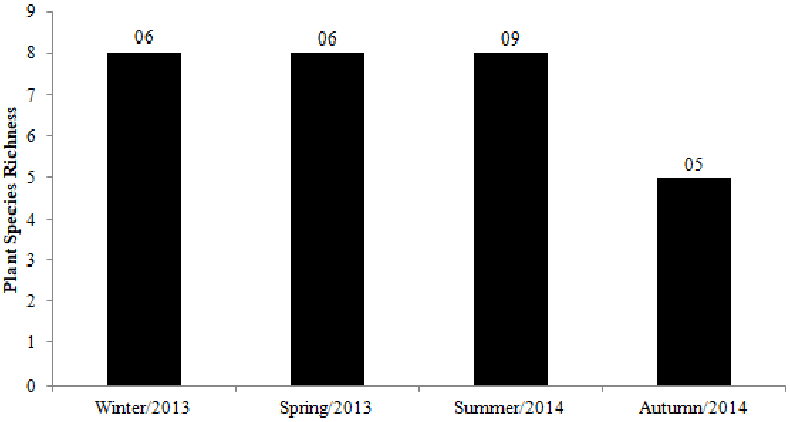

Figure 2. Richness of fruit in fecal samples and number of marsupial *Didelphis albiventris* captures from June 2013 to May 2014 in a remnant of Semideciduous seasonal forest in South Brazil, RS.

test group (PG = 39%). Germination velocity also differed significantly (t = 3.455; DF = 5; p = 0.018) between control (GVI = 0.06) and test groups (GVI = 0.44); velocity in the test group was 7.33 times greater than in the control group (Figure 3B). The second repetition also exhibited significant differences in percentage germination (G = 24.670; DF = 1; $p < 0.0001$) between the control (PG = 12.72%) and test groups (PG = 57%). Germination velocity was 1.53 times faster in the test group (GVI = 0.29) than in the control group (GVI = 0.19).

The germination tests with *Psidium* sp. also revealed significant differences. Percentage germination for *Psidium* sp. and *P. guajava* (control) differed significantly (G = 4.935, DF = 1, p = 0.026) between control (PG = 31.21%) and test groups (PG = 80%) (Figure 3A). Germination velocity was also around 1.2 times greater in the test group (GVI = 0.72) than in the control group (GVI = 0.61) (Figure 3B). However, *Psidium* sp. and *P. cattleianum* (control) did not differ significantly (G = 0.112, DF = 1, p = 0.738), although percentage germination was higher in the control group (PG = 100%) than in the test group (PG = 80%). However, germination velocity was 4.1 times greater in the test group (GVI = 0.726) than in the control group (GVI = 0.177). In turn, *Cereus hildmannianus*, did not exhibit significant differences in percentage germination (*n* = 10, p = 1.0) between the control and test groups. However, the small sample size should be taken into consideration in relation to this result. In contrast, percentage germination of *Banara parviflora* did differ significantly (*n* = 181, p = < 0.0001) between control and test groups, with higher percentage germination in the first group (40%) than in the second (2%).

Passiflora amethystina and Solanaceae seed samples did not germinate during the experimental period (70 days), which may be related to the size of the seeds, the plants' life forms, climatic variations, and/or the length of time the seeds spent in the animals' intestines (Cantor et al. 2010).

Overall, the germination tests indicated that both germination percentage (Figure 3A) and germination velocity (Figure 3B) of *Ficus cestrifolia* and *Psidium* sp. seeds increased after passage through the animals' digestive tracts. Cáceres (2006) states that the majority of seeds that pass through marsupials' digestive tracts remain viable for germination, because their masticatory behavior does not damage seeds, particularly not smaller ones. The action of gastric acids is also insufficient to damage seeds, according to the same author. Notwithstanding, since *B. parviflora* seeds exhibited a higher germination percentage in the control group, it appears that passage through the intestinal tract of *D. albiventris* may compromise their viability. Cantor et al. (2010) investigated the success of germination of five plant species after passage through the digestive tract of *D. albiventris*, observing that the viability of seeds varied across different species. For three species, the germination percentage in the control group was medium to high (*Cecropia pachystachya*, *Psidium guajava* and *Morus nigra*), whereas *Piper amalago* L. did not exhibit significant differences between groups. Only *Passiflora edulis* Sims exhibited a higher germination percentage in the test group.

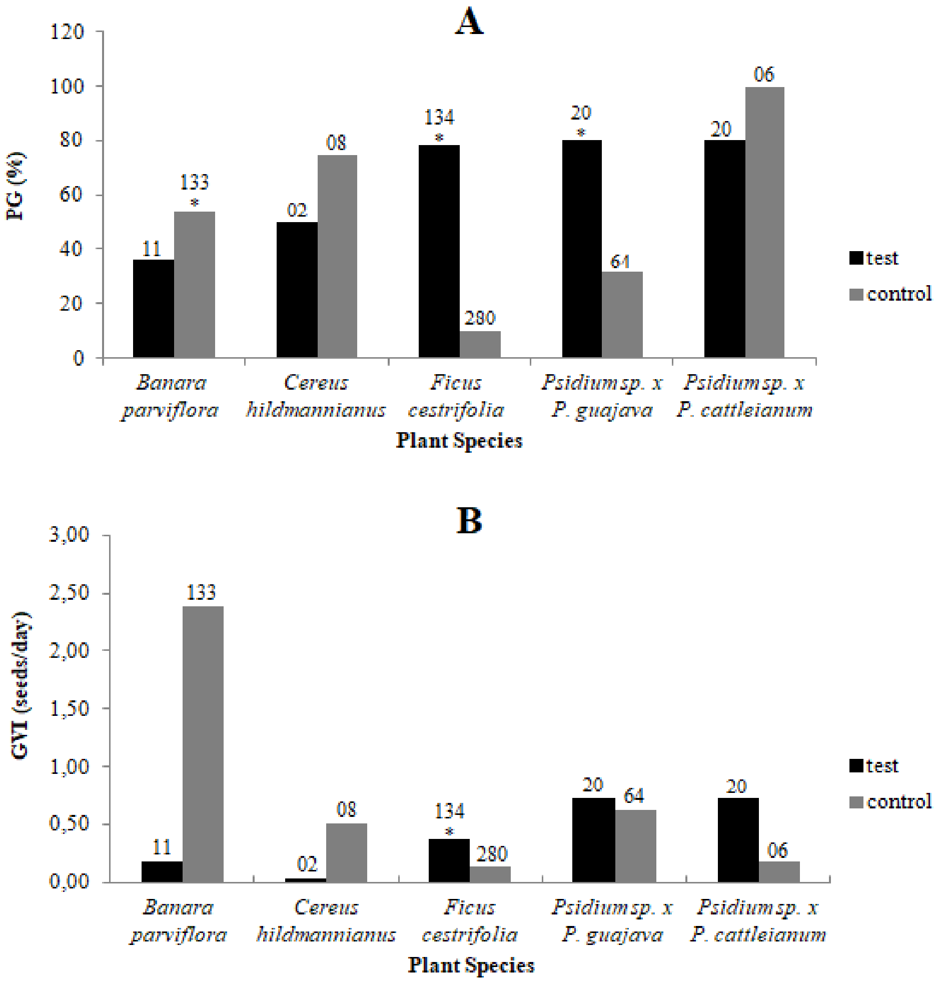

Figure 3. (A) Percentage germination (PG) and (B) germination velocity index (GVI) for seeds in test groups and control groups (above vertical bars are the numbers of tested seeds) in experiments with marsupial *Didelphis albiventris* from a remnant of Semideciduous seasonal forest in South Brazil, RS. (*) indicates significant differences ($p<0.05$).

In turn, Oliveira and Leme (2013) tested the germination potential of *Rapanea ferruginea* (Ruiz et Pav.) after passage through the digestive tract of *D. albiventris* and reported that both germination percentage and germination velocity were higher than for other treatments and a control group. As a result, they concluded that the animal is a potential disperser of *R. ferruginea* in conserved areas and in anthropized areas. Camargo et al. (2011) tested the viability of seeds of three species of the genus *Miconia* found in fecal content from *Gracilinanus agilis* (Burmeister,1854) and in addition to not detecting significant differences between the groups tested, they found that percentage germination was higher in the control group for *Miconia cuspidata*. These authors suggested that the seeds had been damaged by the animals' gastric acids, because of their small size, contradicting Lessa & Costa (2010), who claim that small seeds (< 1 mm) remain intact when eaten by small marsupials. Cáceres & Monteiro-Filho (2007) tested germination of 14 plant species and the majority of seeds did not exhibit significant differences between control and test groups, with the exception of *Rubus rosifolius* Stokes (the viability of which has been confirmed previously after passage through the intestines of bats and primates). The authors highlighted the influence of the dormancy period of each species' seeds, which is essential to understanding the potential of an effective disperser. In contrast, Cáceres (2006) argues that the length of dormancy does not impact on dispersal, since a seed from a pioneer plant deposited in a site that is inappropriate for germination (such as the forest interior), may spend a long period of time in the soil seed bank until favorable conditions appear, such as a clearing opening, for example. Notwithstanding, Lessa et al. (2013) point out that even if germination after passage through the intestines of these animals is irrelevant, the impact is still positive because of the increased distance of dispersal and maintenance of the soil seed bank.

Conclusions

Didelphis albiventris can be considered a frugivore-omnivore, since fruit is an important item in the diet of both males and females, and was found in all fecal samples and all seasons of the year.

Didelphis albiventris can disperse large quantities of small endozoochorous seeds, increasing both germination percentage and germination velocity of some species, such as *Ficus cestrifolia* and *Psidium* sp. Many small seeds pass through the digestive tracts of these didelphids without being damaged, retaining germination viability and, consequently, resulting in dispersal of plant species between forest fragments. They therefore contribute to regeneration of forests and to maintaining the soil seed bank, both in conserved and in degraded areas, where specialized frugivores are often absent as a result of localized extinctions.

Since not enough is yet known about the mutualistic relationships between marsupials and plants, further studies are needed to better understand the germination dynamics of the many different plant species whose fruit attract this group of mammals. The role that these animals play in the process of seed dispersal, whether they act as occasional or effective dispersers, whether or not they damage the seeds and the part didelphids play in regeneration of damaged environments are just some of the subjects that merit investigation.

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Author Contributions

Susana de Oliveira Junges and Cristina Vargas Cademartori: substantial contribution to the concept and design of the study; contribution to data collection, data analysis and interpretation; contribution to manuscript preparation and critical revision.

Eduardo Périco: substantial contribution to data analysis and interpretation; contribution to manuscript critical revision.

Elisete Maria de Freitas and Sérgio Augusto de Loreto Bordignon: contribution to data collection, data analysis and interpretation; contribution to manuscript critical revision.

Guilherme Consatti: substantial contribution to data collection and data analysis.

Conflicts of Interest

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

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Short Communication

Hepatic steatosis in six-banded armadillo (*Euphractus sexcinctus* Linnaeus, 1758)

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Abstract: The six-banded armadillo (*Euphractus sexcinctus*) stands out among wild mammals due to the rare occurrence of spontaneous metabolic diseases. When altered, the liver, which is important in metabolism, may trigger a dysfunctional cascade, leading to hepatic steatosis. Here we describe a case of hepatic steatosis in a six-banded armadillo living in captivity. The female specimen was captured and donated to the Federal University of Piaui under SISBIO authorization nº53303. The animal was first referred for a veterinary clinical evaluation, and then euthanized following the ethical standards of the Federal Council of Veterinary Medicine. At the start of the dissection, the abdominal cavity was accessed and sections of all *ex situ* liver lobes, spleen and mandibular lymph node were subjected to routine histological processing; the results were photo documented. The anatomic and histopathological analysis confirmed the diagnosis of hepatic steatosis, which we propose is related to an infectious process as a result of the changes observed in the organs of the lymphoid system. This report of fatty liver disease in armadillo suggests an acute infectious process with lymphoid system involvement.

Keywords: *Euphractus sexcinctus, infectious, liver, six-banded armadillo, steatosis.*

Esteatose hepática em tatu-peba (Euphractus sexcinctus Linnaeus, 1758)

***Resumo*:** O tatu-verdadeiro (*Euphractus sexcinctus*) se destaca entre os mamíferos selvagens devido à rara ocorrência de doenças metabólicas espontâneas. Quando alterado, o fígado, que é importante no metabolismo, pode desencadear uma cascata disfuncional, levando a esteatose hepática. Foi descrito um caso de esteatose hepática em um tatu-verdadeiro vivendo em cativeiro. O espécime feminino foi capturado e doado para a Universidade Federal do Piauí sob a autorização SISBIO nº53303. O animal foi encaminhado para uma avaliação clínica veterinária e, depois, submetido à eutanásia seguindo os padrões éticos do Conselho Federal de Medicina Veterinária. No início da dissecção, acessou-se a cavidade abdominal e as seções de todos os lóbulos do fígado ex situ, do baço e do linfonodo mandibular foram submetidos ao processamento histológico de rotina. Os resultados foram fotomicrografados e documentados. A análise anatômica e histopatológica confirmou o diagnóstico de esteatose hepática, que está relacionada a um processo infeccioso como resultado das mudanças observadas nos órgãos do sistema linfóide. Este relatório de doença hepática gordurosa no armadillo sugere um processo infeccioso agudo com comprometimento do sistema linfático.

Palavras-chaves: *Euphractus sexcinctus, infecção, fígado, tatu-verdadeiro, esteatose.*

Introduction

Among wild mammals, the six-banded armadillo (*Euphractus sexcinctus* Linnaeus, 1758) stands out for its wide territoriality as well as having varied eating habits, including dietary fruit, leaves and insects (Abba & Superina 2010). The cases of death involving armadillos in nature are related to poaching, unlike those in captivity, which are due to cage injuries, illnesses associated with food, and respiratory infections (Diniz et al., 1997). Spontaneous diseases are rarely reported in these animals, except for a single case of squamous cell carcinoma (Bo-ram et al., 2015). Most agents already isolated in these animals do not cause symptoms; it is not yet known whether the immune system is effective or deficient, making them potential reservoirs of fungal, bacterial, parasitic and viral infections (Capellao et al., 2015).

The liver is an important organ that carries glandular endocrine and exocrine functions in the body, which allows it to perform many vital functions essential to maintaining homeostasis. Among its main activities, the following stand out: the regulation of nutrient metabolism, the immune role, the synthesis of proteins and other molecules, the storage of vitamins and iron, hormone degradation, and the excretion of drugs and toxins

(Rouiller, 2013). It also confers, control the cell cycle and hepatic cells regeneration by compensatory mechanisms involved in hyperplasia of cells affected in tumor processes (Jesus et al., 2000, Beier, 2016).

When external factors culminate in disruption of liver function, serial reactions are initiated which modify the dynamics of the body. The accumulation of triglycerides inside the liver, for example, leads to the situation referred to as "fatty liver", in which the organ is yellowish and engorged with fat, characteristic of hepatic steatosis (Brunt & Tiniakos 2010). This is due to changes in the metabolism of fatty acids, possibly related to an increase in its synthesis, and/or insufficient catabolism or transport and excretion from the liver (Silva & Goncalves 2008).

Hepatic steatosis is frequently found in domestic animals, especially cats, most often animals deprived of food or undergoing periods of appetite loss (Silva, 2012). Potential causes of abnormal hepatic function include viral hepatitis, autoimmune liver diseases, hepatobiliary diseases and systemic infection, including agents such as *Mycobacterium spp.* and *Leishmania spp* (Shimizu, 2008). Liver disease is also reported in fattened animals (pigs and geese), in females in late pregnancy (especially cows), and in bulls, chickens and cuttlefish (Santos, 1975). In wild animals, reports of this syndrome can be found in the literature; cases of hepatic steatosis have also been described in birds (Couto, 2007), lagomorphs, rodents (Spinelli, 2014), carnivores (Armstrong & Blanchard 2009, Martins et al., 2016) and six banded armadillo (Batista et al., 2016).

This study describes a case of hepatic steatosis in a six-banded armadillo (*Euphractus sexcinctus* Linnaeus, 1758) that lived illegally in captivity and was seized by the Chico Mendes Institute for Biodiversity and donated to the Federal University of Piaui.

Material and Methods

A six-banded female armadillo, of the species *Euphractus sexcinctus* (Linnaeus, 1758), was seized on a farm in the municipality of São Raimundo Nonato - PI, where she had been held captive. The animal was donated to the Federal University of Piaui, with the authorization of SISBIO No. 53303, for scientific purposes supported by the ethics committee on animal experiments (protocol 136/15).

The six-banded armadillo was sent to the Histotechnique and Embryology Laboratory at the Morphology Department for the first clinical examination, where it was found to be weak and to have numerous injuries of the limbs, head and tail (Figure 1). The animal was apathetic, with normal colored mucous. Upon radiological examination, it was found to be a young animal due to incomplete closure of the epiphysis with the diaphysis.

Thus, the specimen was euthanized following the ethical standards of the Federal Council of Veterinary Medicine. After complete disruption of vital signs, dissection of the neck was initiated to access the jugular vein; this was followed by cannulation and fixation by perfusion with 10% buffered formaldehyde followed by immersion in a tank with the same solution for 48 hours. After this period, the specimen was dissected and the observations and macroscopic findings were recorded.

The abdominal cavity was accessed by incision in the alba linea. Macroscopically, pathological liver changes were observed, including curved edges, increased size, and intense yellowish color with hyperemic and necrotic foci (Figure 2).

After macroscopic evaluation *in situ* and *ex situ*, liver, lymph node and spleen sections of about 0.5 cm thick were submitted to routine histological processing and stained with hematoxylin-eosin, toluidine blue and ziehl-neelsen (used 1% aqueous hydrochloric acid) for the detection of acid-fast bacilli.

Liver histopathological analysis revealed moderate congestion of the sinusoid vessels, clear and microvacuolar degeneration of peri-sinusoidal hepatocytes, a large number of macrophages (Kupffer cells), inflammatory infiltrates diffuse with the presence of lymphocytes, plasma cells and neutrophils, and necrosis of hepatocytes. All of the above hepatic characteristics were compatible with hepatic steatosis and acute hepatitis. Acid-fast bacilli and intracellular pathogens were not detected via Toluidin Blue stain (Figure 3).

Mandibular lymph nodes showed reactive interfollicular hyperplasia, with histiocytes and mast cells surrounding the follicles. The histopathologic characteristics of the spleen indicated reactive hyperplasia of both red and white pulp (Figure 3).

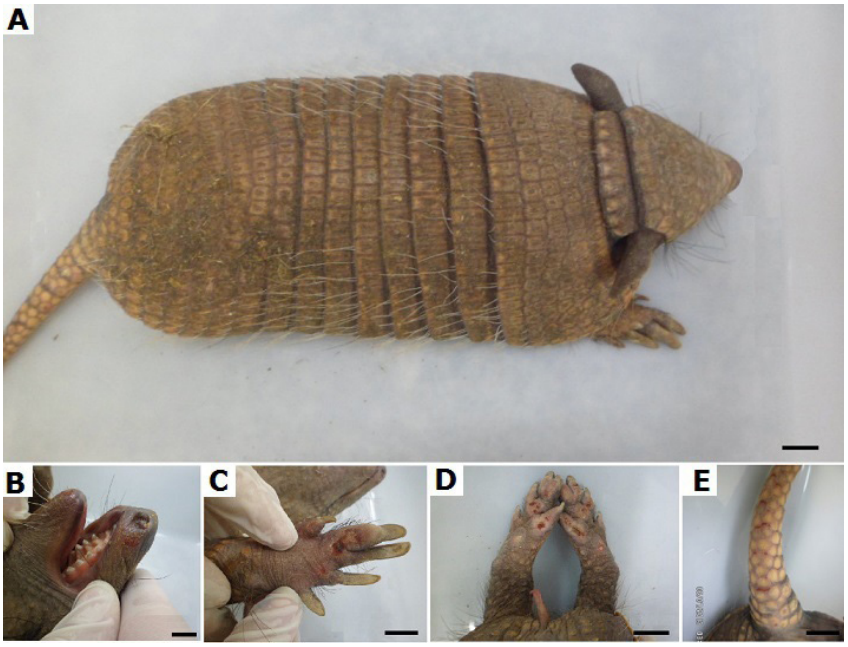

Figure 1: Photomicrographs of six-banded armadillo (*Euphactus sexcinctus*) **s**howing the animal in dorsal view (A), and the various lesions found in the mouth (B), the forelimbs (C), the hind limbs (D) and tail (E). Bar: 1cm.

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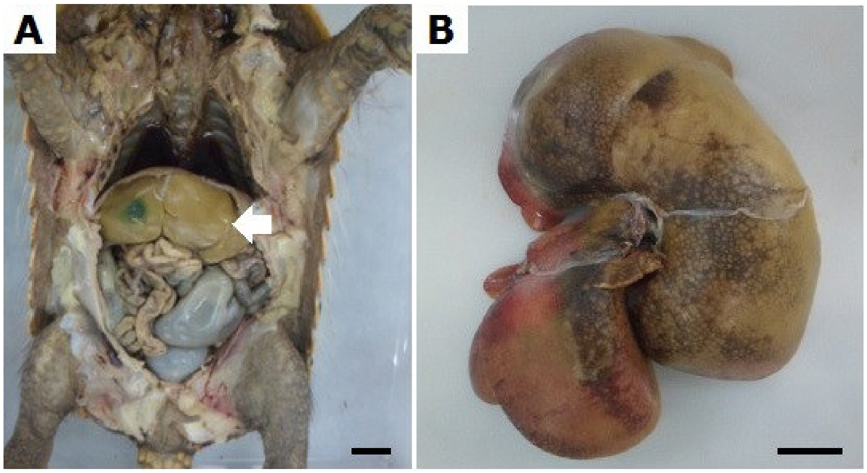

Figure 2: Photomicrograph of the six-banded armadillo (*Euphractus sexcinctus*) abdominal cavity after the incision and during anatomical dissection (A) showing lipemic appearance of the liver (white arrow). The right *ex situ* organ diaphragmatic face can be noted (B). Bar: 1cm

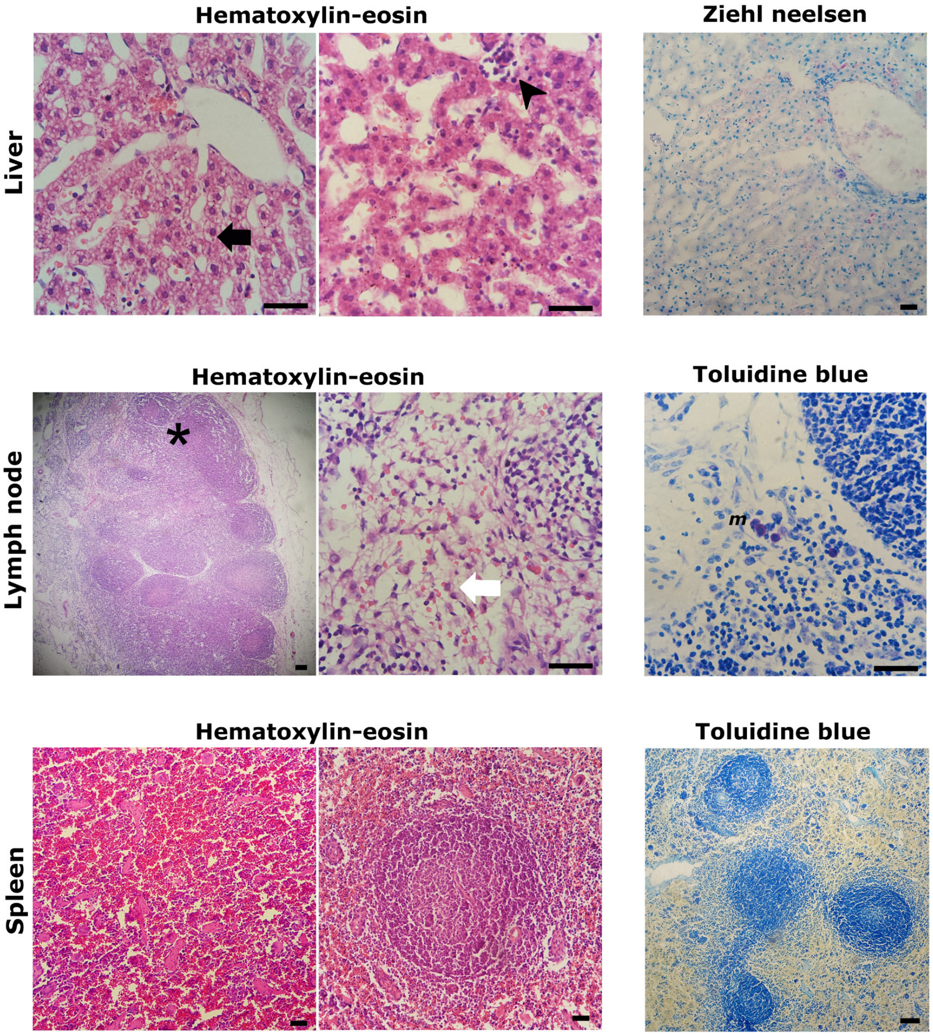

Figure 3: Photomicrograph of the liver, mandibular lymph node and spleen of the six-banded armadillo (*Euphractus sexcinctus*)**.** Top left and middle images show microvacuolar degeneration around the centrolobular vein (black arrow; 500x; 50µm), accompanied by diffuse infiltrate with focal clusters of inflammatory cells (arrowhead; 500x; 50µm). Negative diagnosis for acid-fast bacilli via Zihel-Neelsen staining is shown in the top-right image (200x; 50µm). Middle images show lymph nodes with interfollicular reactive hyperplasia (asterisk; 40x; 200µm), the presence of histiocytes in the medullary sinus (white arrow; 500x; 50µm), and mast cells around paracortical lymphoid follicles (*m*; 500x; 50µm - Toluidine Blue). Bottom images show reactive hyperplasia of the white and red pulp in the spleen (100x, 200x – H&E; 100x - Toluidine Blue).

Results and Discussion

The six-banded armadillo in this report presents clinical characteristics indicative of mistreatment in captivity, which was suggested by the apathy and the injuries found, mainly on peripheral areas. The anatomical changes in the liver, indicative of fatty degeneration, may reflect a metabolic state induced by environmental factors such as stress or systemic infection.

However, a study envolved Dasypus novencinctus and Euphractus sexcinctus infected by Micobacterium leprae were full of dermal ulcers in nodular aspect (Frota et al., 2012). Lesions found in armadillo in this experiment may suggest to nerve damage subsequent to sensitivity loss caused by the etiological agent of leprosy, since they are animals that have a habit of underground digging in search of their food, but the injuries are inconclusive to diagnosis. Similar lesions can be found in the humans foots diagnosed with leprosy and experiencing metabolic diseases, such as diabetes (Boulton, 2012).

Typically, triglycerides are ingested with food and cleaved by the lipase enzyme into fatty acids and glycerol, which are stored in the liver (Jensen-urstad & Semenkovich 2012). Since the body needs energy, the liver triglycerides are converted into free fatty acids in the circulation and oxidized to produce metabolic energy. Several factors require the production of free fatty acids, among which are the hormones cortisol, adrenaline, and noradrenaline (released in situations of stress) (Moon et al., 2002), and toxins produced by pathogenic microorganisms, for example, *Mycobacterium spp*, *Leishmania spp* and *Trypanosoma spp* (Coash et al., 2012).

Stress can be considered a triggering factor of mobilization of lipids to the liver, as it causes the chronic release of free fatty acids into the bloodstream and storage in the liver in the form of triacylglycerides (Rodrigues, 2009). The proposed stress-related mechanism for liver steatosis is based on the notion that the release of catecholamines (adrenalin and nor-adrenaline) promotes the activity of lipases (specifically, hormone sensitive lipase, which preferentially hydrolyses diacylglicerol generated by the activity of adipose triglyceride lipase or ATGL) (Jaworsku et al., 2007, Nieminen et al., 2009). This in turn results in mobilization of fatty acids from adipose tissue to the blood, ultimately favoring the accumulation of hepatic triglycerides (Rodrigues, 2009, Silva, 2012).

Alternatively or complementing the above described hormone-dependence mechanism, chronic stress might lead to liver steatosis via an inflammation-mediated process. This possibility is supported by observations of an enhanced expression of different pro-inflammatory cytokines by visceral adipose tissue in a rodent experimental model of stress-induced steatohepatitis (Liu et al., 2014). In addition to stress, liver steatosis can be related to endocrine, nutritional, or metabolic disorders, as well as other inflammatory diseases of the liver, pancreas and adjacent structures (Richter, 2005).

Recently, researchers reported an occurrence of hepatic lipidosis in six banded armadillo bred in captivity. However, the pathogenesis is a consequence of the caloric diet that animals receive leading to obesity and the diffuse intracytoplasmic vacuolation of hepatocytes without compromising the lymphoid system organs (Batista et al., 2016).

Among other inflammatory processes that can cause liver disease are those associated with pathogen infection. In support of this possibility, we observed reactive hyperplasia in submandibular lymph nodes and spleen. Reactive interfollicular hyperplasia with a high component of cells of the monocyte-phagocyte lineage has been associated with active presentation of pathogen-derived antigens (Keller et al., 1972, Ozawa, 2015). Whether the observed reactivity is the result of a viral, bacterial or parasitic infection remains to be determined. We were not able to detect intracellular pathogens via Zihel-Neelsen or Toluidin blue, stains useful in detecting pathogens of the genus *Leishmania* (Tryphonas, 1977), and *Mycobacterium* (Kumar, 2015). More specific tests for these and other pathogens will be required to establish a definitive diagnoses.

In wild small animals, cases of hepatic steatosis have been diagnosed in tiger cats (Martins et al., 2016), mustelids (Nieminen et al., 2009), birds, rodents (Couto, 2007, Silva & Goncalves, 2008, Spinelli et al., 2014) and armadillos (Batista et al., 2016). The majority of these cases were associated to fasting accompanied by environmental stress, although some cases involved a high-calorie diet or inflammatory disease, as was the case of cholangiohepatitis diagnosed in tiger cat (*Leopardus tigrinus* Schreber, 1775) (Martins et al., 2016) and the hepatic lipidosis in armadillos bred in captivity (Batista et al., 2016). To the best of our knowledge, this is the first report of fatty liver disease in an armadillo suggests an acute infectious process with lymphoid system involvement. Armadillos are endangered animals, which have been heavily exploited for hunting, food and popular culture, and at the same time are involved in numerous cases of zoonosis (Richini-Pereira et al., 2014, Elsayed et al., 2015). Clinical case reports describing spontaneous pathological states in these animals are key for the improvement of natural and, when necessary, captivity environments, for both the conservation of these species and the development of zoonosis-related research.

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Author's Contributions

- ABSS: Substantial contribution in the concept and design of the study and contribution to data collection;
- MSR: Contribution to data analysis and interpretation;
- AVP: Contribution to manuscript preparation;
- AMCJ: Contribution to critical revision, adding intelectual content

Conflicts of interest

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

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article

Evaluation of collared anteaters (*Tamandua tetradactyla*) presented in a wildlife health reference center of São Paulo state, Brazil

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Abstract: Deforestation and road construction have inflicted negative effects on wildlife populations. To contribute to the comprehension and preservation of wildlife species, this study aimed to evaluate collared anteater presented to a reference center located in São Paulo state, Brazil. Furthermore, the locations where the animals were most frequently found were determined. Data on collared anteaters (*Tamandua tetradactyla*) examined at the Center for Medicine and Research in Wild Animals (CEMPAS) were evaluated retrospectively for a period of 13 years. A total of 46 collared anteaters was examined, comprised of 14 males, 10 females, and 22 without identifying information. On average, adult males weigh 4.41 kg while adult females weigh 4.95 kg. The most common admission circumstance was vehicular strikes (36.96%), followed by dog bites (21.74%); 23.91% were considered clinically healthy. Animal outcomes were 36.96% (n=17) reintroduced, 34.78% (n=16) fatalities, 15.22% (n=7) transferred to other institutions, and 6.52% (n=3) escaped from the enclosure; and 6.52% (n=3) presented no medical record information. The collared anteaters were found mainly in the municipal areas of Botucatu (21.73%), São Manuel (17.39%), Lençóis Paulista (6.52%), and Bauru (4.34%), with SP-300 being the main highway associated with the occurrences. In conclusion, the main cause of lesion in the collared anteaters evaluated in this study was vehicular strike that that led to death. However, including all collared anteaters at least half of them had a positive outcome.

Keywords: *Wild animal, highway, clinical evolution, anteater.*

Avaliação de tamanduás-mirins (*Tamandua tetradactyla)* atendidos em um centro de referência de animais selvagens do Estado de São Paulo, Brasil

Resumo: O desmatamento e a construção de estradas têm promovido uma série de problemas à fauna. Visando contribuir com informações que podem ser usadas na compreensão e preservação das espécies, o trabalho teve por objetivo avaliar tamanduás-mirins atendidos em um centro de referência localizado no Estado de São Paulo, Brazil. Foram avaliados retrospectivamente dados referentes a tamanduás-mirins (*Tamandua tetradactyla*), que foram trazidos ao Centro de Medicina e Pesquisa em Animais Selvagens (CEMPAS), durante um período de 13 anos. De um total de 46 tamanduás-mirins atendidos, 14 foram machos e 10 foram fêmeas, e 22 não possuíam identificação quanto ao sexo. Em média, os machos adultos pesaram 4,41 kg, enquanto as fêmeas adultas pesaram 4,95 kg. Com relação à circunstância que levou ao atendimento, as mais importantes foram o atropelamento (36,96%) e a mordedura de cães (21,74%), sendo que 23,91% foram considerados clinicamente saudáveis. No tocante à evolução dos casos, 36,96% (n=17) foram reintroduzidos, 34.78% (n=16) foram a óbito, 15,22% (n=7) foram transferidos para outras instituições, 6,52% (n=3) evadiram do recinto e 6,52% (n=3) não há informação. Os animais foram provenientes principalmente dos municípios de Botucatu (21,73%), São Manuel (17,39%), Lençóis Paulista (6,52%) e Bauru (4,34%), sendo a SP-300 a principal rodovia relacionada as ocorrências. Foi possível concluir que dos tamanduás-mirins avaliados a principal causa de lesão foi o atropelamento, que teve por evolução o óbito; porém ao se incluir os tamanduás-mirins saudáveis e demais, ao menos metade evoluíram positivamente.

Palavras-chave: *Animal selvagem, rodovia, evolução clínica, tamanduá.*

Introduction

Tamandua tetradactyla, also known as the collared anteater or lesser anteater, is a mammal that belongs to the Xenarthra magnorder, Mymecophagidae family (Naugher 2004; Hayssen 2011), and is considered an arboreal and terrestrial opportunist (Naugher 2004; Medri et al. 2006). In South America, the species is distributed over the northern and central regions (Hayssen 2011). The species is found in all types of biomes in Brazil, but in the Pantanal it lives mainly at the edge of the forest (Desbiez & Medri 2010; Miranda 2014).

The collared anteater has a head-body length of approximately 47 to 77 cm, body mass from 2 to 7 kg, with a long prehensile tail used as a fifth limb (Nowak 1999; Naugher 2004; Medri et al. 2006), and tubular mouth without teeth (Naugher 2004; Haysen 2011). The color of the fur is usually pale tan or golden, and may or may not have a black vest or collar (Hayssen 2011; Miranda et al. 2014). Color variation and morphological differences may occur according to territorial origin; for example, in Brazil, individuals from the northern portion of the country present a darker color than those from the Atlantic Forest (Miranda 2014).

Feeding can occur on the ground or on trees and consists of termites, ants, honey and bees (Medri et al. 2006; Smith 2007; Miranda et al. 2014; Gallo et al. 2017). The collared anteater has a well-developed sense of smell that assists in locating food and compensates for poor vision and hearing (Smith 2007; Miranda 2014). Feeding items adhere to the sticky substance secreted by the submaxillary glands on the tongue, which is cylindrical and 40 cm long (Smith 2007).

Deforestation and road construction have caused negative effects on wildlife populations, a fact observed in Amazon state where animals, especially mammals of the order Pilosa, have been struck by vehicles (Costa 2014). This has also occurred in the central region of Brazil (Miranda 2014); furthermore, there are reports of animals injured or killed in the south and southeast of the country (Vieira 1996; Prada 2004; Cherem et al. 2007; Costa, 2011; Orlandin et al. 2015; Preuss 2015).

Aiming to contribute to the comprehension and preservation of wildlife species, the purpose of this study was to evaluate collared anteaters presented to a reference center located in São Paulo state. In addition, the locations where the animals were most frequently found were determined.

Materials and Methods

This study was approved by Ethics Committee for Animal Care and Use of the School of Veterinary Medicine and Animal Science, UNESP Botucatu (no. 33/2016 –CEUA).

Data in relation to collared anteaters (*Tamandua tetradactyla*) examined at the Center for Medicine and Research in Wild Animals (CEMPAS) of the School of Veterinary Medicine and Animal Science, UNESP Botucatu, Brazil, were evaluated retrospectively for a period of 13 years (2003–2016).

Identifying data on collared anteaters (sex, body mass), municipal area where animal rescue occurred, circumstances of admission, time of hospitalization, and case outcome (reintroduction, place of destination, death) were analyzed. A map of São Paulo state enabled estimation of the locations where the largest numbers of animals were found.

A descriptive analysis was made of the admission circumstances, case outcome and hospitalization duration. Spearman correlation test was used to assess the correlation between sex (male/female) and circumstances of admission, number of hospitalization days and case outcome (reintroduction, place of destination, death, escape from the enclosure). The correlation between survival/death and cause of hospitalization, number of hospitalization days and case outcome (reintroduction, place of destination, death, and escape of the enclosure) were also evaluated by Spearman correlation test. The Spearman correlation coefficient was interpreted as follows: r 0.1-0.3 = weak; 0.4-0.7 = moderate; 0.8-1.0 =strong (Bhuyan et al. 2016). Incomplete data in the medical record that created inconsistency were excluded from statistical analysis.

The comparison of the collared anteater divided into survival versus death groups was carried out using Mann-Whitney test for non-parametric data (cause of hospitalization and sex), and unpaired *t* test for parametric data (days until outcome). For comparison of the animals allocated into two groups according to sex (males vs. females), the Mann-Whitney test for nonparametric data (cause of hospitalization, outcome and days until outcome), and unpaired *t* test for parametric data (body mass) were used. The animals that survived were also allocated into groups according to the disease to compare days until outcome by one-way analysis of variance (ANOVA). Differences were considered significant at $P < 0.05$.

Results

A total of 46 collared anteaters was examined, comprised of 14 males, 10 females and 22 without identifying information. On average, adult males weigh 4.41 kg (SD = 1.83) while adult females weigh 4.95 kg (SD = 1.53). As to the admission circumstances: 36.96% (n=17) of the collared anteaters had been struck by vehicles; 23.91% (n=11) were considered clinically healthy; 21.74% (n=10) had been bitten by dogs; 4.34% (n=2) had suffered burns; 6.52% (n=3) were hospitalized due to other causes (gastroenteritis, eye injury, prostration); and for the remaining 6.52% (n=3), there was no information in the medical record (Fig. 1). The days of hospitalization associated with admission circumstances were: from 0 to 300 days (Mean ± SD = 48.73 ± 92.64) for those struck by vehicles; from 1 to 300 days for animals bitten by dogs (Mean ± SD = 28.56 ± 46.33); from 8 to 10 days for those that suffered burns (Mean ± SD = 9 ± 1.41); from 1 to 105 days for those hospitalized due to other cause (Mean ± SD = 35.67± 60.04); from 1 to 9 days (Mean ± SD = 2.4 ± 2.84) for healthy animals.

In relation to animal outcomes, 36.96% (n=17) were reintroduced, 34.78% (n=16) died, 15.22% (n=7) were transferred to other institutions, and 6.52% (n=3) escaped from the enclosure; there was no information in the medical record of the remaining 6.52% (n=3). Among the anteaters that had been struck by vehicles, 47.60% (n=8) died, 23.53% (n=4) were transferred to other institutions, 23.53% (n=4) were reintroduced, and 5.88% (n=1) escaped from the enclosure. All the anteaters that had suffered burns died (n=2). Considering the collared anteaters that had been bitten by dogs, the outcomes were: 40% (n=4) reintroduced, 30% (n=3) fatalities, 20% (n=2) transferred to

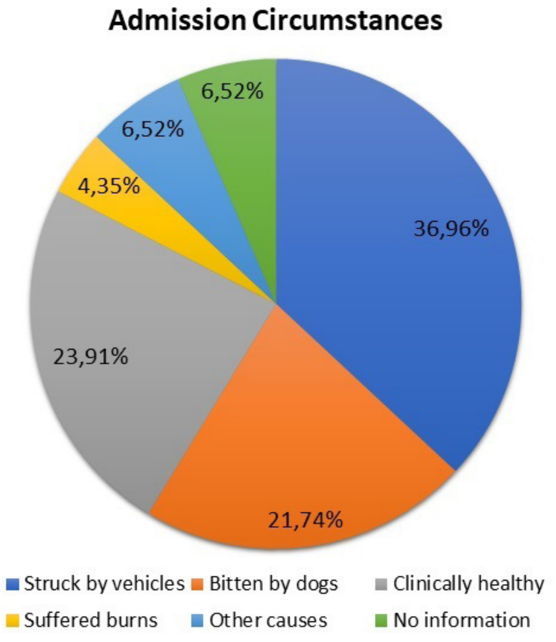

Figure 1. Admission circumstances of collared anteaters presented in a wildlife health reference center.

other institutions, and 10% (n=1) without information in the medical record. Among the animals considered clinically healthy anteaters, 72.73% (n=8) were reintroduced, 9.09% (1) escaped from the enclosure, and 9.09% (n=1) died; for the remaining 9.09% (n=1), there was no information. As to the animals hospitalized due to other causes (n=3), the outcomes were one death (33.33%; n=1), one reintroduction and one transference to another institution. Of those animals without information about admission circumstances, 67.7% (n=2) died, while the outcome of 33.33% (n=1) was not registered.

The statistical analysis did not show correlation between sex (male/female) and cause of hospitalization (r = -0.026; P = 0.9032), days of hospitalization (r = 0.043; P = 0.8483) or outcome of the cases (r = -0.257; P = 0.2374). No correlation was observed between survival/death and cause of hospitalization (r = 0.3635; P = 0.195), days of hospitalization (r = 0.1365; P = 0.3948), or sex (r = 0.2390, P = 0.2606). There was a significant correlation between survival/death and case outcome (r = 0.8914, P = 0.0001).

No statistical differences were detected between survival versus death groups or sex (P = 0.3503), body mass (P = 0.3402) and days of hospitalization (P = 0.1043). However, a significant difference occurred in relation to cause, given that every animal that died had been struck by a vehicle (P = 0.0287). No statistical differences were detected between groups according to sex (males vs. females) and cause of hospitalization (P = 0.9314), outcome (P = 0.7088), days until outcome (P = 0.8718) or body mass (P = 0.3402).

The collared anteaters were found mainly in the municipal areas of Botucatu (21.73%; n=10), São Manuel (17.39%; n=8), Lençóis Paulista (6.52%; n=3), and Bauru (4.34%; n=2). Only one collared anteater was found in each of the following municipal areas: Agudos, Areiópolis, Assis, Avaí, Bofete, Cabrália Paulista, Cerqueira César, Conchal, Pardinho, and Tupã, (2.17%). There was no information in the medical record of 13 (28.26%) animals.

The São Paulo state map indicated that the main highway associated with the findings of these animals was SP-300 (Fig. 2).

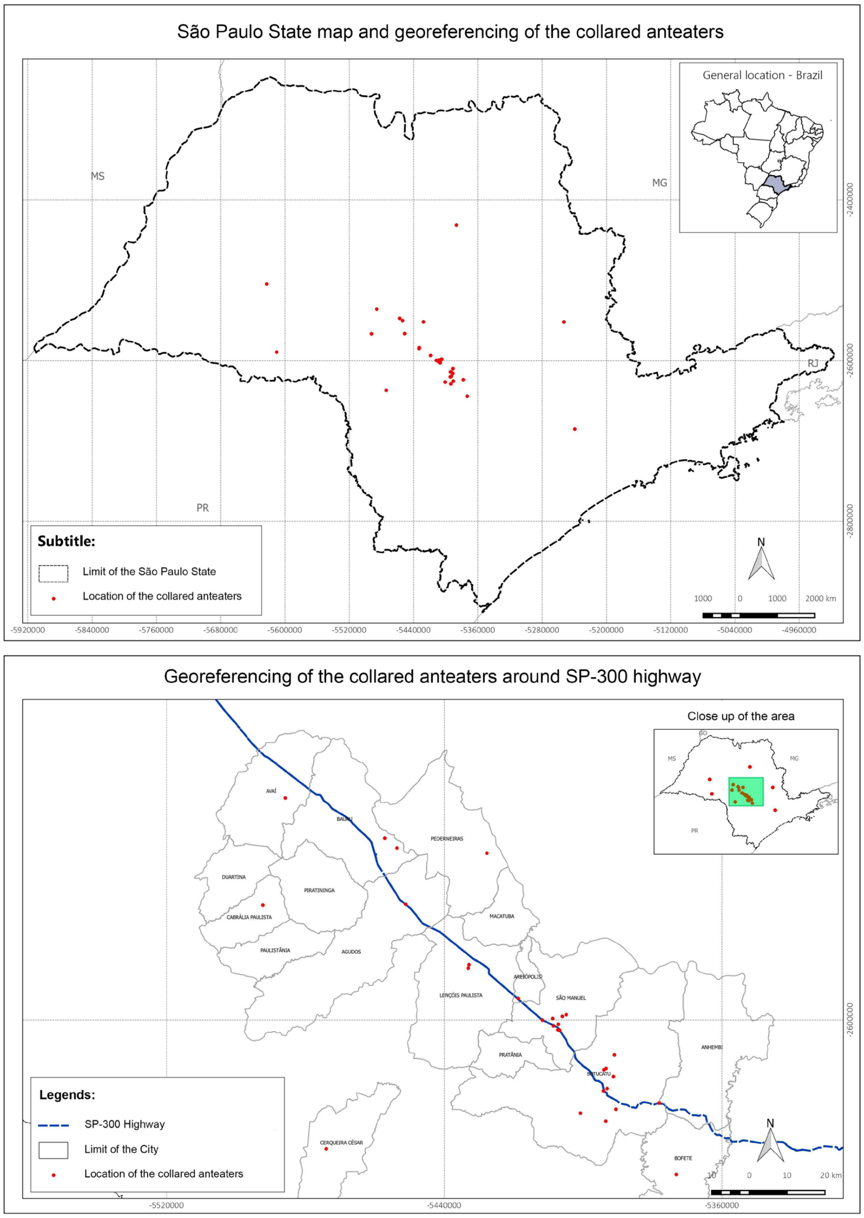

Figure 2. Map of São Paulo State showing the places where collared anteaters were found. Note that the SP-300 was the main highway associated with the occurrences.

Discussion

Due to their broad distribution, the collared anteaters are ranked as Least Concern on the red list of Threatened Species by the IUCN (International Union for Conservation of Nature and Natural Resources) (Miranda et al. 2014). Nevertheless, the reduction of the species is associated with vehicular accidents, fire, habitat loss, hunting and dog attacks (Medri et al. 2006; Hayssen 2011; Miranda 2014). In the present study, except for the collared anteaters that were considered clinically healthy, vehicular strikes and dog bites were the main causes of admission.

One of the reasons for these findings may be related to the solitary habits of collared anteaters that are found together only during the mating season (Naugher 2004; Rezende et al. 2013; Miranda et al. 2014). This makes them more vulnerable to being attack by other animals, despite taking refuge in tree-holes, as well as in ground nests (Nowak 1999; Smith 2007). Moreover, although captive collared anteaters may be active during the day, their nocturnal to diurnal-crepuscular activities are the most common in nature (Hayssen 2011), which corroborates their being hit by cars. In addition, these animals present slow-moving and placid behavior (Smith 2007; Miranda 2014).

The present study has found a significant correlation between survival/death and case outcome, given that most of the animals that died had been struck by vehicles. Thus, injury severity and fatality risk are very high when collared anteaters are struck by vehicles. In addition, the distribution of locations where the animals were found on the São Paulo state map revealed the importance of the SP-300 highway. Several authors have reported the negative consequences of constructing highways or roads on wildlife, since they affect, directly and indirectly, the populations of numerous species of wildlife, constituting an important cause of habitat loss and fragmentation (Cherem et al. 2007; Costa 2014; Miranda 2014). In studies related to the number of mammals species hit by cars on roads and highways in Brazil, the following percentages were found related to the collared anteaters: 1.4%, 1.51%, 5.4%, 8.6%, 10.12%, and 21% respectively, in relation to six roads in the northeast region of São Paulo state (Prada 2004), five highways in western Santa Catarina (Orlandin et al. 2015), three roads in Santa Catarina state (Cherem et al. 2007), Brasilia-Campinas highway (Vieira 1996), the highway BR-282 located in the west of Santa Catarina state (Preuss 2015). and BR 101 road along of the cost of Santa Catarina state (Costa 2011). Thus, more studies are necessary to determine the roads impact in wild animal populations in each State of Brasil.

Although there was no correlation between sex and cause of hospitalization, days of hospitalization, or outcome of the cases, it should be considered that only 24 out of the 46 collared anteaters had their sex identified in the medical record (14 males and 10 females). There are citations that the estrous cycle of collared anteaters lasts approximately 42 days, whereas the gestation period may be 85-190 days (Hayssen 2011; Rezende et al. 2013). In general, the female gives birth to one offspring per year (Miranda et al. 2014), then carries the newborn on her back for several months, or even for a year (Smith 2007; Miranda 2014). Thus, the death of collared anteaters could cause significant loss of species.

On the other hand, it is important to emphasize that including all healthy and unhealthy or injured collared anteaters (n=46) presented for examination, 17 were reintroduced (36.95%) and seven were sent to another institution (15.21%), indicating that at least half of the cases had a positive outcome. The reintroductions were performed with the authorization of the competent authorities, and was done in fragments of forest near the region where the animal was found, or in places registered by the Ministry of the Environment.

In conclusion, the main cause of lesion in the collared anteaters evaluated in this study was vehicular strike that that led to death. However, including all collared anteaters at least half of them had a positive outcome.

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Author Contributions

Agda Maria Bernegossi - Substantial contribution in the concept and design of the study, Contribution to data collection

Sheila Canevese Rahal - Substantial contribution in the concept and design of the study

Alessandra Melchert - Contribution to data analysis and interpretation

Carlos Roberto Teixeira, Fábio Henrique de Lima - Contribution to critical revision, adding intelectual content

Raphael Duarte Medeiros, Aline Alves da Silva - Contribution to manuscript preparation

Conflicts of Interest

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

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Trends and gaps of the scientific literature about the effects of fire on Brazilian Cerrado

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ARRUDA, F. V., SOUSA, D. G., TERESA, F. B., PRADO, V. H. M., CUNHA, H. F., IZZO, T. J. **Trends and gaps of the scientific literature about the effects of fire on Brazilian Cerrado.** Biota Neotropica. 18(1): e20170426. http://dx.doi.org/10.1590/1676-0611-BN-2017-0426

Abstract: Fire management is an important issue in the Brazilian Cerrado, since both anthropogenic high intensity fires and complete fire suppression can reduce the biodiversity in this biome. In this paper, we highlight the trends in scientific literature about fire effects in the Cerrado, aiming to detect possible gaps and to indicate directions of future scientific research. We searched for articles in the periodic database Web of Knowledge from 1991 to 2016, and observed an increase in the number of publications throughout the years. Most articles were associated with Brazilian institutions (58%), followed by those with collaboration between Brazilian and international institutions (33%), and those published by authors exclusively from international institutions (9%). Most articles addressed the effects of fire on biodiversity (77%), followed by articles about abiotic environment (19%), and then biotic interactions or interactions between organisms and environment (4%). The most studied taxonomic group was plants (75%), followed by mammals (8%) and insects (6%), with the remaining taxa comprising about 11% of publications. The Federal District was the federative unit with the greatest number of studies (31%). The majority of studies was conducted in areas with fewer fire events, whereas areas with major incidence of fires are poorly studied. Our data shows that studies on the effect of fires on the Brazilian Cerrado are geographically and taxonomically biased. This lack of knowledge limits the extrapolations about the effects of fire on this biome. Therefore, we emphasize the need for investment in research in areas with high fire frequency and also for an increase in knowledge about these effects on the biota, especially on the fauna. This action is fundamental to support the development of public policies for effective and directed fire management in the Cerrado.

Keywords: *Burning, Scientometrics, Biodiversity Hotspots, Savanna, Perturbation.*

Tendências e lacunas na literatura científica sobre os efeitos do fogo no Cerrado Brasileiro

Resumo: O manejo do fogo é uma questão importante no Cerrado brasileiro, pois tanto os incêndios antropogênicos de alta intensidade, quanto a sua supressão completa podem reduzir a biodiversidade neste bioma. Neste artigo, destacamos as tendências na literatura científica sobre os efeitos do fogo no Cerrado, com o objetivo de detectar possíveis lacunas e indicar direções de futuras pesquisas científicas. Buscamos artigos na base de periódicos Web of Knowledge no período de 1991 a 2016, e observamos um aumento no número de publicações ao longo dos anos. A maioria dos artigos foi associada a instituições brasileiras (58%), seguidos por colaborações entre instituições brasileiras e internacionais (33%) e publicações de autores exclusivamente de instituições internacionais (9%). A maioria dos artigos abordou os efeitos do fogo na biodiversidade (77%), seguido de artigos sobre o ambiente abiótico (19%), e interações bióticas ou interações entre organismos e o ambiente (4%). O grupo taxonômico mais estudado foi plantas (75%), seguido de mamíferos (8%) e insetos (6%). Os táxons restantes incluem 11% das publicações. O Distrito Federal foi a unidade federativa com o maior número de estudos (31%). A maioria dos estudos foi realizada em áreas com menos queimadas, enquanto as áreas com maior incidência de fogo são mal estudadas. Nossos dados mostram que os estudos sobre o efeito do fogo no Cerrado brasileiro são enviesados do ponto de vista geográfico e taxonômico. Esta falta de conhecimento limita as extrapolações sobre o efeito do fogo neste bioma. Portanto, enfatizamos a necessidade de investimentos para pesquisas em áreas com alta freqüência de queimadas e a necessidade em aumentar o conhecimento sobre estes efeitos na biota, especialmente na fauna. Esta ação é fundamental para apoiar o desenvolvimento de políticas públicas para uma gestão efetiva e direcionada do fogo no Cerrado.

Palavras-chave: *Queimadas, Cienciometria, Hotspots de Biodiversidade, Savana, Perturbação.*

Introduction

The Brazilian Cerrado comprises an area of 2 million km^2 and is home to a rich and diverse fauna and flora (Ribeiro & Walter 2008). In this biome, there are more than 1,000 terrestrial vertebrates and 12,000 plant species (Myers et al. 2000, Mendonça et al. 2008), and about 80% of those plant species are endemic (Lenthall et al. 1999). Given its wide range, the Cerrado is constituted by different phytophysiognomies that vary from open areas to forest formations (Oliveira-Filho & Ratter 2002, Lenthall et al. 1999). Therefore, we cannot assume that the Brazilian Cerrado is homogeneous since it hosts a large variation in soil structure, geomorphological formation, drain basins and associated vegetation (Silva et al. 2006, Furley 1999).

The Cerrado exhibits remarkable characteristics such as (i) poor, acidic soil with a high concentration of aluminum (Queiroz-Neto 1982, Reatto et al. 1998), (ii) climatic seasonality with well-defined rainy and dry seasons, and (iii) resistance to fire (Coutinho 1990). In fact, fire has been considered the prevalent force driving the evolution of biota in this biome (Simon et al. 2009). Fire can influence community composition (Oliveras et al. 2012, Vieira & Briani 2013, Silvério et al. 2015, Abreu et al. 2017), soil properties and regional climate (Bustamante et al. 2012, Wendling et al. 2014). In fact, fire can act as either a natural or an anthropic disturbance in the Cerrado. In non-protected areas, the frequency and intensity of fires has increased due to recent expansion of agriculture and livestock, with potential negative effects on the biota (Bowman et al. 2011). However, this issue is controversial since some studies have shown that fire frequency is reduced in protected areas of the Cerrado, with negative consequences for biodiversity (Moreira 2000, Roitman et al. 2008, Cardoso et al. 2009, Pinheiro & Durigan 2009, Pinheiro et al. 2010). Thus, not only the increase but the changes in the frequency of fires are of great concern for the conservation of the Cerrado's biodiversity (Pivello 2011, Abreu et al. 2017).

Several studies have been conducted to understand the effects of fire on different environmental aspects of the Cerrado (e.g. Tubelis et al. 2009, Fagundes et al. 2015, Abreu et al. 2017). Indeed, the understanding of the effects of fire and also of fire suppression on biodiversity and ecosystem function is fundamental to a satisfactory establishment of public policies focusing on natural resource management (Durigan & Rattter 2016). Nevertheless, due its wide range, the Cerrado is also a spatially heterogeneous biome (Silva & Bates 2002). Considering that fire or fire suppression can have different effects in different regions, there should be studies conducted across a wide geographical range, and spatially replicated across the biome. The predominance of studies in the vicinity of traditional research centers (Nabout et al. 2015) or the non-equitable spatial distribution of infrastructure providing unequal access to many areas may generate a geographic asymmetry on the development of studies in the Cerrado. Also, the occurrence and frequency of wildfires are not spatially homogeneous (Pereira Júnior et al. 2014). Thus, the spatial variation in fire events may also benefit the development of studies, given the urgency in obtaining parameters for fire management in those environments.

In this study, we performed a scientometric evaluation of the studies related to fire in the Cerrado biome, seeking to understand the temporal and spatial trends in publications about this theme. Specifically, we (i) evaluated the temporal increment of the number of articles; (ii) highlighted the main taxonomic groups or themes addressed in the studies; (iii) determined the journals with the most publications about fire in the Cerrado; (iv) evaluated the possible biases in the location where studies were conducted and the fire frequency in the Cerrado; and (v) indicated directions for future studies.

Material and Methods

We used the Web of Science database (www.isiknowledge.com) to access articles about the influence of fire in the Cerrado biome. We considered publications from 1991 to 2016, since abstracts are only available in this database from 1991 to 2016, and this research is based on abstracts. The search was restricted to articles using the words ("fire*") AND (("savanna*") OR ("cerrado*")) AND ("Brazil*") in the topics.

For each article, we evaluated the title, keywords and abstract, to collect the following information: (1) publication year; (2) the institution, the nationality and state of the institution where the authors were associated at the time the manuscript was written; (3) taxonomic group (i.e. humans, mammals, amphibians, birds, reptiles, fishes, insects, non-insect invertebrates, microorganisms, plants and fungi); (4) journal in which it was published; and (5) geographic location(s) of the research.

To assess the temporal trend in the number of articles, we used Pearson correlation between the year of the publication and the number of articles published in that given year. The same test was performed separately for each taxonomic group to check the temporal trend in articles by taxonomic group. We also used Pearson correlation to test separately the relation of the number of authors and first authors by state with the frequency of fire occurrences in that given state. In this case, the states were considered as sample units since data on fire occurrence are easily available on the *Instituto Nacional de Pesquisas Espaciais* (INPE) website (https://queimadas.dgi.inpe.br/queimadas). The Federal District is not a formal state, but was considered a sample unit in the present study. These data refer to fire events from 1992 to 2015. We then built maps with the number of fire events as well as with the number of published papers within this period for each state. One limitation of the INPE dataset is that it does not distinguish fire occurrences between natural vegetation and crops or pastures. We recognize this limitation but we acknowledge that fire events in crops and pastures pose a high risk for the natural vegetation of the Cerrado. Therefore, these limitations do not detract our inferences about the relation between fire frequency and number of studies in the states of this biome.

Results

We found 288 articles from 1991 to 2016 addressing fire in the Brazilian Cerrado. Among these articles, 166 (58%) have authors associated only with a Brazilian institution. We counted a total of 96 articles with collaboration between Brazilian and international institutions (33%) and a small part (26 articles, 9%) published only by authors affiliated with international institutions.

We found an increasing temporal trend in the number of articles published over the years ($r = 0.86$; $p < 0.001$) (Figure 1). Most articles (223, totaling 77%), addressed the effect of fire on biodiversity. Another 53 (18%) focused on the association between fire and abiotic variables. Among the studies about abiotic factors, the highlighted factor

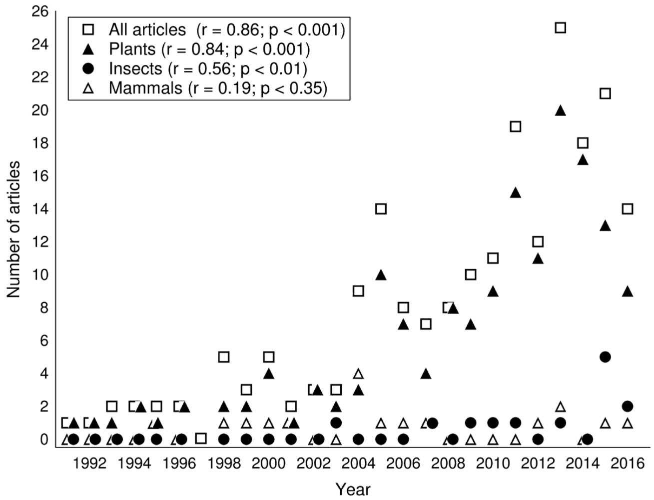

Figure 1. Temporal tendency of the total number of articles and number of articles involving insects, mammals and plants related to fire in the Cerrado from 1991 to 2016

was the effect of fire on soil and nutrients. Only 12 articles (4%) reported interactions (biotic interactions or interaction between organisms and the abiotic environment).

We identified the studied taxa in 207 articles and observed the predominance of studies involving plants, 155 publications (75%), followed by mammals with 17 (8%) and insects with 13 (6%) publications (Figure 2). The remaining taxa comprise 22 publications (11%) and each one has less than 10 publications (Figure 2). Among the taxa with the highest number of publications, plants ($r = 0.84$; $p < 0.001$) and insects ($r = 0.56$; $p < 0.01$) showed an increase in the number of articles across the years (Figure 1). We did not observe any temporal trend in the number of publications for mammals ($r = 0.19$, $p = 0.35$) (Figure 1). Due to the low number of studies, we did not perform inferential analyses to microorganisms, lichens, reptiles, humans, invertebrates (except insects), and birds.

We found 115 journals with articles addressing fire in the Cerrado. However, the number of articles published in these journals is asymmetric, since about 40% of them were published in only 13 journals. The journal *Acta Botanica Brasilica* published the highest number of articles (20 articles) about this theme, representing 7% of the total (Figure 3).

We identified the state in 285 articles and 14% of these studies were performed in more than one state (39 studies). For studies restricted to just one Brazilian political unit, the major part of them (89 or 31%) was done in the Federal District (Figure 4). The states of Minas Gerais and Goiás (35 studies or 12% each), Mato Grosso and São Paulo (28 or 10% each) are where most of the research was conducted (Figure 4). About 11% of the publications were performed in the remaining states (Tocantins, Roraima, Pará, Mato Grosso do Sul, Bahia, Amapá, Maranhão, Piauí and Rondônia) and each State has less than 5% of all publications (Figure 4).

The number of studies performed in each state was positively correlated with the number of first authors with an institutional address associated with that state ($r = 0.80$, $p < 0.001$). The fire frequency in each state was not correlated with either the number of articles per state ($r = 0.08$, $p = 0.73$) nor with the number of first authors affiliated to an institution located in the state ($r = -0.13$, $p = 0.59$). The spatial distribution of these three variables

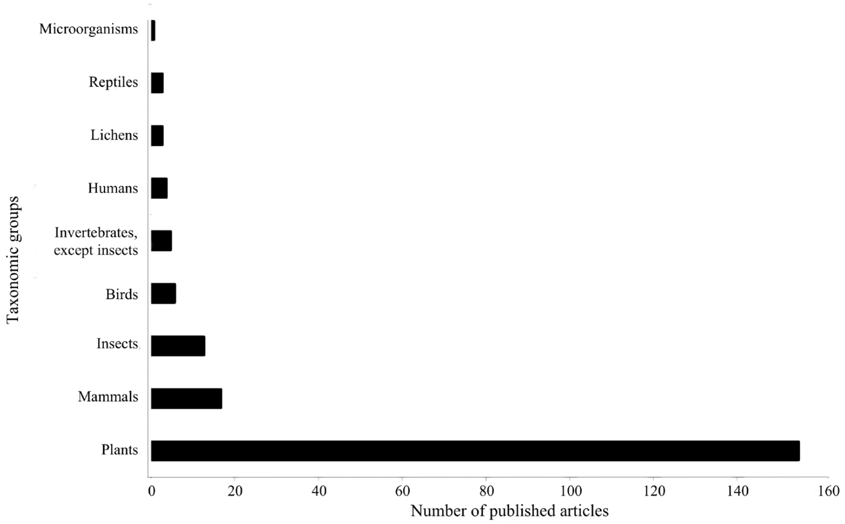

Figure 2. Taxonomic groups addressed in scientific articles about fire in the Cerrado from 1991 to 2016

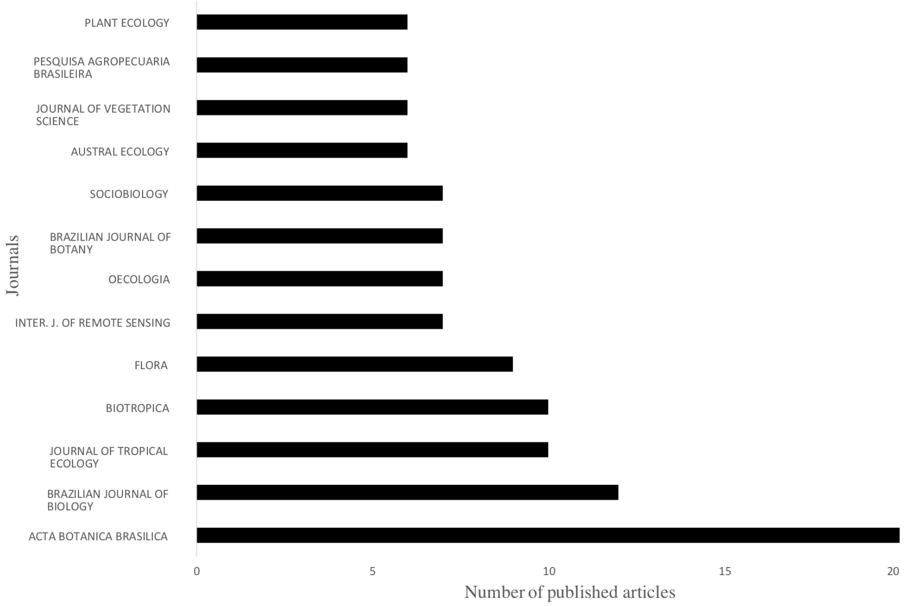

Figure 3. Journals with the highest number of articles about fire in the Cerrado from 1991 to 2016

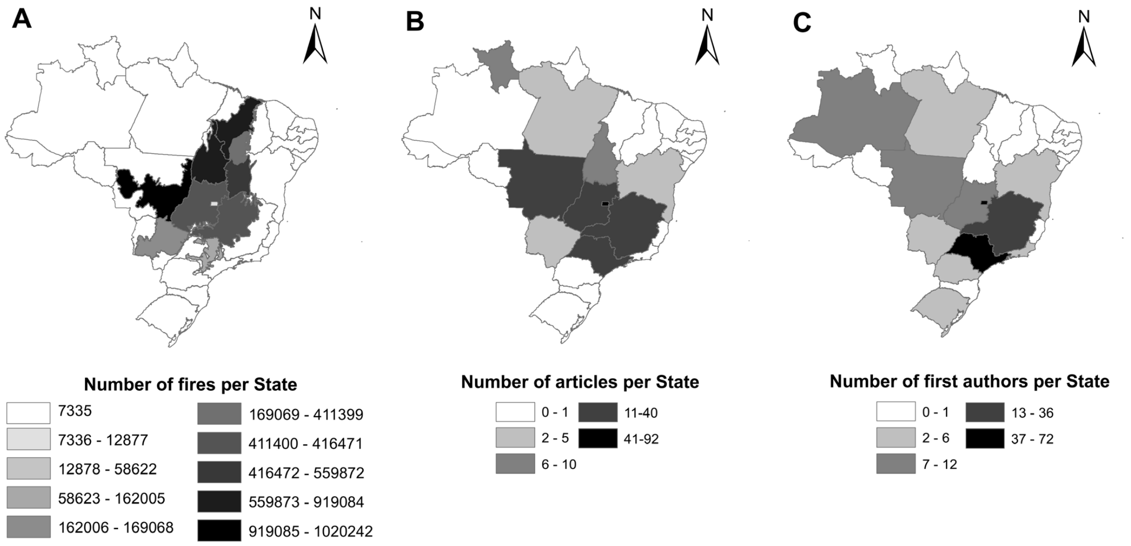

Figure 4. Geographic distribution per Brazilian State of (A) number of fires events (from 1992 to 2015 available at https://queimadas.dgi.inpe.br/queimadas), (B) number of articles and (C) number of first authors affiliated

demonstrates asymmetry in the distribution of fire occurrence, studies location and researchers' institutional affiliation (Figure 4). Fire occurrence is more frequent in the Cerrado areas bordering the Amazon rainforest (Figure 4), while location where research was conducted and affiliation of researchers are mainly in the southeast and central-west states of Brazil (Figure 4).

Discussion

We found that the number of publications about fire in the Cerrado has increased over the years and this trend was about the same for the literature involving the Cerrado (r = 0.86 in the present study *vs.* r = 0.89 in Borges et al. 2015). Thus, the interest about fire in the Cerrado reflects the interest in the biome by itself. Additionally, the growing interest in the effects of fire in the Cerrado may be due to the biome complexity: both high and low frequencies of fire can have negative effects on biodiversity (Anjos et al. 2016, Maravalhas & Vasconcelos 2014, Abreu et al. 2016), and proper fire management programs based on scientific knowledge are still in development **(**Durigan & Ratter 2016). Our results also indicate that most publications were about the effects of fire on plants, and were performed in states with fewer fire events. These asymmetries limit generalizations of the observed patterns that may not be applicable to the whole biome. In Brazil, there is a growing demand to create fire policies to conserve the Cerrado (Durigan & Ratter 2016). Thus, an unbiased knowledge about the effects of fire on different taxa and regions of the biome is desirable to create clear guidelines for future fire management policies.

Even though fire has become an international issue due to its biodiversity and atmospheric consequences (Miranda et al. 2009, Bustamante et al. 2012), most studies were conducted by researchers from Brazilian institutions. These results demonstrate the importance of Brazilian universities and research institutions in this field. Thus, the recent cuts in Brazilian research investments (Fearnside 2016, Gibney 2015, Angelo 2017) may directly impact the generation of knowledge applied to Cerrado preservation, especially due to the lack of scientific background in fire management policies and consequences of both fire effects and fire suppression.

Although the number of journals publishing articles related to fire in the Cerrado biome is high, the distribution of the theme among them is clearly asymmetrical. A small number of journals is responsible for the great majority of publications. The Brazilian journals *Acta Botanica Brasilica* and *Brazilian Journal of Biology* stand out. Nevertheless, besides these two journals, international journals dominate the articles publication assessing fire in the Cerrado. This demonstrates the global interest in this subject, which is directly related to greenhouse gas emissions and climate change, which are currently two of the greatest issues facing humanity (Moraes et al. 2011). Moreover, similar to the Cerrado, there are other biomes where fire exerts a constant pressure. This demonstrates that the issue of fire is not only applicable to a regional context.

Among the different studied taxa, plants were the most frequent in articles about fire in Cerrado (Borges et al. 2015). Since the journal *Acta Botanica Brasilica* has a botanical perspective, this may be a possible reason for the higher number of publications about fire and vegetation in the Cerrado in this journal. Furthermore, the great vegetation diversity, the high endemism rates and the high risk of species extinctions (Myers et al. 2000) seem to explain the higher interest about the effects of fire in plants. Also, the plant's adaptations to fire are diverse and attract interest in this field (*e.g.* Eiten 1972, Ratter et al. 1997, Simon et al. 2009). Moreover, there are fewer studies performed on other taxa, which demonstrates the insufficient knowledge regarding the effects of fire on the general biodiversity. By reviewing the studies regarding the effect of fire on the Brazilian savanna fauna, Frizzo et al. (2011) found a great shortage of studies, which according to them, besides limiting knowledge, might induce generalizations that do not reflect the real effects of fire on animal communities. The fact that some animal taxa have a different number of specialists, demands more complex logistic and infrastructure to be studied, may have an influence on the low number of published articles about them (Agostinho et al. 2005, Borges et. al. 2015). Therefore, investment in research on other animal groups in the Cerrado is necessary for a better comprehension about the effects of fire on biodiversity.

We gathered evidence showing that the studies involving the effects of fire in the Cerrado are not evenly distributed. There is a tendency for studies to be regionally concentrated, with a particular concentration in the Federal District. A great part of this production occurred in the IBGE (Brazilian Institute of Geography and Statistics) Ecological Reserve – RECOR. Created in 1975, the RECOR has been established as an important research center of the Cerrado biome, mainly by the creation of a Long-Term Ecological Research – LTER (PELD in Portuguese) developed in collaboration between the IBGE and the University of Brasilia – UnB (http://recor.org.br). This concentration of studies brings some limitations to summarize what we currently know about the effects of fire on the Cerrado. Although a large number of species have broad distributions, the plant communities of the Cerrado *sensu stricto* harbor a high number of locally restricted species, as a consequence of climatic and geomorphological heterogeneity (Felfili & Silva Júnior 2005, Françoso et al. 2016). In fact, different studies have confirmed high beta diversity among regions within the Cerrado (Lindoso & Felfili 2007, Lopes et al. 2011). Furthermore, the Cerrado is surrounded by Pantanal, Amazon rainforest, Caatinga and Atlantic Forest biomes with distinct floristic composition in the transition areas (ecotones) (Maracahipes et al. 2011, Lima et al. 2015).

Fire can also affect the strata of the flora in different ways. Frequent burnings can damage the woody strata and make the areas more open, decreasing diversity over time (Libano & Felfili 2006). Absence of fire in turn may reduce the diversity of herbaceous savanna specialists plants due to the low light availability promoted by forest canopy (Abreu et al. 2017). The fauna can also be affected in different ways, depending on the taxonomic group and the intensity of the fires. For instance, arthropod abundance can be lower in burned rupestrian fields over two years after a fire event (Anjos et al. 2016). On the other hand, Xenarthrans (*e.g.* armadillos and anteaters) probably use burned areas to obtain food resources such as termites and ants (Prada & Marinho-Filho 2004). Ant diversity is reduced in a fire suppression scenario (Abreu et al. 2017) and lizard abundance responds negatively to both fire suppression and high intensity fires (Sousa et al., 2015). Considering the great heterogeneity within the Cerrado, caution is needed when generalizing conclusions about the effects of fire on the whole biome from studies carried out in restricted regions. This issue is particularly important once studies that evaluate the effect of fire on biotic and abiotic components can promote public policies of fire management within and nearby legally protected areas.

Lastly, the most studied locations are not those where higher fire frequency is observed. Most study sites are in the region of the study author's institution, usually far from the agricultural frontier (e.g., Deforestation Arch and region called MATOPIBA), where the major proportion of fires is observed. This geographic bias in research is concentrated in regions with higher Gross Domestic Product (GDP), such as São Paulo and Federal District, where universities are concentrated (Moerman & Estabrook 2006, Pautasso & McKinney 2007, Boakes et al. 2010, Nabout et al. 2012). Nevertheless, in states where fire events are more frequent (e.g. Tocantins e Maranhão) studies are scarce. Therefore, we assert the need for investment in research in areas with high fire frequency and the need for an increase in knowledge about these effects on the biota.

Conclusion

Despite the increase in the number of articles published about the effects of fire on the Cerrado over the years, the main focus of these studies is on the impacts of fire on vegetation, with an evident gap in knowledge related to animal groups. Most studies are concentrated in few states, particularly those with a lower fire incidence. This shows a clear imbalance in our knowledge about the effects of fire on the Cerrado. In addition, evidence from the literature has shown that the response of biodiversity to fire is context-dependent. In this scenario, the development of public policies for effective and specific fire management would depend on research efforts driven to overcome the spatial bias in knowledge about the effects of fire on the Cerrado.

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Author Contributions

Filipe Viegas de Arruda and Diego Guimarães de Sousa participated in all stages of the research.

Fabrício Barreto Teresa, Vitor Hugo Mendonça do Prado, Hélida Ferreira da Cunha, and Thiago Junqueira Izzo participated in the analysis of data, writing and revisions of the article.

Conflicts of interest

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

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short communication

Antifungal potential of crude extracts of *Trichoderma* spp.

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Abstract: Antibiosis is the mechanism by which certain microorganisms respond to the presence of others, secreting compounds or metabolites capable of inhibiting or impeding their development. The crude extract of *Trichoderma* contains a mixture of secondary compounds, which may show antibiotic effect, and has been used for the prospect of this fungus for biological control and other industrial purposes. Faced with the increasing demand of agriculture for ecologically compatible alternatives for the management of diseases, this work aimed to investigate the spectrum of action of Non-Volatile Metabolites (NVMs) of *Trichoderma* isolates against different plant pathogenic fungi. The antagonistic potential of NVMs was evaluated through the incorporation method of the filtered liquid extract in PDA medium. The assays showed that all the NVMs produced inhibited the fungus *Sclerotinia sclerotiorum* similarly. On the other hand, strains CEN1245 and CEN1274, both belonging to the species *Trichoderma brevicompactum*, showed broad spectrum against *Sclerotium rolfsii, Colletotrichum gloesporioides*, *Verticillium dahliae*, *Fusarium oxysporum* and *Cylindrocladium* sp. The present study describes isolates producing non-volatile metabolites with broad spectrum of antifungal action, as well as pathogen-specific. The *Trichoderma* spp. NVMs obtained from different soil samples cultivated with vegetables, cassava and maize were efficient in inhibiting plant pathogenic fungi belonging to other patossystems, such as forest or fruit, which could increase their potential application in biological control of plant diseases. In addition, these antagonistic fungi should be studied in greater depth for the identification of bioactive molecules of industrial interest or in commercial formulations of products for biological control of plant pathogens.

Keywords: *secondary metabolites, antagonism, inhibition of mycelial growth, plant pathogenic fungi.*

Potencial antifúngico de extratos brutos de *Trichoderma* spp.

Resumo: Antibiose é um mecanismo pelo qual certos microrganismos respondem à presença de outros, secretando compostos ou metabólitos capazes de inibir ou impedir o seu desenvolvimento. O extrato bruto de *Trichoderma* contém uma mistura de compostos secundários e tem sido utilizado na prospecção deste fungo para o controle biológico e demais fins industriais. Diante da crescente demanda da agricultura por alternativas ecologicamente compatíveis para o manejo de doenças, este trabalho teve como objetivo investigar o espectro de ação de Metabólitos Não Voláteis (MNVs), produzidos por isolados de *Trichoderma,* contra diferentes fungos fitopatogênicos. O potencial antagônico dos MNVs foi avaliado através do método de incorporação do extrato líquido filtrado em meio BDA. Os ensaios mostraram que todos os MNVs produzidos inibiram de forma semelhante o fungo *Sclerotinia sclerotiorum*. Por outro lado, os isolados CEN1245 e CEN1274, ambos *Trichoderma brevicompactum*, mostraram um amplo espectro de ação, atuando contra *Sclerotium rolfsii, Colletotrichum gloesporioides*, *Verticillium dahliae*, *Fusarium oxysporum* e *Cylindrocladium* sp. O presente estudo descreve isolados que produziram metabólitos não voláteis com amplo espectro de ação antifúngico, assim como patógeno-específico. Os MNVs de *Trichoderma* spp. obtidos de diferentes amostras de solo cultivadas com vegetais, mandioca e milho, foram eficientes na inibição de fungos fitopatogênicos pertencentes a outros patossistemas, como os de espécies florestais e frutíferas, o que poderia aumentar sua potencial aplicação no controle de doenças de plantas. Adicionalmente, estes fungos antagonistas devem ser mais bem estudados para identificação de moléculas bioativas de interesse industrial ou formulação de produtos para o controle biológico de patógenos de plantas.

Palavras-chave*:* *metabólitos secundários, antagonismo, inibição do crescimento micelial, fungos fitopatogênicos.*

Introduction

Trichoderma Persoon is a hyperparasite fungus that uses different mechanisms of biological control, which include: parasitism, antibiosis, competition, induction of resistance and growth promotion (Kumar 2013). Antibiosis is the mechanism by which certain microorganisms respond to the presence of others, secreting compounds or metabolites capable of inhibiting or preventing their development (Benítez et al. 2004). According to Dennis & Webster (1971a, b), antibiotic compounds may be Volatile Metabolites (VMs) and Non-Volatile Metabolites (NVMs). Although these molecules are not essential, in some cases they are important for the selective survival of organisms under certain conditions (Donidio & Monciardini 2002), eliminating microbial competition and, consequently, leading to greater availability of scarce food sources in the soil (Benítez et al. 2004). Raw metabolites consist of mixtures of compounds resulting from primary or secondary metabolism (Yamaguchi 1996). Primary metabolites (polysaccharides, fatty acids and nucleic acids) are common to all biological systems and are produced in the *log* phase of growth. Secondary metabolites (SMs) are produced during the stationary phase and in lesser quantities than the primary ones, encompassing a diversified class of low molecular weight substances and produced by specific groups of organisms (Hanson 2003), which include antibiotics.

Weidling (1941) described gliotoxin, isolated from the fungus *Gliocladium fimbriatum* Gilman & Abbott, which was one of the first SM described and associated with antifungal action. At the present time, several SMs have been reported, and these are the main groups found in *Trichoderma* isolates: polyketides, peptaibols, terpenoids/steroids; pyrones and daucanes (Zeilinger et al. 2016). The SMs may play an antibiotic function against plant pathogens (Daoubi et al. 2009, Takhiro et al. 2013), act as a plant growth regulator, and have pharmacological properties for use as antiaging agents, cholesterol lowering agents, cancer cell fighters, immunological suppressors (Keswani et al. 2014) and flavoring for the food industry (Fadel et al. 2015).

Levels of biological control exerted on a target organism vary according to the antagonistic isolate, and therefore vary both intra and interspecifically (Dennis & Webster 1971a, b, Brodeur 2012). In the context of the selection of these biocontrol agents, *in vitro* assays are used as indicative of the mode of action of the antagonist (Carvalho Filho et al. 2008). The evaluation of NVMs produced by fungi under laboratory conditions, as well as dual culture, is an initial step in the prospection of metabolites for both biological control and other purposes and can not be overlooked. It can be performed by the technique of cellophane paper (Khalili et al. 2012, Jeyaseelan et al. 2014, Barari 2016) or also from the liquid culture, filtration and incorporation in the substrate method. Numerous scientific studies have used the methodology of incorporation of filtrates into culture medium for the evaluation of antagonism in different plant pathogenic fungi species, such as *Verticillium dahliae* Kleb. (Jamdar et al. 2013), *Sclerotium rolfsii* Sacc. (Darvin et al. 2013, Isaias et al. 2014, Srinivasa et al. 2014), *Sclerotinia sclerotiorum* Lib. De Bary and *Fusarium oxysporum* Schlecht, Snyder & Hansen (Jaspal et al. 2009, Castillo et al. 2011, Carvalho et al. 2011, Saxena et al. 2014), *Colletotrichum* spp. (Martins et al. 2007, Ajith & Lakshmidevi 2010, Farah & Nasreen 2013) and *Cylindrocladium* sp. (Carvalho Filho et al. 2008).

Although this biocontrol agent has been studied for a long time, including in Brazil, the industry still needs new isolates with potential for use in Integrated Pest Management programs (IPM). Nowadays, according to the Brazilian Ministry of Agriculture Livestock and Food Supply (MAPA), only five bioproducts, formulated with either *T. asperellum* and *T. harzianum*, are available.

Faced with the increasing demand of agriculture for ecologically compatible alternatives for the management of diseases, this work aimed to investigate the spectrum of action of non-volatile metabolites of *Trichoderma* isolates against different plant pathogenic fungi.

Material and methods

1. Origin of fungal isolates

Eight isolates of *Trichoderma* from the Collection of Fungi for the Control of Plant Pathogens and Weeds of EMBRAPA (Brazilian Agricultural Research Corporate), already characterized and identified in previous studies (Marques et al. 2016), were used. According to the study cited, these isolates exhibited antifungal activity in dual culture tests *in vitro* against *S. sclerotiorum,* and the identification was performed based on the sequencing of regions ITS1/2 of ribosomal DNA (rDNA). In addition to *S. sclerotiorum*, six other plant pathogenic fungal isolates were used in the present study, all described in Table 1.

2. Evaluation of the antifungal activity

The methodology described by Dennis & Webster (1971a) was used to evaluate the potential of NVMs (crude extract or filtered from cultures) produced by *Trichoderma* isolates against plant pathogenic fungi. Routine multiplications of both antagonists and pathogens was performed on plastic Petri dishes containing commercial Potato-Dextrose-Agar (PDA) medium. Incubation of the cultures occurred at 25 ºC with

Table 1. Description of biocontrol agents and plant pathogenic fungi used in this study

Collection code	Origin of the isolates	Fungi
CEN1242	Maize (*Zea mays* L.)	*T. harzianum*
CEN1245	Tomato (*Solanum lycopersicum* L.)	*T. brevicompactum*
CEN1251	Maize (*Z. mays*)	*T. harzianum*
CEN1255	Tomato (*S. lycopersicum*)	*T. harzianum*
CEN1256	Tomato (*S. lycopersicum*)	*T. harzianum*
CEN1258	Cassava (*Manihot esculenta* Crantz)	*T. spirale*
CEN1270	Cassava (*M. esculenta*)	*T. spirale*
CEN1274	Kale (*Brassica oleracea* var. *acephala*)	*T. brevicompactum*
CEN216	Common bean (*Phaseolus vulgaris* L.)	*S. rolfsii*
CEN419	Passion fruit (*Passiflora edulis* Sims)	*C. gloeosporioides*
CEN1273	Chickpeas (*Cicer arietinum* L.)	*F. oxysporum*
CEN494	*Eucalyptus* (*Eucalyptus grandis* Hill ex. Maiden)	*Cylindrocladium* sp.
CEN788	Strawberry (*Fragaria* sp.)	*V. dahliae*
CEN1147	Common bean (*P. vulgaris*)	*S. sclerotiorum*

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Table 2. Inhibition Index of Mycelial Growth (IMG %) of plant pathogenic fungi after being confronted with filtrates from cultures of isolates of *Trichoderma*

Collection code	Plant pathogenic fungi					
	Sclerotinia sclerotiorum	***Fusarium oxysporum***	***Colletotrichum gloeosporioides***	***Sclerotium rolfsii***	***Verticillium dahliae***	***Cylindrocladium* sp.**
CEN1242	9.7 a*	1.0 b	1.0 d	1.0 c	2.4 e	5.7 b
CEN1245	9.2 a	8.2 a	6.8 bc	9.5 a	10.2 a	5.7 b
CEN1251	8.9 a	1.0 b	1.0 d	5.0 b	2.4 e	5.0 b
CEN1270	8.9 a	1.0 b	5.1 c	5.6 b	5.5 c	5.3 b
CEN1255	9.4 a	1.0 b	1.0 d	1.0 c	1.0 f	11.0 a
CEN1256	9.5 a	1.0 b	1.0 d	1.0 c	5.2 cd	5.4 b
CEN1258	8.9 a	1.0 b	8.7 ab	1.0 c	4.3 d	5.7 b
CEN1274	9.1 a	7.7 a	9.2 a	5.7 b	9.1 b	5.9 b
CV%**	5.2	6.5	17.3	11.5	6.5	4.6

* Means followed by the same letter do not differ statistically by Tukey test ($P < 0.05$). ** CV: coefficient of variation.

a 12 h photoperiod for seven days. To obtain the liquid phase with the non-volatile metabolites, the *Trichoderma* isolates were grown in PD medium (Potato-Dextrose, without addition of agar) in an orbital shaker at 150 rpm at 25 °C with absence of light for seven days. After this period, the liquid part was collected by filtration on filter paper and centrifuged at 6.081 *x g* for removal of fungal spores that could hinder membrane sterilization. The liquid phase was filtered through cellulose membranes of 0.45 µm diameter and incorporated into the PDA medium (~ 50 °C) in a proportion of 25% (v / v). Three replicates were prepared with agar discs (5 mm diameter) taken from pathogen cultures. The mycelial agar disks were deposited in the center of each Petri dish containing PDA medium, supplemented with the respective antagonist culture filtrates. Control plates consisted of mycelial agar disks of each pathogen deposited in PDA medium, with sterile distilled water added.

The evaluation of the radial mycelial growth of the pathogen was carried out by taking the measurements of the diameter of the colonies, in millimeters. These measurements were used to calculate the inhibition Index of Mycelial Growth (Menten et al. 1976), using the equation: $\text{IMG} (\%) = [(D_{ctreat} - D_{treat}) / D_{ctreat}] \text{x}100$, where D_{ctreat} = diameter of the radial mycelial growth of the pathogen in the control treatment without filtrates; D_{treat} = diameter of the radial mycelial growth of the pathogen in the treatment with the filtrates. These evaluations were performed when the entire surface of the medium, in the control treatment, was colonized by the pathogen.

3. Statistical analysis

IMG data were converted to $\sqrt{x+1}$ and used in analysis of variance (ANOVA), followed by Tukey test, at a level of 5% probability of significance, using the program Assistat 7.6 beta (Silva & Azevedo 2016).

Results

All *Trichoderma* isolates tested produced NVMs that were effective against *S. sclerotiorum*, with no significant statistical difference between them (Table 2). Against *F. oxysporum*, only isolates CEN1245 and CEN1274 produced non-volatile compounds capable of inhibiting this fungus. Different inhibition indices of mycelial growth of *C. gloesporioides* were observed in the treatments with NVMs produced by the isolates CEN1245, CEN1270, CEN1258 and CEN1274, but without significant statistical difference between them. The other isolates did not produce effective secondary compounds against this plant pathogenic fungus. Among the isolates tested against *S. rolfsii*, four produced antifungal substances; however, it was isolate CEN1245 that stood out. *V. dahliae* had mycelial growth inhibited by NVMs produced by almost all isolates tested except for CEN1255. The highest IMGs observed were, once again, produced by isolates CEN1245 and CEN1274, with significant statistical difference between them. Finally, all isolates produced SMs with activity against *Cylindrocladium* sp., although isolate CEN1255 stood out with the highest IMG (Table 2).

Discussion

Since all isolates produced active NVMs against *S. sclerotiorum*, the results were compared with those obtained in previously performed dual culture tests (Marques et al. 2016), and a greater inhibition was observed with the use of the culture filtrates than in the direct comparison between pathogen and antagonist. This fact suggests that the main mechanism of action of these isolates is antibiosis, that is, the production of secondary compounds with antibiotic properties. It was observed that the three different species, *T. harzianum, T. spirale* and T. *brevicompactum* were effective against the fungus that causes white mold (Figure 1). Saxena et al. (2014) reported maximum inhibition rates against this plant pathogenic fungus, using culture filtrates from *T. harzianum* and *T. atroviride* (Jaspal et al. 2009), while for Castillo et al. (2011) this index was higher with *T. asperellum NVM*. It is postulated, therefore, that this action by antibiosis is variable intra and interspecifically, corroborating the findings of Dennis & Webster (1971a, b) and Brodeur (2012).

The same was verified with the other plant pathogenic fungi confronted. Thus, the isolate that produced metabolites with the greatest capacity for inhibition of *V. dahliea* was CEN1245, belonging to the species *T. brevicompactum* (Table 2), while Jamdar et al. (2013) and Isaias et al. (2014) observed a greater inhibitory effect against this pathogen, using filtrates from *T. harzianum, T. koningii* and *T. koningiopsis*. With *F. oxysporum*, the NVMs that led to the greatest inhibition of mycelial growth in this work were also those produced by *T. brevicompactum* (CEN1245 and CEN1274). There are reports of greater inhibition of *F. oxysporum* with metabolites of *T. viride* (Farah & Nasreen 2013) and *T. harzianum* (Farah & Nasreen 2013, Saxena et al. 2014). In addition to the filtrates from the last two of the aforementioned isolates of *T. brevicompactum*, another one also filtered from *T. harzianum* (CEN1258) inhibited the mycelial growth of *C. gloesporioides*. Several studies, such as those developed by Ajith et al. (2010), Jaspal et al. (2009) and Martins et al. (2007) reported the production of *T. viride* and *T. harzianum* metabolites as those with the highest antifungal potential of different species of *Colletotrichum*, among several other species tested.

In the tests with *Cylindrocladium* sp., the isolate CEN1255, from *T. harzianum*, was highlighted as to the potential inhibition of growth of this pathogen. The same was verified by Carvalho Filho et al. (2008) who, however, also selected a *T. pseudokoningii* isolate as highly active against this pathogen in NVMs production tests. As for *S. rolfsii*, the culture filtrate with the highest antifungal effect was *T. brevicompactum* (CEN1245), while in the literature the filtrates were *T. harzianum* (Saxena et al. 2014), *T. viride* (Darvin et al. 2013) and *T. virens* (Srinivasa et al. 2014), corroborating, once again, with Dennis & Webster (1971a, b) and Brodeur (2012).

In the present study the intra and interspecific variation of the antifungal activity of *Trichoderma* isolates in relation to different plant pathogenic

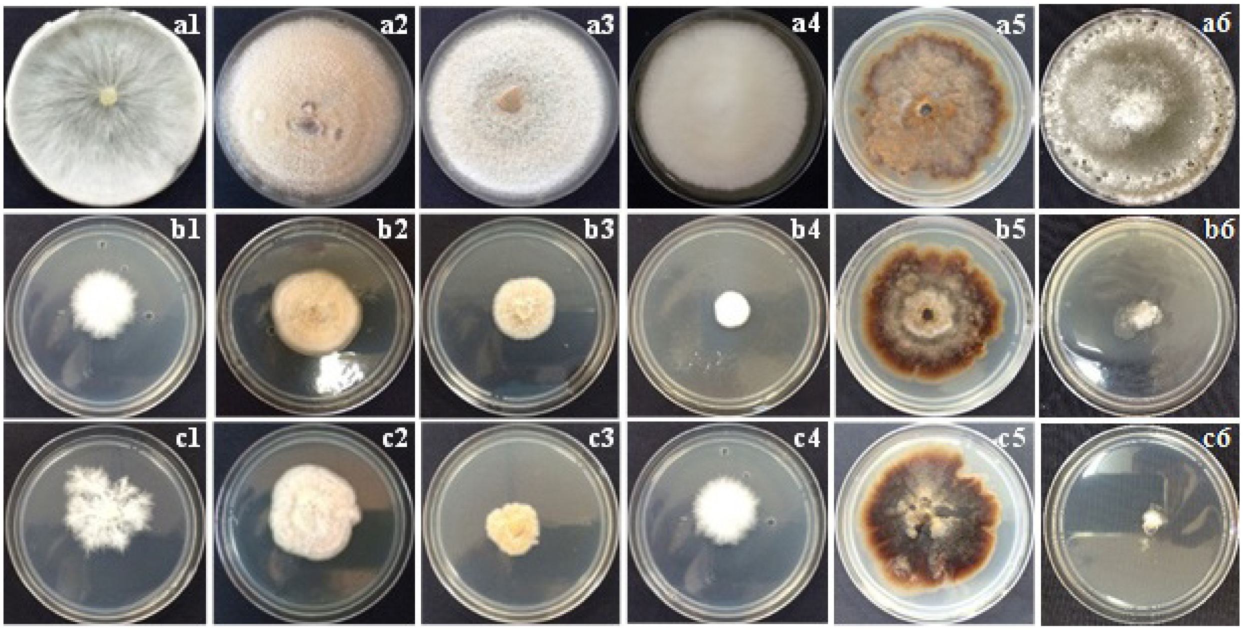

Figure 1. Effect of filtrates from cultures of *Trichoderma* spp. that stood out in the mycelial growth of plant pathogenic fungi, where a1-a6: Control treatments, b1-b6: treatments with the isolate CEN1245 and c1-c5: treatments with isolate CEN1274 and a1, b1 and c1: *S. rolfsii* (CEN216), a2, b2 and c2: *F. oxysporum* (CEN1273), a3, b3 and c3: *C. gloesporioides* (CEN419), a4, b4 and c4: *V. dahliae* (CEN788), a5, b5 and c5: *Cylindrocladium* sp. (CEN609) and a6, b6 and c6: *S. sclerotiorum* (CEN1147), respectively.

fungi was confirmed. Filtered cultures of the isolates of *T. brevicompactum*, CEN1245 and CEN1274, presented a broad antifungal spectrum, although isolates from other species were also prominent in the production of active metabolites with specificity against the plant pathogenic fungi used in the experiments. The *Trichoderma* metabolites obtained from different soil samples cultivated with vegetables, cassava and maize were effective to plant pathogenic fungi belonging to other patossystems such as forest or fruit trees, which could broaden their application in the biological control of plant diseases. In addition, the antagonistic fungi should be studied in greater depth for the identification of bioactive molecules of industrial interest or in commercial formulations of products for biological control of plant pathogens.

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Author Contributions

Sueli Correa Marques de Mello: substantial contribution in the concept and design of the study; contribution to manuscript preparation and critical revision.

Irene Martins: substantial contribution to data collection; contribution to data analysis and interpretation.

Eder Marques: 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.

Conflicts of interest

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

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Going extinct before being discovered? New lichen fungi from a small fragment of the vanishing Atlantic Rainforest in Brazil

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Abstract: In the frame of an ongoing lichen inventory of Atlantic Rainforest remnants in Northeast Brazil, five new species of Graphidaceae were discovered in a small forest fragment, Mata do Cipó, in Sergipe state, the smallest state of Brazil and among those with the highest deforestation rate in the country. An additional new species had already been collected in Panama before and was now also found in the Mata do Cipó and is described here as well. In total, 40 species of Graphidaceae are reported for this remnant, including a large number of taxa indicative of well-preserved rainforest. The new species are: *Fissurina atlantica* T.A. Pereira, M. Cáceres & Lücking, *sp. nov.*, *Graphis subaltamirensis* Passos, M. Cáceres & Lücking, *sp. nov.*, *Ocellularia cipoensis* L.A. Santos, M. Cáceres & Lücking, *sp. nov.*, *O. sosma* T.A. Pereira, M. Cáceres & Lücking, *sp. nov.*, *O. submordenii* Lücking, *sp. nov.* (also known from Panama), and *Pseudochapsa aptrootiana* M. Cáceres, T.A. Pereira & Lücking, *sp. nov.* The findings are discussed in the context of the strong fragmentation of the Atlantic Rainforest, with individual remnants apparently serving as refugia for residual populations of rare species of lichen fungi that were more widely distributed in the past, but currently seem to occur only in isolated fragments.

Keywords: *deforestation, fragmentation, lichen diversity, Graphidaceae, refugia, Sergipe*

Indo extinto antes de ser descoberto? Novos fungos liquenizados da evanescente Mata Atlântica no Brasil

Resumo: No âmbito de um inventário atual de liquens dos remanescentes da Mata Atlântica, no Nordeste do Brasil, cinco espécies novas de Graphidaceae foram descobertas em um pequeno fragmento florestal, Mata do Cipó, no estado de Sergipe, o menor estado do Brasil, o qual está entre aqueles com maior taxa de desmatamento no país. Uma nova espécie adicional já havia sido coletada no Panamá, anteriormente, e agora também foi encontrada na Mata do Cipó, e está sendo descrita aqui também. No total, 40 espécies de Graphidaceae são relatadas para este remanescente, incluindo um grande número de táxons indicadores de floresta bem preservada. As novas espécies são: *Fissurina atlantica* T.A. Pereira, M. Cáceres & Lücking, *sp. nov.*, *Graphis subaltamirensis* Passos, M. Cáceres & Lücking, *sp.nov.*, *Ocellularia cipoensis* L.A. Santos, M. Cáceres & Lücking, *sp. nov.*, *O. sosma* T.A. Pereira, M. Cáceres & Lücking, *sp. nov.*, *O. submordenii* Lücking, sp. nov. (também conhecido do Panamá), e *Pseudochapsa aptrootiana* M. Cáceres, T.A. Pereira & Lücking, *sp. nov.* Os achados são discutidos no contexto da forte fragmentação da Mata Atlântica, com remanescentes aparentemente servindo de refúgio para populações residuais de espécies raras de fungos liquenizados que, provavelmente, tiveram uma distribuição mais amplia no passado, mas que atualmente parecem ocorrer apenas em fragmentos isolados.

Palavras-chave: *Desmatamento, fragmentação, diversidade de liquens, Graphidaceae, refúgios, Sergipe*

Introduction

In the past centuries, extensive and continuing land use change has led to the so-called ‘sixth mass extinction’ or ‘holocene extinction’ (Leakey & Lewin 1992; Wake & Vredenburg 2008; Barnosky et al. 2011). Largely perceived through documented extinctions of charismatic megafauna, this phenomenon extends to many other organisms including bryophytes, lichens, and fungi. Small organisms in particular are likely to contain large numbers of undescribed species in danger of extinction before being discovered (Dirzo & Raven 2003).

The Atlantic Rainforest is one of the most diverse ecosystems on Earth, with a high level of endemism (Mittermeier et al. 1998). Mostly restricted to Brazil but extending into Argentina and Uruguay, and within Brazil found in 17 states, the Atlantic Rainforest originally covered approximately 13% of the Brazilian territory, while its current extension has been reduced to about 1%, with a high level of degradation and fragmentation of the remaining forest (Brooks & Balmford 1996; Ranta et al. 1998; Ribeiro et al. 2009; Fundação SOS Mata Atlântica & INPE 2009, 2016; Tabarelli et al. 2010). In the state of Sergipe, the smallest state in Brazil, nearly half of the territory belongs to the domain of the Atlantic Rainforest, but only about 11% of its original cover remains as more or less natural areas, dispersed into small fragments in numerous municipalities (Fundação SOS Mata Atlântica & INPE, 2016). One of these fragments is the Mata do Cipó, a 64 ha large piece of comparatively intact forest found in the municipalities of Capela and Siriri (Fig. 1). These are among the ten municipalities with the highest deforestation rate in the state in the past two decades, out of 75 municipalities in total; together, both have about 5,000 ha forest cover left, corresponding to about 10% of their area (Fundação SOS Mata Atlântica & INPE 2015).

The family Graphidaceae is the largest family of tropical lichens (Staiger 2002; Rivas Plata et al. 2012, 2013; Lücking et al. 2014). Its representatives occur mainly in forests in the tropical and subtropical regions of the world, mostly corticolous on tree trunks and branches (Rivas Plata et al. 2008; Lücking et al. 2014). There are currently some 2,100 species of Graphidaceae in the world, but up to 3,500 are predicted to exist (Lücking et al. 2014). The use of molecular methods has led to many taxonomic and systematic changes in the family, which now also includes Thelotremataceae, previously treated as family of its own (Staiger et al. 2006; Mangold et al. 2008; Rivas Plata et al. 2012, 2013). On the other hand, Gomphillaceae, for a while included in Graphidaceae (Rivas Plata et al. 2012), has now been shown to be its sister family (Lücking & Lumbsch, in prep.).

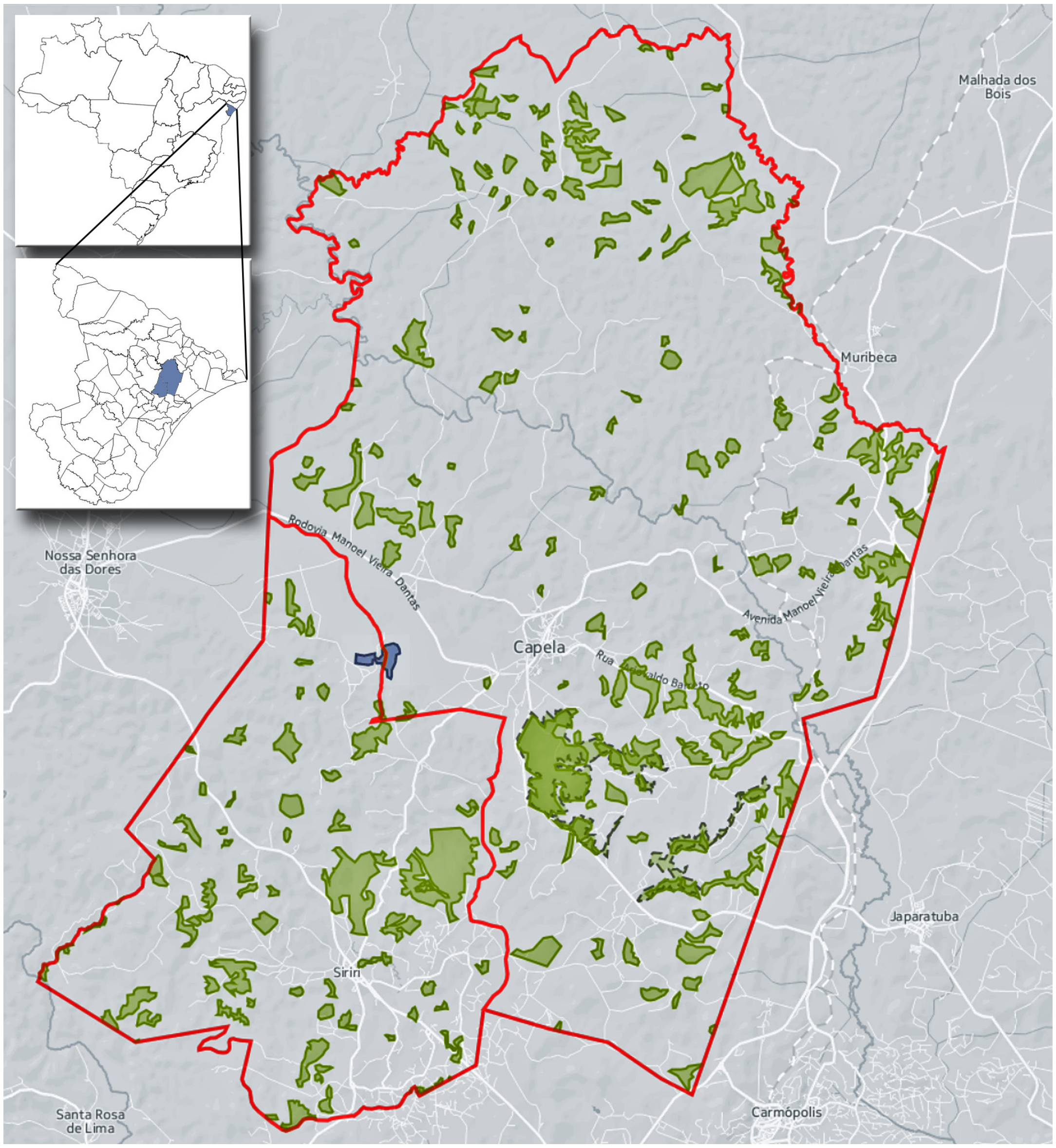

Figure 1. Location of the study site in Sergipe state, Brazil. Atlantic rain forest fragments for the two municipalities of Capela and Siriri are indicated as green areas and the study site is indicated in blue. Detailed map based on the "Aqui tem Mata?" online application [http://aquitemmata.org.br (accessed 17-11-2017)], with data from the "Atlas da Mata Atlântica", the Fundação SOS Mata Atlântica and the Instituto Nacional de Pesquisas Especiais (INPE).

In recent years, the number of species in the Graphidaceae family has rapidly increased worldwide (Lücking et al. 2014). Many new species were recently described from Brazil, including the south (Dal Forno & Eliasaro 2010; Käffer et al. 2010, 2014), north (Cáceres et al. 2014a), and northeast, including Sergipe state (Menezes et al. 2011; Cáceres & Lücking 2013; Cáceres et al. 2014b). Many Graphidaceae, especially in the tribes Ocellularieae and Thelotremateae, are excellent bioindicators of intact rain forest (Rivas Plata et al. 2008), and hence specific assessments of this family can help to evaluate the conservation status of Atlantic Rainforest fragments.

The present work aimed at an inventory of Graphidaceae in the small Atlantic Rainforest fragment of the Mata de Cipó in the municipalities of Capela and Siriri in the state of Sergipe, northeast Brazil. The objective was to assess the value of such small fragments for the conservation of unique lichen species richness.

Materials and Methods

Sampling took place at the ARIE (Area of Relevant Ecological Interest) Mata do Cipó, which is located across the municipalities of Siriri and Capela, 10º61'41" S and 37 º11'87", in Sergipe state (Fig. 1). The Mata do Cipó comprises approximately 64 ha of Atlantic Rainforest, situated at 80–100 m altitude, characterized as a semideciduous seasonal rainforest, with some parts in regeneration (Umbelino 2012). Lichens were collected using an opportunistic sampling method along the trails through forest (Gradstein et al. 1996; Cáceres et al. 2008).

Identification of the material was done using a LEICA EZ4 stereomicroscope and a LEICA DM500 compound microscope at the Laboratório de Liquenologia (LALIQ), Departamento de Biociências da Universidade Federal de Sergipe, Campus Professor Alberto Carvalho, in Itabaiana, Sergipe. Voucher specimens were deposited in the herbarium of the Laboratório de Liquenologia (ISE). The following taxonomic works were consulted to identify the genera and species: Staiger (2002; graphidoid Graphidaceae), Kalb et al. (2004; *Diorygma*), Frisch et al. (2006; thelotremoid Graphidaceae), Lücking et al. (2009, 2016; *Graphis*, *Myriotrema*), Rivas Plata et al. (2010; thelotremoid Graphidaceae), Gaswick & Lücking (2012; thelotremoid Graphidaceae), Lücking (2014; *Ocellularia*), and Feuerstein et al. (2014; *Diorygma*). All identifications were verified by comparison with data from authentic type material, in part consulting types digitized through the *Global Plants Initiative* [http://gpi.myspecies.info; https://plants.jstor.org]. Thin-layer chromatography was performed using standard methods as described in Orange et al. (2010).

Results and Discussion

Of the lichen fungi thus far identified in material collected in the Mata do Cipó, 40 species belong to the family Graphidaceae, distributed in 15 genera (Table 1). Six new species are described below, five of these based on material from the study site and one previously collected in Panama (Fig. 2–4). Four species are newly reported for Brazil and further six are new records for Sergipe state (Fig. 5–6).

At first glance, a number of 40 Graphidaceae appears to be fairly low for an area of tropical rain forest. For instance, 131 species were reported from the Surumoni crane station in Venezuela (Komposch & Hafellner 1999), 116 from Los Amigos Biological Station in Amazonian Peru (Rivas Plata & Lücking 2013), and 111 from Fakahatchee Strand Park Preserve in Florida (Lücking et al. 2011). However, these numbers are not directly comparable, as the latter areas are by several orders of magnitude larger and embedded into vast regions of intact tropical rain forests; for instance, Los Amigos covers 145,000 ha and Fakahatchee 25,000 ha, compared to the 64 ha of Mata do Cipó. Of the 116 species from Los Amigos, 18 (15.5%) were new to science, 13 of which (11.2%) represented thelotremoid genera; at Fakahatchee, 13 out of the 111 species (11.7%) were new, with no thelotremoid taxon. Of the 40 species found at Mata do Cipó, 15% were new to science, 10% representing thelotremoid genera, thus with proportions comparable to the much larger and better preserved areas. Most notably, 23 Atlantic rain forest fragments have now been studied in six states in northeastern Brazil by our working group (Cáceres et al. 2017), and yet this very small fragment revealed so far the largest number of new Graphidaceae. The total number of 20 thelotremoid species, representing eight different morphotypes in the classification provided by Rivas Plata et al. (2008), suggests the Mata do Cipó to be a rather well preserved, near-intact piece of Atlantic Rainforest worth further protecting. Five years ago, it was actually proposed to transform this fragment into a legally protected area (SEMARH 2012); however, we are unaware of the status of this process.

The discovery of six new species in a small area of Atlantic rain forest at first glance might suggests a very local distribution of these species, that is, a high degree of local endemism. However, given that the Atlantic Rainforest originally formed a broader, continuous strip along the coast in this part of Brazil, we hypothesize that the current distribution patterns are a relict of originally broader distributions. Most of these lichen fungi are very rare and hence, a high degree of fragmentation will stochastically eliminate many regional and local populations, with few or single populations remaining in particular fragments in more or less random patterns. As a consequence, each fragment harbors a unique component of the overall remaining diversity of the Atlantic Rainforest. One could consider these refugia, similar to Pleistocene refugia (Grazziotin et al. 2006), although the nature of the environmental changes is quite different, and these changes occur much faster, not allowing for evolutionary adaptations.

Since lichen communities adapted to intact forest, in particular thelotremoid Graphidaceae (Rivas Plata et al. 2008), are unable to adjust to fast environmental changes, forest fragmentation basically results in depauperation, with randomly dispersed relict populations. As a consequence, fragment size is not necessarily a predictor for species richness or occurrence of unique taxa. For instance, the Refugio de Vida Silvestre Mata do Junco, the second largest fragment of Atlantic Rainforest in Sergipe state with 894 ha (SEMARH 2013), yielded thus far 28 species of Graphidaceae, one of them new to science (Cáceres et al. 2017). The overlap in species composition of Graphidaceae between both fragments is extremely low, with just one species (*Graphis glaucescens*) in common. This appears to be contrast to findings for vascular plants and animals, in which community formation usually responds to disturbances through community changes, correlated both with fragment size and degree of isolation, with ruderal species becoming increasingly dominant in smaller, more isolated fragments (Tabarelli et al. 1999; Uezu et al. 2005; Galetti et al. 2006). However, one should not underestimate sampling artifacts concerning small organisms such as lichens, since only the more common species will be regularly collected, so even thorough sampling may not detect all taxa and hence differences in composition might be in part due to this effect. Also, the material collected at Mata do Junco has not been fully evaluated and might contain more species of Graphidaceae.

Taxonomic treatment of the new species

Fissurina atlantica* T.A. Pereira, M. Cáceres & Lücking, *sp. nov. (Fig. 2A)
MycoBank MB 823652

Diagnosis: Differing from other species of *Fissurina* in the sorediate thallus forming maculate soralia.

***Holotype*:** Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36371).

Etymology: Referring to the Atlantic Rainforest.

***Description*:** Thallus corticolous, epiperidermal, 1–5 cm diam., continuous; surface uneven-verrucose, white-grey to pale yellowish grey or cream-colored;

Table 1: List of Graphidaceae found in the ARIE Mata do Cipó, Sergipe state, Brazil. New species found so far only at this locality are highlighted in boldface; a further new species (*O. submordenii*) was first discovered in Panama.

Species	Morphotype	Novelty
Carbacanthographis subalbotecta Staiger & Kalb	graphidoid	new for Sergipe
Diorygma junghuhnii (Mont. & Bosch) Kalb, Staiger & Elix	graphidoid	
Diorygma minisporum Kalb, Staiger & Elix	graphidoid	new for Sergipe
Diorygma poitaei (Fée) Kalb, Staiger & Elix	graphidoid	
Fissurina amazonica M. Cáceres, Aptroot & Lücking	graphidoid	new for Sergipe
***Fissurina atlantica* T. A. Pereira, M. Cáceres & Lücking**	graphidoid	**new species**
Fissurina bothynocarpa (Redinger) Lücking	graphidoid	
Graphis conferta Zenker	graphidoid	
Graphis glaucescens Fée	graphidoid	
Graphis miniata Redinger	graphidoid	new for Sergipe
Graphis oxyclada Müll. Arg.	graphidoid	
***Graphis subaltamirensis* Passos, M. Cáceres & Lücking**	graphidoid	**new species**
Graphis subtenella Müll. Arg.	graphidoid	new for Brazil
Phaeographis brasiliensis (A. Massal.) Kalb & Matthes-Leicht	graphidoid	
Phaeographis flavescens Dal-Forno & Eliasaro	graphidoid	new for Sergipe
Phaeographis haematites (Fée) Müll. Arg.	graphidoid	
Phaeographis scalpturata (Ach.) Staiger	graphidoid	
Platythecium grammitis (Fée) Staiger	graphidoid	
Sarcographa labyrinthica (Ach.) Müll. Arg.	graphidoid	
Thalloloma hypoleptum (Nyl.) Staiger	graphidoid	new for Sergipe
Chapsa diploschistoides (Zahlbr.) Frisch	thelotremoid	new for Brazil
Chapsa elabens (Müll. Arg.) Rivas Plata & Mangold	thelotremoid	
Chapsa thallotrema Lücking & N. Salazar	thelotremoid	
Glaucotrema costaricense (Müll. Arg.) Rivas Plata & Lumbsch	thelotremoid	
Leucodecton fissurinum (Hale) Frisch	thelotremoid	
Leucodecton subcompunctum (Nyl.) Frisch	thelotremoid	
Melanotrema platystomum (Mont.) Frisch	thelotremoid	
Myriotrema microporum (Mont.) Hale	thelotremoid	
Ocellularia cavata (Ach.) Müll. Arg.	thelotremoid	
Ocellularia aff. *cavata* (Ach.) Müll. Arg.	thelotremoid	
***Ocellularia cipoensis* L.A. Santos, M. Cáceres & Lücking**	thelotremoid	**new species**
Ocellularia crocea (Kremp.) Overeem & D. Overeem	thelotremoid	
Ocellularia dolichotata (Nyl.) Zahlbr.	thelotremoid	
Ocellularia aff. *mordenii* Hale	thelotremoid	
Ocellularia obturascens (Nyl.) Hale	thelotremoid	
***Ocellularia sosma* T. A. Pereira, M. Cáceres & Lücking**	thelotremoid	**new species**
***Ocellularia submordenii* Lücking**	thelotremoid	**new species**
Ocellularia violacea Räsänen	thelotremoid	new for Brazil
Ocellularia zamorana Sipman, Lücking & Chaves	thelotremoid	new for Brazil
***Pseudochapsa aptrootiana* M. Cáceres, T. A. Pereira & Lücking**	thelotremoid	**new species**

prothallus absent; soralia present and abundant, well-delimited, rounded to slightly irregular in outline, erumpent, crateriform to excavate, 0.3–0.6 mm diam.; soredia mealy-granular, white. Thallus in section 30–50 µm thick, with loose upper cortex, 5–10 µm thick, and irregular photobiont layer 25–40 µm thick. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 8–10 × 6–9 µm. Ascomata lirelliform, flexuose, more or less stellately branched, erumpent, with thin but distinct, gaping labia and complete thalline margin, 0.5–1.5 mm long, 0.1–0.2 mm wide, 0.07–0.1 mm high; disc partially exposed, flesh-colored, thinly white-pruinose; proper margin indistinct, labia entire; thalline margin whitish. Excipulum 50–10 µm wide, pale yellowish; laterally covered by algiferous thallus including clusters of crystals in basal parts; hypothecium prosoplectenchymatous, 5–10 mm high, colorless; hymenium 50–60 µm high, colorless, clear; epithecium granulose, 5–10 µm high, grey. Asci fusiform, 50–60 × 6–8 µm. Ascospores 8 per ascus, oblong-ellipsoid, 3(–4)-septate, 10–12 × 3–4 µm, 3–3.5 times as long as wide, colorless. Secondary chemistry: No substances detected by TLC.

Remarks: This new species is the first within the genus *Fissurina* to form soralia. Without the ascomata, the sterile thallus would probably have been classified tentatively within a different genus, such as *Chapsa* or *Ocellularia*, which include several sorediate species. However, this find shows that the formation of soralia is more widespread within the family, although generally still rare. The verrucose thallus is also unusual for a species of this genus.

Graphis subaltamirensis* Passos, M. Cáceres & Lücking, *sp. nov. (Fig. 2B)
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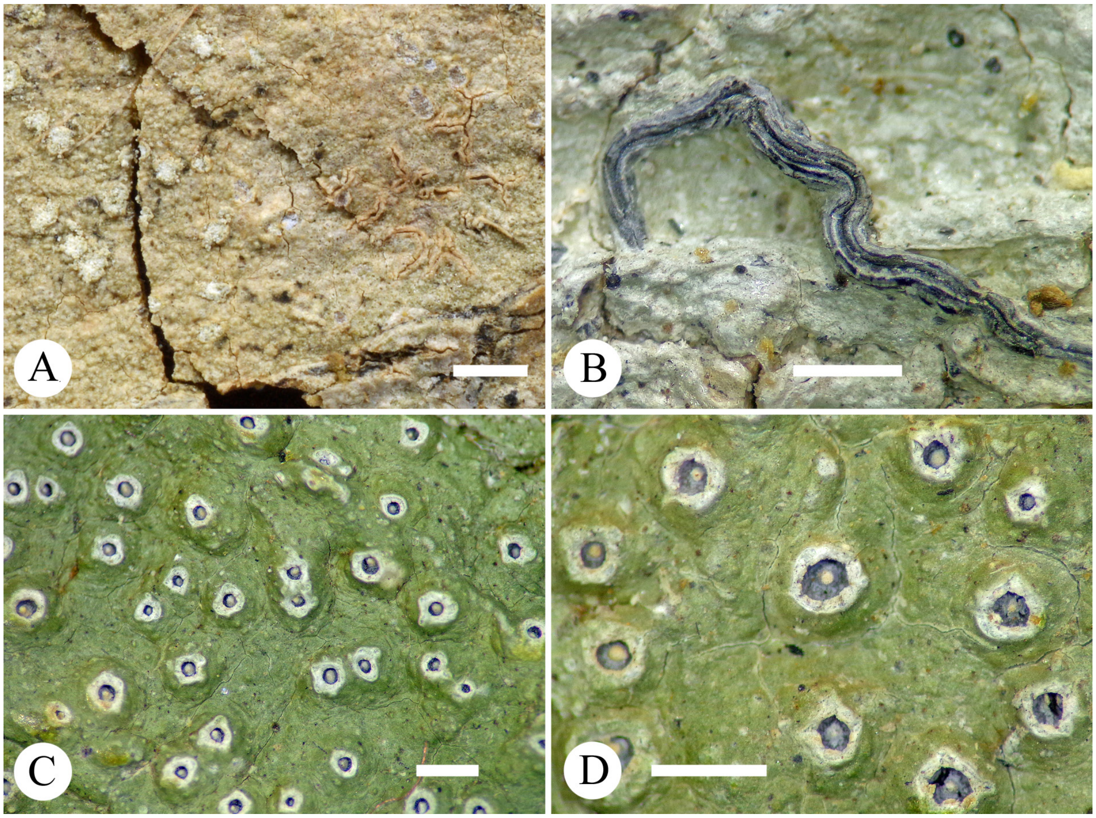

Figure 2. Habit of new species. A. *Fissurina atlantica* (holotype), thallus with soralia and clusters of lirellae. B. *Graphis subaltamirensis* (holotype), thallus with lirella. C–D. *Ocellularia cipoensis* (C, holotype; D, ISE 36119), thallus with ascomata. Scale bar = 1 mm.

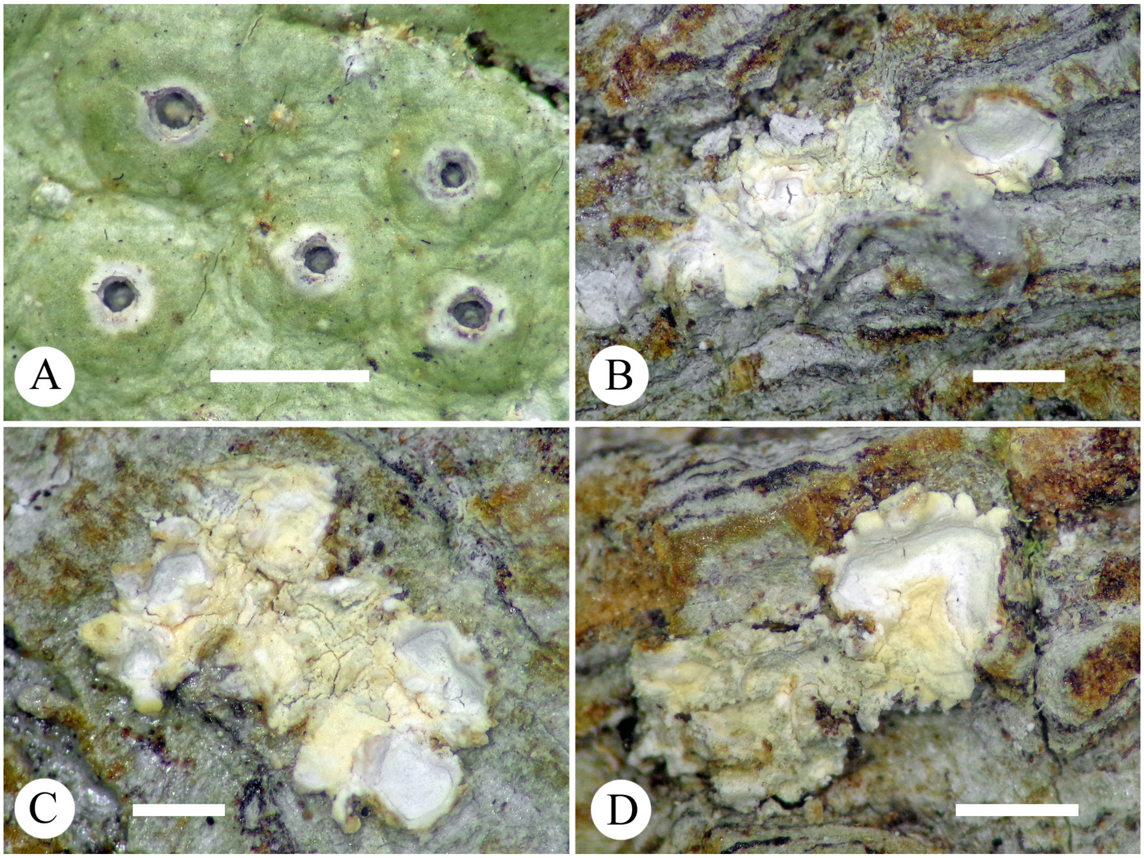

Figure 3. Habit of new species. A. *Ocellularia sosma* (ISE 36537), thallus with ascomata. B–D. *Pseudochapsa aptrootiana* (holotype), thallus with ascomata. Scale bar = 1 mm.

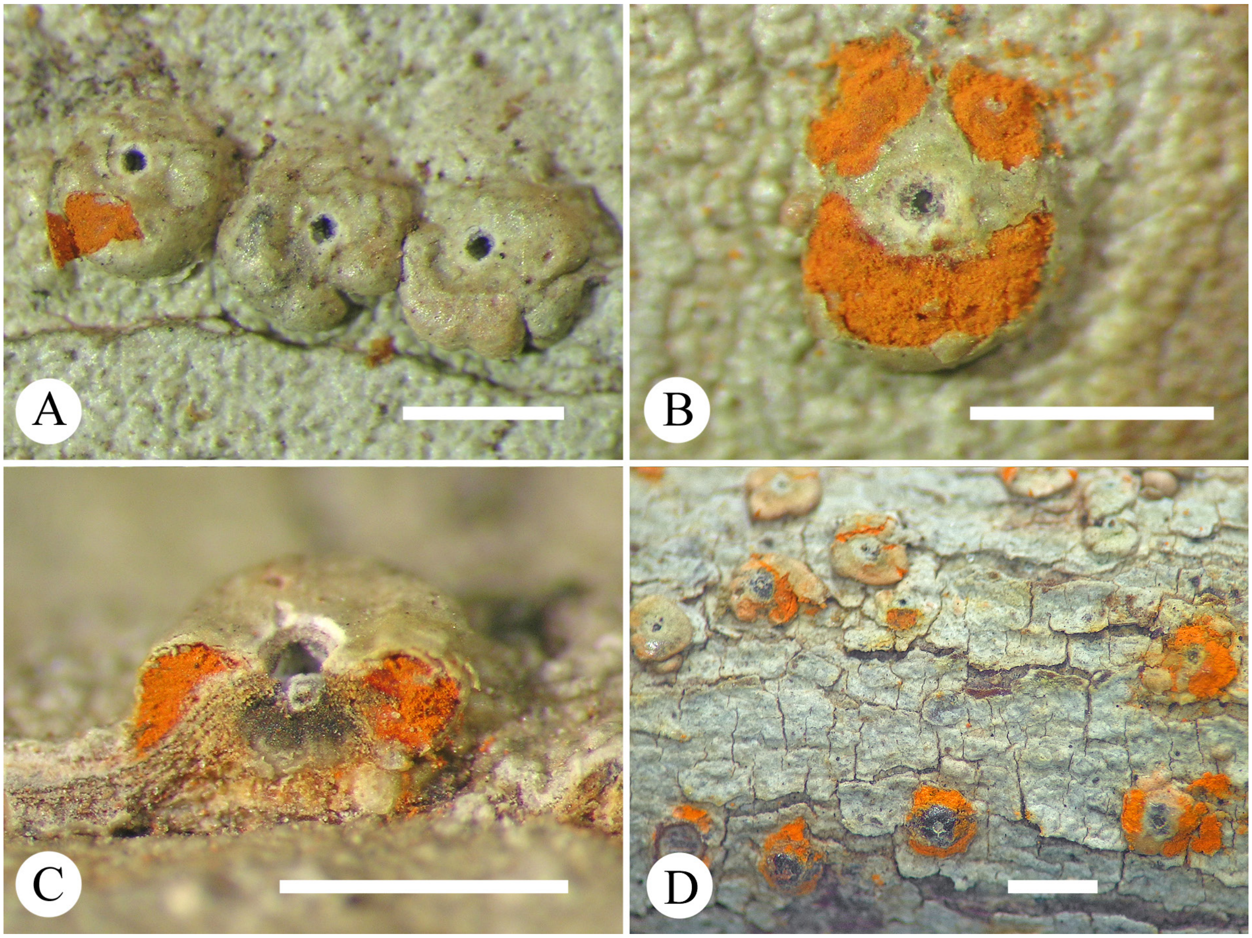

Figure 4. Habit of new species. A–D. *Ocellularia submordenii* (A–C, holotype from Panama; D, ISE 36125), thallus with ascomata, in B showing exposed medulla, in C showing section. Scale bar = 1 mm.

Diagnosis: Differing from *Graphis altamirensis* in the shorter ascospores.

Holotype: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36083).

Etymology: Referring to the similarity with *Graphis altamirensis*.

Description: Thallus corticolous, epiperidermal, 1–5 cm diam., continuous; surface uneven, white-grey; prothallus absent. Thallus in section 100–150 µm thick, with cartilaginous upper cortex, 5–10 µm thick, and irregular photobiont layer 50–100 µm thick, with large clusters of calcium oxalate crystals. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 8–12 × 6–9 µm. Ascomata lirelliform, flexuose, unbranched to sparsely branched, prominent to almost sessile, with thick labia and apically thin complete thalline margin, 3–8 mm long, 0.4–0.5 mm wide, 0.3–0.5 mm high; disc concealed; proper margin very thick, labia striate, dark gray to gray-black with white lines; thalline margin laterally thick, gray to white, apically thin (cortex only), gray. Excipulum apically crenulate, completely carbonized, 130–170 µm wide, black; laterally covered by corticate algiferous thallus including clusters of crystals in basal parts; hypothecium prosoplectenchymatous, 15–25 mm high, colorless; hymenium 130–170 µm high, colorless, strongly inspersed (paraphyses and asci hardly visible), inspersion rapidly disappearing in KOH (type B); epithecium granulose, 5–15 µm high, olive brown. Asci fusiform, 120–150 × 25–30 µm. Ascospores (4–)8 per ascus, oblong, submuriform with 15–19 transverse and 0–1 longitudinal septa per segment, 70–100 × (10–)12–17 µm, 5–6 times as long as wide, colorless. Secondary chemistry: No substances detected by TLC.

Remarks: This species is most similar to *Graphis altamirensis*, *G. cinerea*, and *G. pseudocinerea*, agreeing in the robust, prominent (to sessile), striate lirellae with fully and massively carbonized excipulum and strongly inspersed hymenium, lacking secondary substances. *Graphis altamirensis* differs in the much longer ascospores (120–180 × 10–15 µm, 10–14 times as long as wide), whereas *G. pseudocinerea* has smaller (50–70 × 9–12 µm), regularly muriform ascospores with numerous longitudinal septa. *Graphis cinerea* lacks longitudinal septa in the much larger and broader ascospores (100–200 × 18–30 µm).

Additional specimens examined: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36035, 36058, 36063).

Ocellularia cipoensis **L.A. Santos, M. Cáceres & Lücking, *sp. nov.*** (Fig. 2C–D)

MycoBank MB 823654

Diagnosis: Differing from *Ocellularia rondoniana* in the verrucose instead of ridged thallus, the slightly larger pore of the ascomata, and the slightly larger ascospores.

Holotype: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36116).

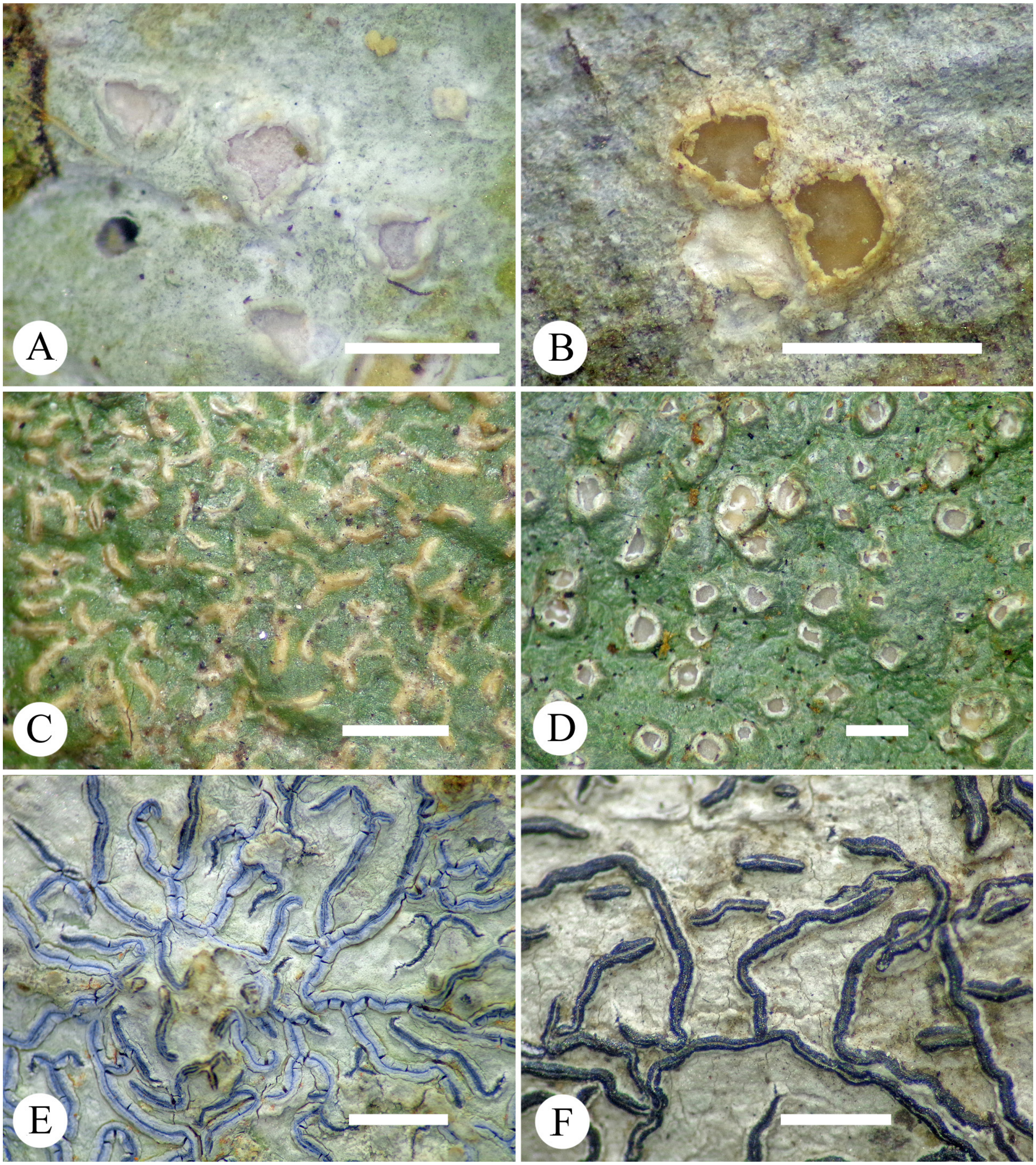

Figure 5. Habit of new or otherwise interesting records of Graphidaceae from Mata do Cipó. A. *Chapsa diploschistoides* (ISE 36046), thallus with ascomata. B. *C. elabens* (ISE 36049), thallus with ascomata. C. *Fissurina amazonica* (ISE 36521), thallus with lirellae. D. *Glaucotrema costaricense* (ISE 36117), thallus with ascomata. E. *Graphis glaucescens* (ISE 36104), thallus with lirellae. F. *G. oxyclada* (ISE 36063), thallus with lirellae. Scale bar = 1 mm.

Etymology: Referring to the type locality, the Mata do Cipó.

Description: Thallus corticolous, epiperidermal, up to c. 5 cm diam., continuous; surface verrucose, light green-grey; prothallus absent. Thallus in section 100–150 µm thick, with prosoplectenchymatous cortex, 10–15 µm thick, photobiont layer 30–80 µm thick, and irregular medulla, 30–50 µm thick, strongly encrusted with numerous small, grey crystals that partially dissolve in K, and large clusters of calcium oxalate crystals forming the verrucae. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 8–11 × 7–9 µm. Ascomata rounded to angular, erumpent, with complete thalline margin, 0.7–1.1 mm diam., 0.2–0.3 mm high; disc covered by 0.2–0.4 mm wide pore; proper margin distinct, entire, visible as 0.1–0.2

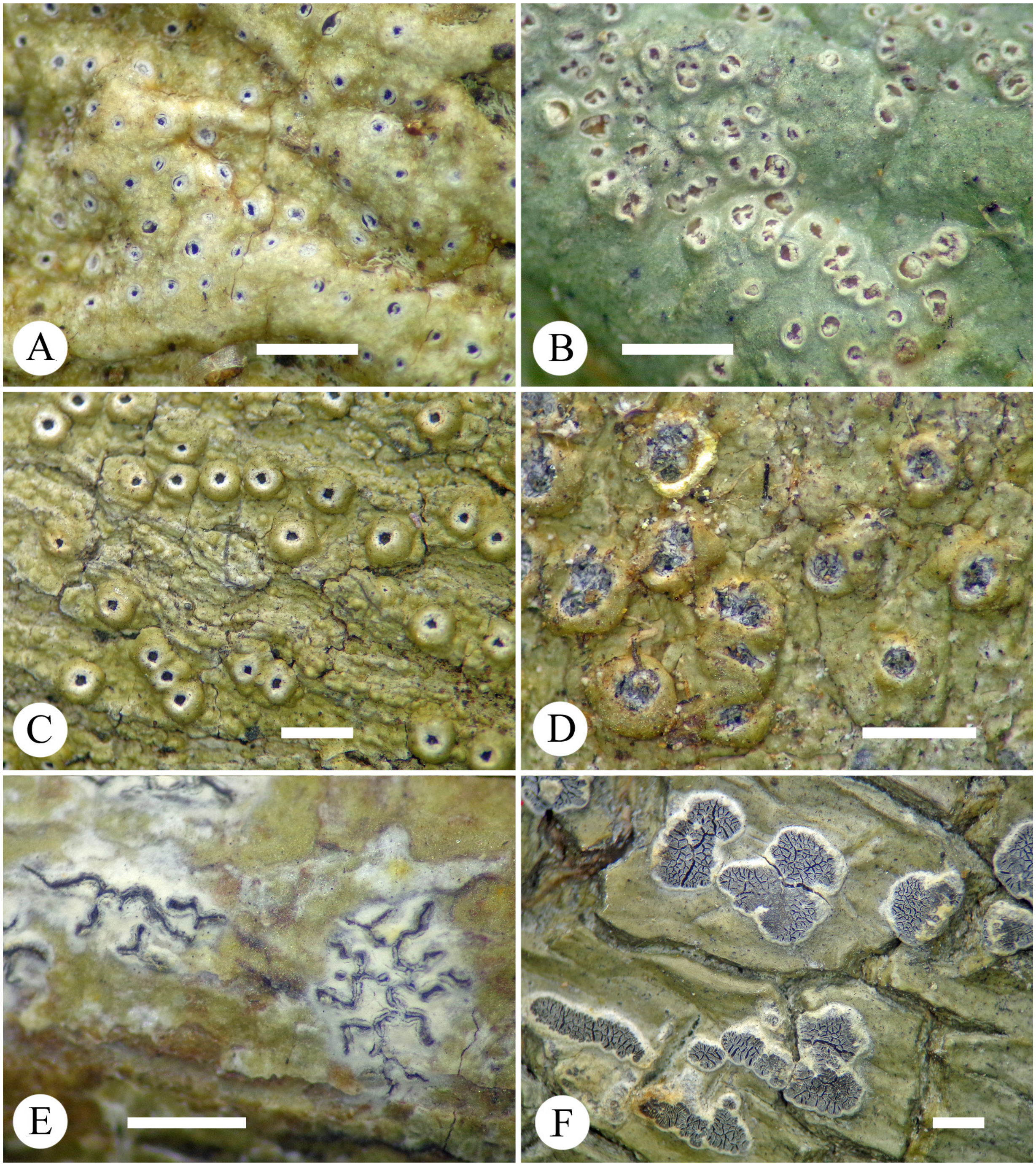

Figure 6. Habit of new or otherwise interesting records of Graphidaceae from Mata do Cipó. A. *Leucodecton subcompunctum* (ISE 36069), thallus with ascomata. B. *Myriotrema microporum* (ISE 36534), thallus with ascomata. C. *Ocellularia cavata* (ISE 36067), thallus with ascomata. D. *O. crocea* (ISE 36124), thallus with ascomata. E. *Phaeographis brasiliensis* (ISE 36057a), thallus with lirellae. F. *Sarcographa labyrinthica* (ISE 36211), thallus with lirellae. Scale bar = 1 mm.

mm broad, white, angular rim around the pore; thalline margin entire, verrucose, light green-grey. Excipulum entire, light yellow-brown to orange-brown (difficult to separate from modified periderm), together with periderm 70–150 µm wide, fused with thalline margin; laterally covered by algiferous, corticate thallus containing periderm layers; columella present, finger-like, 100–150 µm broad, thinly carbonized; hypothecium prosoplectenchymatous, 10–15 µm high, hyaline; hymenium 100–120 µm high, hyaline, clear; epithecium indistinct, 10–15 µm high, hyaline. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical, 95–110 × 8–10 µm. Ascospores 8 per ascus, ellipsoid, 5–7-septate, 20–30 × 6–8 µm, 3–4 times as long as wide, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Secondary chemistry: Psoromic (major), subpsoromic (minor), and 2’-O-demethylpsoromic acids (minor to trace); medulla P+ yellow, microscopic section K–.

Remarks: This new species agrees with the recently described *Ocellularia rondoniana* from the Brazilian Amazon in most features, including ascoma morphology and anatomy, but differs in the verrucose instead of ridged thallus, the larger pore of the ascomata (0.15–0.25 mm in *O. rondoniana*) and the slightly larger ascospores (17–23 × 5–7 µm in *O. rondoniana*). Both species are known from several well-developed collections and the differences are consistent.

Additional specimens examined: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36119); ibid., 17 March 2016, *T.A. Pereira & P.O. Passos* (ISE 36114).

Ocellularia sosma* T. A. Pereira, M. Cáceres & Lücking, *sp. nov. (Fig. 3A)

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Diagnosis: Differing from *Ocellularia rondoniana* in the thallus lacking ridges and the columella forming thin, irregular strands.

Holotype: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 17 March 2016, *T.A. Pereira & P.O. Passos* (ISE 36519).

Etymology: The epithet honors the work of the foundation SOS Mata Atlântica (SOSMA) in conserving the Atlantic Rainforest.

Description: Thallus corticolous, epiperidermal, up to c. 7 cm diam., continuous; surface uneven to shallowly verrucose, light grey-green; prothallus absent. Thallus in section 50–100 µm thick, with prosoplectenchymatous cortex, 10–20 µm thick, photobiont layer 40–80 µm thick, and indistinct medulla, photobiont layer encrusted with numerous small, gray crystals that partially dissolve in K, and scattered, large clusters of calcium oxalate crystals forming the verrucae. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 8–12 × 7–10 µm. Ascomata rounded, erumpent, with complete thalline margin, 0.7–1.2 mm diam., 0.2–0.3 mm high; disc covered by 0.1–0.2(–0.3) mm wide pore; proper margin distinct, entire, visible as white rim around the pore; thalline margin entire, smooth to uneven, light grey-green. Excipulum entire, yellow-olive, externally covered by dark brown periderm, together with periderm 70–120 µm wide, fused with thalline margin; laterally covered by algiferous thallus; columella present in part of the ascomata, absent in others, when present forming one to several, thin strands, 25–50 µm broad, carbonized; hypothecium prosoplectenchymatous, 10–15 µm high, hyaline; hymenium 90–100 µm high, hyaline, clear; epithecium distinct, 10–15 µm high, gray-granular. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical, 85–95 × 8–10 µm. Ascospores (few mature ones seen) 8 per ascus, ellipsoid, 5–7-septate, 18–25 × 6–8 µm, 3–4 times as long as wide, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Secondary chemistry: Psoromic (major), subpsoromic (minor), and 2'-O-demethylpsoromic acids (minor to trace); medulla P+ yellow, microscopic section K–.

Remarks: This new species belongs in the difficult *Ocellularia terebrata* complex (Lücking 2014), within which it is characterized by a non-carbonized excipulum combined with a carbonized columella. The species somewhat resembles *O. rondoniana* M. Cáceres, Aptroot & Lücking, but differs in thallus structure and especially in the columella forming thin, irregular strands, while other species in this group have finger-like to broad-stump-shaped columellas.

Additional specimens examined: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 17 March 2016, *T.A. Pereira & P.O. Passos* (ISE 36537).

Ocellularia submordenii* Lücking, *sp. nov. (Fig. 4A–D)

MycoBank MB 823656

Diagnosis: Differing from *Ocellularia mordenii* in the more delicately verrucose thallus and the orange instead of cinnabar-red medullary pigment, as well as the regularly columellate apothecia.

Holotype: Panama. Colón: Santa Rita Ridge, about 6 km from Roosevelt Highway; 350 m; logged primary forest and remnant stands; 1974, *M. Hale 43501* (US).

Etymology: The epithet refers to the similarity with *Ocellularia mordenii*.

Description: Thallus corticolous, epiperidermal, up to c. 7 cm diam., continuous; surface verrucose, light greyish olive; prothallus absent. Thallus in section 100–150 µm thick, with loose, irregular, cartilaginous cortex, 10–15 µm thick, photobiont layer 50–100 µm thick, and indistinct medulla, partially encrusted orange pigment granules. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 9–12 × 8–10 µm. Ascomata rounded, prominent, with complete but often eroding thalline margin, 0.7–1.2 mm diam., 0.3–0.4 mm high; disc covered by 0.1–0.2 mm wide pore filled with white-pruinose columella; proper margin thin, entire, visible as white rim around the pore; thalline margin entire, verrucose, light greyish olive. Excipulum entire, apically carbonized, laterally covered by a thick layer of brown periderm and above with a massive layer of orange medulla, together with periderm 200–300 µm thick, fused with thalline margin; laterally covered by corticate algiferous thallus; columella present, finger-like, 50–100 µm broad, upper half carbonized; hypothecium prosoplectenchymatous, 10–20 µm high, hyaline; hymenium 150–200 µm high, hyaline, clear; epithecium distinct, 10–15 µm high, gray-granular. Paraphyses unbranched, apically smooth; periphysoids absent; asci cylindrical, 130–170 × 30–40 µm. Ascospores 1–2 per ascus, oblong, 21–29-septate, 70–150 × 15–20 µm, 5–8 times as long as wide, hyaline, distoseptate with lens-shaped lumina, I+ violet-blue. Secondary chemistry: unidentified orange anthraquinone, K+ purple.

Remarks: This new species was first collected 1974 in Panama by Mason Hale and identified with *Ocellularia mordenii* Hale, described previously from Dominica (Hale 1974, 1978). However, the latter differs in the much more coarsely verrucose-areolate thallus and in the cinnabar-red instead of orange medullary pigment and most apothecia lack a columella. With the additional discovery of this undescribed taxon in the Atlantic Rainforest fragment studied here, the time has come to formally describe it.

The new species was reported in the supplementary material (data spreadsheet) in Cáceres et al. (2017: Appendix A: line 512) as "Ocellularia submardinis" [sic], an unfortunate typographical error.

Additional specimens examined: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36126).

Pseudochapsa aptrootiana* M. Cáceres, T.A. Pereira & Lücking, *sp. nov. (Fig. 3D–F)

MycoBank MB 823657

Diagnosis: Differing from *Pseudochapsa phlyctidioides* in the endoperidermal thallus and the aggregate-pseudostromatic ascomata with thick, cream-white pruina, and the slightly larger ascospores.

Holotype: Brazil. Sergipe: Capela e Siriri, ARIE Mata do Cipó; 10º61'41" S, 37 º11'87", 80–100 m; Atlantic Rainforest remnant, corticolous in understory; 18 September 2015, *T.A. Pereira & P.O. Passos* (ISE 36059).

Etymology: This new species is dedicated to our esteemed colleague, mentor and friend, André Aptroot, for his invaluable contributions to tropical lichenology.

Description: Thallus corticolous, endoperidermal, up to c. 3 cm diam., continuous; surface uneven, following the relief of the bark, light grey; prothallus absent. Thallus in section 30–50 µm thick, ecorticate, with partially endoperidermal photobiont layer, 10–30 µm thick, and irregular medulla, 10–20 µm thick. Photobiont *Trentepohlia*; cells rounded to irregular in outline, in irregular groups, yellowish green, 7–10 × 6–9 µm. Ascomata 3–10 aggregate into distinct pseudostromata, angular-rounded, immersed-erumpent, chroodiscoid, 0.8–1.2 mm diam., 0.1–0.15 mm high; disc exposed, thickly cream-white-pruinose; proper margin fused with thalline margin, forming numerous recurved, irregular to triangular lobes, sometimes falling off, internal with cream-white to pale yellowish surface. Excipulum entire, light yellow-brown, 20–30 µm wide, fused with thalline margin; columella absent; hypothecium prosoplectenchymatous, 10–15 µm high, pale yellowish; hymenium 80–100 µm high, hyaline, clear; epithecium distinct, 15–25 µm high, grey-granular. Paraphyses unbranched, apically smooth; periphysoids present, 10–20 µm long; asci clavate to cylindrical, 80–90 × 10–12 µm. Ascospores 8 per ascus, oblong-fusiform, 11–15-septate, 30–35 × 7–8 µm, 4–5 times as long as wide, hyaline, distoseptate with more or less lens-shaped lumina, I–. Secondary chemistry: Stictic (major), constictic (submajor to minor), and cryptoctistic, hypostictic, and acetylhypoconstictic acids (minor to trace); ascomata and thallus K+ yellow then orange, P+ orange, microscopic section with K+ persistently yellow efflux.

Remarks: This species is placed in the genus *Pseudochapsa* because of its agreement in several features, including chemistry and ascospore type, with *P. phlyctidioides* (Müll. Arg.) Parnmen, Lücking & Lumbsch and related species. It differs from *P. phlyctidioides* in the endoperidermal thallus and the aggregate-pseudostromatic ascomata with thick, cream-white pruina, and the slightly larger ascospores. The only other ecorticate species with stictic acid and transversely septate ascospores, *P. dilatata* (Müll. Arg.) Parnmen, Lücking & Lumbsch, has larger, angular to elongate ascomata and amyloid ascospores. *Pseudochapsa albomaculata* (Sipman) Parnmen, Lücking & Lumbsch agrees with *P. aptrootiana* in the aggregate-pseudostromatic ascomata, but has an epiperidermal, greenish thallus, smaller ascospores, and constictic acid as predominant substance.

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Author's Contributions

MESC conceived the study. TAP, POP, and MESC carried out the field work and collection of specimens. TAP, POP, LAS, RL and MESC did the taxonomic studies, and TAP, POP and LAS wrote the species descriptions, under guidance by RL and MESC. TAP, POP, LAS, and RL created the images. All authors analyzed the available data on Atlantic rain forest fragments. MESC and RL wrote the draft manuscript and the final version was elaborated by all authors.

Conflicts of interest

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

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Biodiversity of Holocene marine fish of the southeast coast of Brazil

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Abstract: Middens are archaeological sites dating between 8,000 and 1,000 years before present and are commonly found on the Brazilian coast. Data were collected from 68 middens allowing an inventory of 142 fish species, most of them recorded in no more than five sites. Conversely, *Micropogonias furnieri* and *Pogonias cromis* had the highest frequencies of occurrence. The biogeographic, ecological and economic data showed that most of the identified fish are widely distributed in the Western Atlantic (59.72%) and inhabit estuarine environments (53.99%), while most species have a demersal habit (35.92%) and exhibit oceanic migratory behaviour (28.87%). Lastly, the surveyed fish are predominantly carnivorous (72.54%) with some commercial value (96.48%). Chi-squared tests comparing midden inventory and current ichthyofauna checklists failed to show significant differences between them ($p > 0.99$). Thus, the results indicate that zoo-archaeological fish remains are key evidence of Holocene biodiversity and may help the establishment of more complete baselines.

Keywords: *Baselines, Fishermen-Gatherers-Hunters, Ichthyofauna, Middens, Species richness, Zooarchaeology.*

Biodiversidade de peixes marinhos do Holoceno da costa Sudeste brasileira

Resumo: Sambaquis são sítios arqueológicos, datando entre 8,000 e 1,000 anos antes do presente, encontrados na costa brasileira. Neste trabalho, dados ictiológicos referentes a 68 sambaquis da costa Sudeste do Brasil foram compilados e construído um inventário de referência no qual constam 142 espécies, a maioria das quais com registro em não mais que cinco sítios. Por outro lado, as espécies *Micropogonias furnieri* e *Pogonias cromis* apresentaram alta frequência de ocorrência nos sambaquis. Os dados biogeográficos, ecológicos e econômicos mostraram que a maioria das espécies registradas nos sambaquis são de ampla distribuição no Atlântico Ocidental (59.72%), habitam ambientes estuarinos (53.99%), tem um hábito demersal (35.92%) e comportamento oceânico migratório (28.87%). A maioria dos registros diz respeito a peixes carnívoros (72.54%) e com algum valor comercial (96.48%). A comparação da lista de ictiofauna registrada para os sambaquis com uma lista construída com base em inventários atuais de peixes para mesma região não mostrou diferenças significativas (teste de qui-quadrado, $p> 0.99$). Dessa forma, os resultados apresentados indicam que os vestígios de ictiofauna encontrados em sambaquis constituem uma amostra relevante da biodiversidade do Holoceno podendo ser muito úteis na construção de inventários de referências mais completos da fauna ictiológica da costa brasileira.

Palavras-chave: *Ictiofauna, Inventário de referências, Pescadores-Coletores-Caçadores, Riqueza de espécies, Sambaquis, Zooarqueologia*

Introduction

Biodiversity-related research has developed significantly since the 1990s, when ecologists worldwide, concerned with anthropogenic effects on ecosystems, intensified their studies of environmental issues (Amaral & Jablonsky 2005, Lewinsohn & Prado 2005). Biodiversity is defined by the Convention on Biological Diversity as the variability among living beings of all origins, including terrestrial, marine and other aquatic ecosystems, and their associated ecological complexes, including intra- and inter-species as well as ecosystem diversity (Arruda et al. 2000).

Recently, the Census of Marine Life raised the estimated number of known marine species from approximately 230,000 to between 1 and 1.4 million; more than 1,200 new species were identified among specimens collected in known and previously unexplored waters (Costello et al. 2010). Furthermore, in a review of research related to Brazilian biodiversity, Siqueira et al. (2015) found that only 21% of 1,156 references from 2009 to 2014 addressed the richness of aquatic species, indicating that the marine environment remains little studied and is therefore largely unknown. Finally, baselines for long-term studies of marine biodiversity are scarce (Knowlton & Jackson 2008, Pinnegar & Engelhard 2008).

Baselines are reference biodiversity inventories that directly assess the species composition of a specific site for a given spatial extent and time. The data generated by such inventories are one of the most important tools for the conservation and management of natural areas, especially the associated endangered species (Silveira et al. 2010). The establishment of baselines is particularly important for the conservation of marine fish because this group is intensely exploited due to their commercial value and account for a significant share of global fishery production. In Brazil, fisheries have been key to the development of the country, concentrating 70% of the population near the coast, and the sea plays a key role in its history, culture and economy (Rosa & Lima 2008).

To be as accurate as possible, reference inventories of ichthyological fauna should include not only current data but also historic and prehistoric (fossil/sub-fossil) data as well (Furon 1969, Warwick & Light 2002, Willis & Birks 2006, Froyd & Willis 2008, Stahl 2008). However, collecting data on the species compositions of the past is complicated because fossil/sub-fossil records are largely characterised by being incomplete; that is, the biological data (species morphology, richness, diversity and evenness, among others) preserved in those records are influenced by non-linear modifications that occur from the time of death to the final burial of an organism (Ritter & Erthal 2016) and the species preservation potential (Prummel & Heinrich 2005). Therefore, prehistoric records are relatively scarce (Bittencourt et al. 2015).

However, there are some Holocene species composition records from sources including beaches, death assemblages and middens. Beaches and restingas originated in the Holocene, but their characteristics prevent the establishment of a chronology (Lacerda et al. 1984). Conversely, death assemblages allow for accurate chronological estimates and have the great advantage of being natural, thus showing actual tanatocenoses, but these formations are rare along the Brazilian coast (Ritter & Erthal 2016). However, middens, which are archaeological sites dating between 8,000 and 1,000 years before present (BP, according to the convention before 1950), are commonly found on the Brazilian coast and allow a chronology to be established because they show a stratigraphic sequence of different species compositions (Kneip et al. 1988, Gaspar 1998, Scheel-Ybert et al. 2009, Klokler et al. 2010).

Middens were built by groups of prehistoric fishermen-gatherers-hunters, which explains why they are found in estuarine areas at the intersections of rivers and the sea. These sites contain a wealth of resources including sediments, coal, lithic material and, above all, faunal remains. High numbers of molluscs and crustaceans, including sea and sand crabs, as well as echinoderms and fish have been found among the marine zoo-archaeological remains recovered from middens (DeBlasis et al. 2007, Figuti 1993, Lima 2000, Lima et al. 2003).

For fish species, their zoo-archaeological remains indicate their usefulness to midden populations, so most species recorded at these sites have a cultural meaning and show sociocultural and identity relationships and spatiotemporal economic characteristics (Figuti 1998, Barbosa-Guimarães 2013, Wagner & Silva 2014, Lopes et al. 2016). Accordingly, because the ichthyological remains in middens represent the diversity of prehistoric fishes obtained from a selective catch, this data source underestimates the diversity of Holocene fish. That is, the species diversity of the remains found in the middens is lower than that in natural communities (Gonzalez 2005, Costa et al. 2012).

Although midden fish records underestimate natural diversity, they are a key source of information on the ichthyological fauna of the past because prehistoric people could only have caught the fish available in the environments at the time. Furthermore, by-catch occurred; that is, species with no known anthropological relevance were incidentally fished with target species (Reitz & Wing 2008, Villagran & Gianini 2014, Beuclair et al. 2016). Therefore, midden records are a good indicator of Holocene biodiversity, providing data on fish species composition, abundance, distribution and richness as well as cultural information (diet, fishing gear, ritualistic symbols, ornaments and artefacts) (Souza et al. 2010a, Souza et al. 2010b, Faria et al. 2014, Mendes et al. 2014, Rodrigues et al. 2016a, Rodrigues et al. 2016b, Beuclair et al. 2016, Silva et al. 2016).

Thus, although middens are artificial accumulations (that is, built by prehistoric populations) of biological material, the presence or absence of species at these sites provides sufficient data to create a taxonomic list that may be useful for defining a historical record of biological diversity (Stahl 2008), so this study presents an inventory of marine ichthyological biodiversity from southeast Brazilian middens. This list is the first comprehensive inventory of Holocene fish fauna from this region and may help elucidate past natural ocean events, enabling the establishment of more complete baselines to inform conservation and management measures.

Methods

The inventory was constructed from an extensive bibliographical survey of the libraries of universities and institutions with archaeological collections from sites along the southeast coast of Brazil as well as online databases (Web of Knowledge, Scientific Electronic Library Online - SciELO, Google Scholar and the Thesis Database of the Brazilian Federal Agency for the Support and Evaluation of Graduate Education (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES)). The data sources included scientific articles and books, thesis, dissertations, monographs and technical reports.

The list of species was analysed in terms of absolute and relative species richness and absolute and relative frequencies of occurrence. Absolute species richness is the number of species present at a sampled site, while the relative species richness is the ratio between that number and the total number of species. Similarly, the absolute frequency of occurrence is the number of sites at which a specific species occurs, and the relative frequency is the ratio between that number and the total number of sites.

Furthermore, taxonomic, biogeographic, ecological and economic data on the inventoried species were collected from the FishBase database (Froese & Pauly 2017) and categorised by distribution (range of occurrence), environment (habitat), habit (way of life at specific locations in the water column), behaviour (migratory movements), feeding guild (food requirements) and commercial value (demand for the species in the fish market).

The checklists constructed by Bizerril & Costa (2001) for Rio de Janeiro and Menezes (2011) for São Paulo were compared along with the inventory from the midden zoo-archaeological remains from this study to current ichthyological inventories for the same regions, and the species catalogued in *species*Link, a digital information system that integrates primary data from scientific collections in real time, for Espírito Santo were surveyed. Furthermore, a chi-squared test (χ^2) was performed to assess whether the ratio of commercial and non-commercial species in the midden fish inventory differed significantly from the ratios in current fish checklists.

Results

Data were collected from 68 middens distributed in 19 locations along the coast of three states of the Southeast Region of Brazil: Espírito Santo (ES), Rio de Janeiro (RJ) and São Paulo (SP) (Figure 1). The location with the highest number of middens (12 sites, 17.65%) was Paraty in the state of Rio de Janeiro, which also had the highest number of documented sites (53, 77.95%). Conversely, Espírito Santo had the lowest number of middens (2, 2.94%; Table 1). From these total of 68 middens, 49 of them had records of ^{14}C radiocarbonic dates (Figure 2). Occupation time ranged from 8,182 BP (Sambaqui de Camboinhas) to 675 BP (Sítio do Major). However, the majority of them were built and occupied during the period defined by Walker et al. (2012) as Late Holocene.

A total of 142 fish species were inventoried, and most taxa belonged to class Osteichthyes (105, 73.94%). Sciaenidae was the most represented family with 21 species, and *Cynoscion* Gill, 1861 was the genus with the highest number of species, seven in total (Table 2). Of the Chondrichthyes (37, 26.06%), the family with the highest number of species was Carcharhinidae with 17 species, and the most representative genus was *Carcharhinus* Blainville, 1816 with 12 species (Table 3).

The site with the highest species richness was the midden Sambaqui do Algodão in Angra dos Reis with 71 inventoried species and a relative species richness value of 0.5 (Table 1). Most of the inventoried species (63.38%) may be considered rare because they were recorded in no more than five of the 68 study sites. Conversely, *Micropogonias furnieri* (Desmarest, 1823) and *Pogonias cromis* (Linnaeus, 1766) had the highest frequencies of occurrence, being found in 53 (relative frequency = 0.78) and 48 (relative frequency = 0.71) of the sites, respectively.

The biogeographic, ecological and economic data showed that most of the identified fish are widely distributed in the Western Atlantic (59.72%) and inhabit estuarine environments (53.99%), while most species have a demersal habit (35.92%) and exhibit oceanic migratory behaviour (28.87%). Lastly, the surveyed fish are predominantly carnivorous (72.54%) with some commercial value (96.48%; Figure 3).

In comparison to the current fish checklists, the middens contained 17.60% of the species recorded in Rio de Janeiro, 15.57% of the species recorded in São Paulo and 2.13% of the species recorded in Espírito Santo. Overall, 17.57% of the fish recorded in the entire Southeast Region were represented in the middens, but they accounted for 28.07% of the species in the region with some commercial value. Moreover, exclusively historic species were identified including 13 in Rio de Janeiro, 15 in São Paulo, and four in Espírito Santo (Table 4). The results from the χ^2 tests indicated that were no significant differences in the number of commercial species in the historic and current inventories for the states (Rio de Janeiro: $\chi^2 = 4.587 \times 10^{-9}$, degrees of freedom = 3, probability > 0.995; São Paulo: $\chi^2 = 3.549 \times 10^{-12}$, degrees of freedom = 3, probability > 0.995; Espírito Santo: $\chi^2 = 0.106$, degrees of freedom = 3, probability > 0.99) or the entire Southeast Region ($\chi^2 = 6.349 \times 10^{-17}$, degrees of freedom = 3, probability > 0.995).

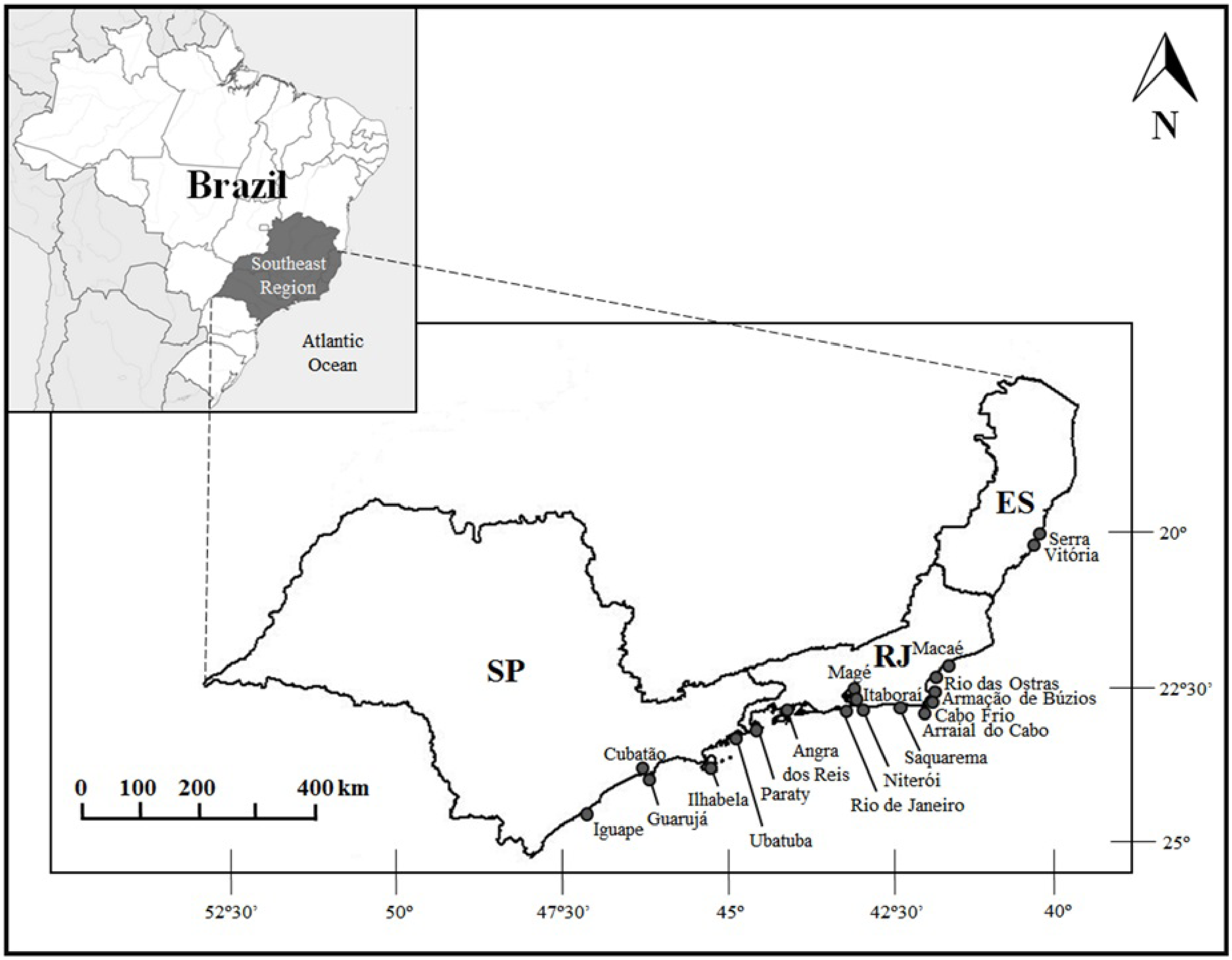

Figure 1. Map of the Southeast Region of Brazil with the locations of the inventoried middens.

Table 1. Middens inventoried in the Southeast Region of Brazil with absolute (Rspp) and relative (relative Rspp) species richness and the associated references from the literature.

Location	Midden	Code	Rspp	Relative Rspp	References
Angra dos Reis	Sambaqui da Caieira	1	27	0.190	Lima 1991, DSc Thesis, FFLCH-USP Lopes et al. 2016, PLoS One 11(6): 1-36
	Sambaqui da Caieira II	2	29	0.204	Lima 1991, DSc Thesis, FFLCH-USP Figuti 1998, Revista Arqueologia 11: 57-70
	Sambaqui do Algodão	3	71	0.500	Lima 1991, DSc Thesis, FFLCH-USP Lopes et al. 2016, PLoS One 11(6): 1-36 Figuti 1998, Revista Arqueologia 11: 57-70
	Sítio do Bigode I	4	38	0.268	Lima 1991, DSc Thesis, FFLCH-USP Lopes et al. 2016, PLoS One 11(6): 1-36 Figuti 1998, Revista Arqueologia 11: 57-70
	Sítio do Major	5	47	0.331	Lima 1991, DSc Thesis, FFLCH-USP Lopes et al. 2016, PLoS One 11(6): 1-36 Figuti 1998, Revista Arqueologia 11: 57-70
	Sítio do Peri	6	39	0.275	Lima 1991, DSc Thesis, FFLCH-USP Lopes et al. 2016, PLoS One 11(6): 1-36 Figuti 1998, Revista Arqueologia 11: 57-70
	Sítio Ilhota do Leste	7	14	0.099	Lima 1991, DSc Thesis, FFLCH-USP Lessa & Carvalho 2015, Bol Mus Para Emílio Goeldi 10: 489-507 Tenório 2003, DSc Thesis, PUC-RS Tenório 2003, Anais XII Congresso da SAB Lessa & Coelho 2010, Rev Mus Arqueol Etnol 20: 77-89
	Sambaqui do Acaiá	8	23	0.162	Lopes et al. 2016, PLoS One 11(6): 1-36
Macaé	Sítio da Ilha de Santana	9	13	0.092	Lima 1991, DSc Thesis, FFLCH-USP
Magé	Sítio Saracuruna	10	5	0.035	Lima 1991, DSc Thesis, FFLCH-USP Mendonça de Souza 1981, Pré-história Fluminense, Instituto Estadual do Patrimônio Cultural, RJ Mello & Mendonça de Souza 1977, Nheengatu 1: 43-58
	Sambaqui Rio das Pedrinhas	11	9	0.063	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui de Sernambetiba	12	2	0.014	Beltão et al. 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 145-155 Perez et al. 1995 In: Arqueologia do Estado do Rio de Janeiro, Arquivo Público do Estado do RJ pp. 29-34
	Sambaqui de Amourins	13	1	0.007	Heredia et al. 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 175-188
Paraty	Abrigo Ponta do Leste I	14	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Abrigo Ponta do Leste II	15	5	0.035	Mendonça de Souza 1981, Pré-história Fluminense, Instituto Estadual do Patrimônio Cultural, RJ
	Sambaqui Olho D'Água	16	3	0.021	Mendonça de Souza 1981, Pré-história Fluminense, Instituto Estadual do Patrimônio Cultural, RJ Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Toca do Cassununga	17	5	0.035	Mendonça de Souza 1981, Pré-história Fluminense, Instituto Estadual do Patrimônio Cultural, RJ
	Toca dos Caboclos I	18	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Abrigo da Ilha Pelada	19	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sítio Trindade III	20	6	0.042	Mendonça de Souza 1981, Pré-história Fluminense, Instituto Estadual do Patrimônio Cultural, RJ
	Sambaqui do Forte	21	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui do Perequê-Açu	22	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui da Ilha Comprida	23	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui do Pouso	24	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui de Mamanguá	25	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131

Table 1. Continued...

Location	Midden	Code	Rspp	Relative Rspp	References
Saquarema	Sambaqui da Beirada	26	21	0.148	Kneip 1994, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 2 Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP Kneip et al. 1988, Revista de Arqueologia 5: 41-54 Kneip et al. 1995 In: Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ 3: 3-12 Barbosa-Guimarães 2012, Sci Plena 8: 1-9
	Sambaqui da Madressilva	27	8	0.056	Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP Kneip 1997, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 4
	Sambaqui da Pontinha	28	17	0.120	Kneip 1994, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 2 Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP Kneip et al. 1995 In: Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ 3: 3-12
	Sambaqui de Saquarema	29	24	0.169	Lopes et al. 2016, PLoS One 11(6): 1-36 Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP
	Sambaqui do Moa	30	15	0.106	Kneip 1994, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 2 Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP Machado 2014, MSc Thesis, PPGG-UFPA Silveira 2001, DSc Thesis, FFLCH-USP Costa et al. 2012, Rev Mus Arqueol Etnol 22: 51-65
	Sambaqui do Saco	31	8	0.056	Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP Kneip 1997, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 4
	Sambaqui do Jaconé	32	9	0.063	Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP
	Sambaqui de Mombaça I	33	3	0.021	Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP
	Sambaqui de Itaúnas	34	5	0.035	Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP
	Sambaqui Manitiba I	35	20	0.141	Lopes et al. 2016, PLoS One 11(6): 1-36 Magalhães et al. 2001, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ, 5 Barbosa-Guimarães 2013, An Acad Bras Ciênc 85: 415-429 Barbosa-Guimarães 2007, DSc Thesis, MAE-USP
	Sambaqui da Ponte do Girau	36	3	0.021	Lopes et al. 2016, PLoS One 11(6): 1-36

Table 1. Continued...

Location	Midden	Code	Rspp	Relative Rspp	References
Cabo Frio	Sambaqui do Forte	37	2	0.014	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131 Costa et al. 2012, Rev Mus Arqueol Etnol 22: 51-65 Kneip 1980, Separata de Pesquisas, Série Antropologia, 31 Kneip et al. 1975, Separata da Revista do Museu Paulista-USP Kneip et al. 1975, An Acad Bras Ciênc 47: 91-97 Gaspar 1991, DSc Thesis, FFLCH-USP Gaspar 2003, Pesquisas, Série Antropologia-IAP 59: 1-163 Souza 2009, Monography, GeoQuater-UFRJ
	Abrigo Arraial do Cabo	38	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
	Sambaqui Boca da Barra	39	10	0.070	Gaspar 1991, DSc Thesis, FFLCH-USP Gaspar 2003, Pesquisas, Série Antropologia-IAP 59: 1-163 Barbosa 1999, MSc Thesis, PGCA-UFF
	Sítio do Meio	40	1	0.007	Gaspar 1991, DSc Thesis, FFLCH-USP Gaspar 2003, Pesquisas, Série Antropologia-IAP 59: 1-163 Gaspar & Scaramella 1992, Anais VI Reunião Científica da SAB
	Sambaqui da Salina Peroano	41	2	0.014	Gaspar 1991, DSc Thesis, FFLCH-USP Gaspar 2003, Pesquisas, Série Antropologia-IAP 59: 1-163 Franco & Gaspar 1992, Anais VI Reunião Científica da SAB
	Sítio Ilha de Cabo Frio	42	15	0.106	Lopes et al. 2016, PLoS One 11(6): 1-36
Armação de Búzios	Geribá II	43	1	0.007	Gaspar 1991, DSc Thesis, FFLCH-USP Gaspar 2003, Pesquisas, Série Antropologia-IAP 59: 1-163
Arraial do Cabo	Sítio Colônia de Pesca	44	2	0.014	Gaspar 1991, DSc Thesis, FFLCH-USP Mendonça de Souza et al. 1983-1984, Arquivos do Museu de História Natural-UFMG 8/9: 107-119 Tenório et al. 2010, Rev Mus Arqueol Etnol 20: 127-145 Tenório et al. 2005, Anais XIII Congresso da SAB
	Sítio Ponta da Cabeça	45	9	0.063	Gaspar 1991, DSc Thesis, FFLCH-USP Tenório et al. 2010, Rev Mus Arqueol Etnol 20: 127-145 Tenório et al. 2005, Anais XIII Congresso da SAB
	Sítío do Condomínio do Atalaia	46	1	0.007	Tenório et al. 2005, Anais XIII Congresso da SAB
	Usiminas	47	24	0.169	Lopes et al. 2016, PLoS One 11(6): 1-36
Mangaratiba	Sambaqui do Saí	48	5	0.035	Mendonça de Souza & Mendonça de Souza 1981-1982, Arquivos do Museu de História Natural-UFMG 6/7: 109-131
Rio das Ostras	Sambaqui da Tarioba	49	15	0.106	Tuna 2015, MSc Thesis, PBMAC-UFF
Niterói	Sambaqui de Camboinhas	50	12	0.085	Lopes et al. 2016, PLoS One 11(6): 1-36 Souza Cunha et al. 1981 In: Pesquisas arqueológicas no litoral de Itaipu, Niterói, RJ, Cia de Desenvolvimento Territorial Souza Cunha et al. 1986, Anais XXX Congresso Brasileiro de Geologia Vogel & Veríssimo 1981 In: Pesquisas arqueológicas no litoral de Itaipu, Niterói, RJ, Cia de Desenvolvimento Territoria Kneip & Pallestrini 1984 In: Restigas: origem, estrutura, processo, CEUFF Kneip 1979, Pesquisas de salvamento em Itaipu, Niterói, Rio de Janeiro, Cia de Desenvolvimento Territorial Kneip 1995, Documento de Trabalho, Série Arqueologia, Museu Nacional-UFRJ 2: 83-102
Itaboraí	Sambaqui Sampaio I	51	1	0.007	Pinto 2009, MSc Thesis, PPGArq-UFRJ
Rio de Janeiro	Sambaqui da Embratel	52	2	0.014	Kneip et al. 1984 In: Restigas: origem, estrutura, processo, CEUFF Kneip et al. 1984, Revista de Pré-História 6: 334-360
	Sambaqui do Zé Espinho	53	12	0.085	Kneip et al. 1984 In: Restigas: origem, estrutura, processo, CEUFF Kneip et al. 1984, Revista de Pré-História 6: 334-360 Vogel 1987 In: Coletores e pescadores pré-históricos de Guaratiba, Rio de Janeiro, EDUFF Kneip et al. 1986, Separata da Rev Mus Pau

Table 1. Continued...

Location	Midden	Code	Rspp	Relative Rspp	References
Cubatão	Sambaqui Piaçaguera	54	63	0.444	Fischer 2012, MSc Thesis, MAE-USP Gonzalez 2005, DSc Thesis, MAE-USP Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Filippini & Eggers 2005-2006, Rev Mus Arqueol Etnol 5-16: 165-180 Garcia 1972, DSc Thesis, IB-USP Garcia & Uchôa 1980, Revista de Pré-História 2: 5-81 Duarte 1968, O sambaqui visto através de alguns sambaquis, Instituto de Pré-História da USP Borges 2015, DSc Thesis, Muséum National D'Histoire Naturelle de Paris
	COSIPA 1	55	35	0.246	Figuti 1998, Revista Arqueologia 11: 57-70 Gonzalez 2005, DSc Thesis, MAE-USP Figuti 1993, Rev Mus Arqueol Etnol 3: 67-80 Figuti 1994-1995, Revista de Arqueologia 8: 267-283
	COSIPA 2	56	16	0.113	Gonzalez 2005, DSc Thesis, MAE-USP Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Figuti 1993, Rev Mus Arqueol Etnol 3: 67-80 Figuti 1994-1995, Revista de Arqueologia 8: 267-283
	COSIPA 3	57	9	0.063	Figuti 1987, Institut de Paleontologie Humaine, Paris Figuti 1989, Revista de Pré-História 7: 112-126
	COSIPA 4	58	36	0.254	Figuti 1998, Revista Arqueologia 11: 57-70 Gonzalez 2005, DSc Thesis, MAE-USP Figuti 1993, Rev Mus Arqueol Etnol 3: 67-80 Figuti 1994-1995, Revista de Arqueologia 8: 267-283
Ubatuba	Sítio Tenório	59	38	0.268	Figuti 1998, Revista Arqueologia 11: 57-70 Gonzalez 2005, DSc Thesis, MAE-USP Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Filippini & Eggers 2005-2006, Rev Mus Arqueol Etnol 5-16: 165-180 Garcia 1972, DSc Thesis, IB-USP Amenomori 1999, MSc Thesis, FFLCH-USP
	Sítio do Mar Virado	60	28	0.197	Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Amenomori 1999, MSc Thesis, FFLCH-USP Amenomori 2005, DSc Thesis, MAE-USP Uchôa 2009, CLIO-Série Arqueológica 24: 7-40 Nishida 2001, MSc Thesis, MAE-USP
	Sítio Couves 1	61	6	0.042	Amenomori 1999, MSc Thesis, FFLCH-USP
Guarujá	Sambaqui do Buracão	62	54	0.380	Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Borges 2015, DSc Thesis, Muséum National D'Histoire Naturelle de Paris Duarte 1968, O sambaqui visto através de alguns sambaquis, Instituto de Pré-História da USP Amenomori 2005, DSc Thesis, MAE-USP
	Sambaqui do Mar Casado	63	48	0.338	Gonzalez 2005, DSc Thesis, MAE-USP Gonzalez & Amenomori 2003, Rev Mus Arqueol Etnol 13: 25-37 Borges 2015, DSc Thesis, Muséum National D'Histoire Naturelle de Paris Duarte 1968, O sambaqui visto através de alguns sambaquis, Instituto de Pré-História da USP
	Sambaqui Maratuá	64	24	0.169	Gonzalez 2005, DSc Thesis, MAE-USP
Ilhabela	Sambaqui Abrigo Sul	65	2	0.014	Bendazzoli 2014, DSc Thesis, MAE-USP
Iguape	Abrigo do Pindú	66	2	0.014	Bendazzoli 2014, DSc Thesis, MAE-USP Bonetti 1997, MSc Thesis, FFLCH-USP
Serra	Sambaqui do Péricles II	67	4	0.028	Rhea Estudos e Projetos Ltda 2009, Salvamento e monitoramento arqueológico na área do Alphaville Jacuhy
Vitória	Sítio Campus 2	68	3	0.021	Perota 1972, Revista de Cultura da UFES 3: 39-45

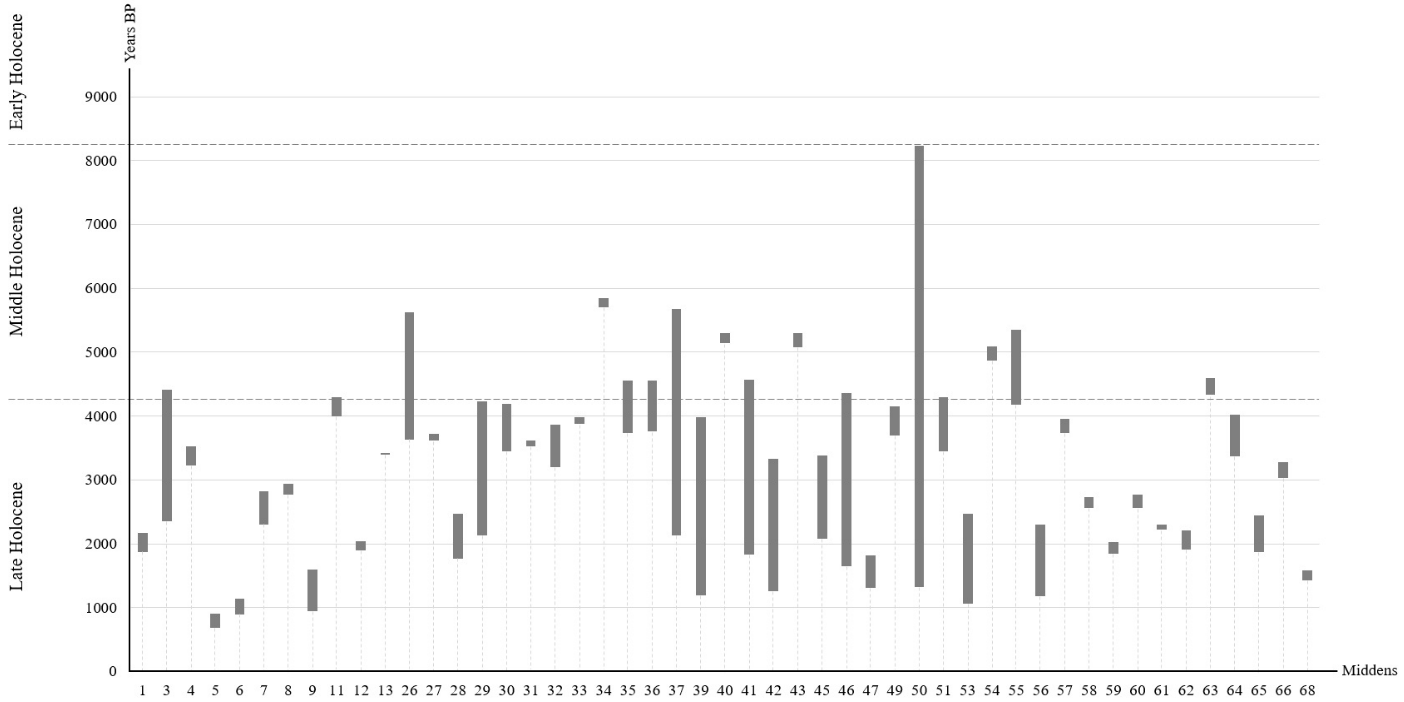

Figure 2. Graphic with the ^{14}C radiocarbonic dates of the 49 inventoried middens of the Southeast Region of Brazil. In the x axis are the middens (codes defined at Table 1) and in the y axis are the ranges of ^{14}C radiocarbonic dating (given in year before present) found in the literature. The different periods of the Holocene were defined based in Walker et al. (2012): Early Holocene (11,700 ~ 8,200 BP), Middle Holocene (8,200 ~ 4,200 BP) and Late Holocene (4,200 BP ~ till today).

Table 2. Marine fish of class Osteichthyes from middens in the Southeast Region of Brazil with their absolute (F) and relative (relative F) frequencies of occurrence as well as the midden(s) in which they were recorded. The codes representing the middens are defined in Table 1.

Family	Species	F	Relative F	Midden(s)
Albulidae	*Albula nemoptera* (Fowler, 1911)	1	0.015	3
Ariidae	*Aspistor luniscutis* (Valenciennes, 1840)	19	0.279	2, 3, 4, 5, 6, 11, 27, 28, 30, 31, 35, 54, 55, 58, 59, 60, 62, 63, 64
	Bagre bagre (Linnaeus, 1766)	23	0.338	1, 2, 3, 4, 5, 6, 9, 10, 11, 20, 21, 22, 23, 24, 25, 48, 50, 54, 55, 58, 59, 62, 63
	Bagre marinus (Mitchill, 1815)	18	0.265	1, 2, 3, 4, 5, 6, 8, 26, 28, 30, 35, 49, 53, 54, 62, 63, 64, 67
	Cathorops spixii (Agassiz, 1829)	18	0.265	2, 3, 4, 5, 6, 26, 28, 29, 30, 35, 54, 55, 57, 58, 59, 62, 63, 64
	Genidens barbus (Lacepède, 1803)	33	0.485	2, 3, 4, 5, 6, 10, 11, 12, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 48, 50, 54, 55, 58, 59, 60, 62, 63
	Genidens genidens (Cuvier, 1829)	19	0.279	2, 3, 4, 5, 6, 8, 26, 28, 29, 35, 49, 50, 54, 55, 58, 59, 62, 63, 64
	Genidens machadoi (Miranda Ribeiro, 1918)	1	0.015	54
	Notarius grandicassis (Valenciennes, 1840)	12	0.176	2, 3, 4, 5, 6, 54, 55, 58, 59, 62, 63, 64
	Potamarius grandoculis (Steindachner, 1877)	2	0.029	54, 63
Balistidae	*Balistes capriscus* Gmelin, 1789	2	0.029	62, 63
	Balistes vetula Linnaeus, 1758	12	0.176	1, 2, 3, 4, 5, 6, 9, 54, 55, 58, 59, 60
Carangidae	*Caranx crysos* (Mitchill, 1815)	4	0.059	29, 54, 62, 63
	Caranx hippos (Linnaeus, 1766)	23	0.338	1, 2, 3, 4, 5, 6, 8, 9, 26, 27, 28, 29, 30, 31, 35, 47, 50, 53, 54, 62, 63, 64, 67
	Caranx ruber (Bloch, 1793)	1	0.015	62
	Chloroscombrus chrysurus (Linnaeus, 1766)	1	0.015	62
	Oligoplites saurus (Bloch & Schneider, 1801)	4	0.059	8, 26, 28, 30
	Selar crumenophthalmus (Bloch, 1793)	1	0.015	8
	Selene vomer (Linnaeus, 1758)	3	0.044	8, 47, 62
	Seriola dumerili (Risso, 1810)	2	0.029	62, 63
	Trachinotus falcatus (Linnaeus, 1758)	1	0.015	65
	Trachurus lathami Nichols, 1920	1	0.015	54
Centropomidae	*Centropomus ensiferus* Poey, 1860	3	0.044	3, 5, 42
	Centropomus parallelus Poey, 1860	6	0.088	35, 47, 54, 62, 63, 64
	Centropomus undecimalis (Bloch, 1792)	16	0.235	3, 11, 26, 27, 28, 29, 30, 32, 35, 54, 57, 62, 63, 64, 67, 69
Coryphaenidae	*Coryphaena hippurus* Linnaeus, 1758	1	0.015	8
Dactylopteridae	*Dactylopterus volitans* (Linnaeus, 1758)	5	0.074	9, 29, 60, 62, 63
Diodontidae	*Chilomycterus spinosus* (Linnaeus, 1758)	12	0.176	1, 2, 3, 4, 5, 6, 47, 49, 54, 55, 58, 59
	Diodon hystrix Linnaeus, 1758	11	0.162	2, 3, 4, 5, 6, 9, 54, 55, 58, 59, 63

Table 2. Continued...

Family	Species	F	Relative F	Midden(s)
Eleotridae	*Dormitator maculatus* (Bloch, 1792)	1	0.015	54
	Eleotris pisonis (Gmelin, 1789)	1	0.015	62
Elopidae	*Elops saurus* Linnaeus, 1766	1	0.015	54
Ephippidae	*Chaetodipterus faber* (Broussonet, 1782)	21	0.309	1, 2, 3, 4, 5, 6, 7, 9, 29, 35, 39, 47, 49, 53, 54, 55, 58, 59, 60, 61, 62
Gerreidae	*Diapterus auratus* Ranzani, 1842	2	0.029	3, 5
	Diapterus rhombeus (Cuvier, 1829)	5	0.074	3, 5, 6, 54, 62
	Eugerres brasilianus (Cuvier, 1830)	2	0.029	54, 62
	Gerres cinereus (Walbaum, 1792)	1	0.015	3
Haemulidae	*Anisotremus surinamensis* (Bloch, 1791)	3	0.044	54, 62, 63
	Anisotremus virginicus (Linnaeus, 1758)	6	0.088	1, 3, 4, 5, 6, 47
	Conodon nobilis (Linnaeus, 1758)	13	0.191	2, 3, 4, 5, 6, 11, 54, 55, 58, 59, 60, 62, 63
	Haemulon aurolineatum Cuvier, 1830	2	0.029	3, 47
	Haemulon plumierii (Lacepède, 1801)	3	0.044	54, 62, 63
	Haemulon sciurus (Shaw, 1803)	1	0.015	3
	Haemulon steindachneri (Jordan & Gilbert, 1882)	3	0.044	1, 3, 47
	Orthopristis ruber (Cuvier, 1830)	10	0.147	1, 2, 3, 4, 5, 6, 54, 55, 58, 59
	Pomadasys crocro (Cuvier, 1830)	1	0.015	63
Hemiramphidae	*Hyporhamphus unifasciatus* (Ranzani, 1841)	2	0.029	1, 3
Holocentridae	*Holocentrus adscensionis* (Osbeck, 1765)	2	0.029	60, 62
Istiophoridae	*Istiophorus albicans* (Latreille, 1804)	1	0.015	47
Kyphosidae	*Kyphosus sectatrix* (Linnaeus, 1758)	3	0.044	9, 49, 60
Labridae	*Bodianus rufus* (Linnaeus, 1758)	4	0.059	3, 8, 42, 47
Lobotidae	*Lobotes surinamensis* (Bloch, 1790)	10	0.147	7, 39, 54, 55, 56, 58, 62, 63, 64, 66
Lutjanidae	*Lutjanus analis* (Cuvier, 1828)	1	0.015	68
	Lutjanus griseus (Linnaeus, 1758)	1	0.015	59
	Lutjanus purpureus (Poey, 1866)	1	0.015	59
	Lutjanus synagris (Linnaeus, 1758)	5	0.074	1, 3, 4, 5, 6
	Ocyurus chrysurus (Bloch, 1791)	1	0.015	47
Malacanthidae	*Caulolatilus chrysops* (Valenciennes, 1833)	1	0.015	53
Megalopidae	*Megalops atlanticus* Valenciennes, 1847	3	0.044	54, 62, 63
Mugilidae	*Mugil liza* Valenciennes, 1836	5	0.074	1, 3, 4, 5, 6
Paralichthyidae	*Paralichthys orbignyanus* (Valenciennes, 1839)	1	0.015	54
Polynemidae	*Polydactylus oligodon* (Günther, 1860)	6	0.088	14, 15, 17, 18, 19, 38
Pomacanthidae	*Pomacanthus paru* (Bloch, 1787)	1	0.015	62
Pomatomidae	*Pomatomus saltatrix* (Linnaeus, 1766)	21	0.309	2, 3, 4, 5, 6, 7, 9, 26, 28, 29, 30, 39, 41, 45, 53, 54, 55, 58, 59, 60, 63
Sciaenidae	*Bairdiella ronchus* (Cuvier, 1830)	13	0.191	1, 2, 3, 4, 5, 6, 32, 49, 54, 55, 57, 58, 59
	Cynoscion acoupa (Lacepède, 1801)	16	0.235	10, 11, 14, 15, 17, 18, 19, 20, 27, 35, 38, 54, 59, 62, 63, 64
	Cynoscion guatucupa (Cuvier, 1830)	1	0.015	64
	Cynoscion jamaicensis (Vaillant & Bocourt, 1883)	12	0.176	1, 2, 3, 4, 5, 6, 8, 32, 34, 49, 55, 58
	Cynoscion leiarchus (Cuvier, 1830)	5	0.074	54, 55, 58, 62, 63
	Cynoscion similis Randall & Cervigón, 1968	6	0.088	1, 3, 4, 5, 6, 36
	Cynoscion striatus (Cuvier, 1829)	3	0.044	28, 29, 35
	Cynoscion virescens (Cuvier, 1830)	19	0.279	11, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 38, 48, 58, 59, 62, 63
	Isopisthus parvipinnis (Cuvier, 1830)	3	0.044	32, 54, 59
	Larimus breviceps Cuvier, 1830	18	0.265	1, 2, 3, 4, 5, 6, 32, 33, 34, 49, 54, 55, 57, 58, 59, 60, 61, 63
	Menticirrhus americanus (Linnaeus, 1758)	2	0.029	49, 59
	Micropogonias furnieri (Desmarest, 1823)	53	0.779	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 38, 39, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64
	Micropogonias undulatus (Linnaeus, 1766)	8	0.118	26, 27, 28, 29, 30, 31, 35, 50
	Ophioscion punctatissimus Meek & Hildebrand, 1925	1	0.015	54
	Pareques acuminatus (Bloch & Schneider, 1801)	1	0.015	35
	Pogonias cromis (Linnaeus, 1766)	48	0.706	2, 3, 4, 5, 6, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 42, 48, 49, 52, 53, 54, 55, 57, 58, 59, 60, 62, 63, 64, 68
	Nebris microps Cuvier, 1830	1	0.015	59
	Stellifer brasiliensis (Schultz, 1945)	1	0.015	54
	Stellifer rastrifer (Jordan, 1889)	2	0.029	32, 54
	Umbrina canosai Berg, 1895	1	0.015	29
	Umbrina coroides Cuvier, 1830	1	0.015	42

Table 2. Continued...

Family	Species	F	Relative F	Midden(s)
Scombridae	*Katsuwonus pelamis* (Linnaeus, 1758)	2	0.029	8, 47
	Scomberomorus cf. *brasiliensis* Collette, Russo & Zavala-Camin, 1978	2	0.029	62, 63
	Thunnus cf. *atlanticus* (Lesson, 1831)	1	0.015	64
Serranidae	*Cephalopholis fulva* (Linnaeus, 1758)	4	0.059	54, 62, 63, 64
	Epinephelus itajara (Lichtenstein, 1822)	4	0.059	54, 62, 63, 64
	Epinephelus morio (Valenciennes, 1828)	6	0.088	3, 47, 54, 62, 63, 64
	Hyporthodus niveatus (Valenciennes, 1828)	2	0.029	26, 29
	Mycteroperca acutirostris (Valenciennes, 1828)	2	0.029	63, 64
Sparidae	*Archosargus probatocephalus* (Walbaum, 1792)	20	0.294	2, 3, 4, 5, 6, 7, 9, 39, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63, 64, 66
	Archosargus rhomboidalis (Linnaeus, 1758)	8	0.118	3, 5, 26, 28, 29, 30, 31, 35
	Calamus pennatula Guichenot, 1868	2	0.029	54, 62
	Diplodus argenteus (Valenciennes, 1830)	2	0.029	9, 60
	Pagrus pagrus (Linnaeus, 1758)	8	0.118	26, 28, 29, 30, 35, 47, 49, 53
Sphyraenidae	*Sphyraena barracuda* (Edwards, 1771)	1	0.015	5
	Sphyraena guachancho Cuvier, 1829	1	0.015	3
Tetraodontidae	*Lagocephalus laevigatus* (Linnaeus, 1766)	24	0.353	2, 3, 4, 5, 6, 7, 8, 9, 26, 28, 35, 39, 42, 49, 54, 55, 56, 58, 59, 60, 61, 62, 63, 67
	Sphoeroides spengleri (Bloch, 1785)	5	0.074	2, 3, 4, 5, 6
	Sphoeroides testudineus (Linnaeus, 1758)	5	0.074	2, 3, 4, 5, 6
Trichiuridae	*Trichiurus lepturus* Linnaeus, 1758	17	0.250	2, 3, 4, 5, 6, 8, 42, 47, 53, 54, 55, 58, 59, 60, 62, 63, 65
Triglidae	*Prionotus punctatus* (Bloch, 1793)	1	0.015	1

Table 3. Marine fish of class Chondrichthyes from middens in the Southeast Region of Brazil with their absolute (F) and relative (relative F) frequencies of occurrence as well as the midden(s) in which they were recorded. The codes representing the middens are defined in Table 1.

Family	Species	F	Relative F	Midden(s)
Alopiidae	*Alopias superciliosus* Lowe, 1841	2	0.029	3, 50
	Alopias vulpinus (Bonnaterre, 1788)	6	0.088	3, 53, 55, 56, 58, 60
Carcharhinidae	*Carcharhinus acronotus* (Poey, 1860)	8	0.118	1, 3, 5, 42, 54, 62, 63, 64
	Carcharhinus altimus (Springer, 1950)	2	0.029	3, 9
	Carcharhinus brachyurus (Günther, 1870)	2	0.029	3, 5
	Carcharhinus brevipinna (Müller & Henle, 1839)	8	0.118	3, 8, 26, 29, 42, 47, 49, 50
	Carcharhinus falciformis (Müller & Henle, 1839)	1	0.015	3
	Carcharhinus leucas (Müller & Henle, 1839)	10	0.147	3, 45, 54, 55, 56, 58, 59, 60, 62, 63
	Carcharhinus limbatus (Müller & Henle, 1839)	11	0.162	1, 3, 4, 5, 6, 8, 45, 54, 62, 63, 64
	Carcharhinus longimanus (Poey, 1861)	2	0.029	3, 45
	Carcharhinus obscurus (Lesueur, 1818)	10	0.147	3, 8, 26, 29, 54, 55, 56, 58, 62
	Carcharhinus perezii (Poey, 1876)	1	0.015	3
	Carcharhinus plumbeus (Nardo, 1827)	10	0.147	1, 3, 5, 6, 26, 45, 47, 55, 56, 58
	Carcharhinus porosus (Ranzani, 1839)	5	0.074	1, 3, 4, 5, 6
	Negaprion brevirostris (Poey, 1868)	9	0.132	1, 2, 3, 4, 5, 6, 8, 29, 42
	Prionace glauca (Linnaeus, 1758)	9	0.132	44, 54, 55, 56, 57, 58, 59, 60, 62
	Rhizoprionodon lalandii (Müller & Henle, 1839)	4	0.059	3, 4, 5, 6
	Rhizoprionodon porosus (Poey, 1861)	3	0.044	3, 4, 5
	Galeocerdo cuvier (Péron & Lesueur, 1822)	23	0.338	1, 2, 3, 4, 5, 6, 7, 8, 30, 42, 44, 45, 46, 47, 54, 55, 56, 58, 59, 60, 62, 63, 64
Dasyatidae	*Bathytoshia centroura* (Mitchill, 1815)	6	0.088	3, 7, 26, 29, 39, 50
	Hypanus americanus (Hildebrand & Schroeder, 1928)	2	0.029	54, 63
	Hypanus guttatus (Bloch & Schneider, 1801)	1	0.015	3
Ginglymostomatidae	*Ginglymostoma cirratum* (Bonnaterre, 1788)	3	0.044	54, 62, 63
Gymnuridae	*Gymnura altavela* (Linnaeus, 1758)	1	0.015	7
Lamnidae	*Lamna nasus* (Bonnaterre, 1788)	7	0.103	1, 3, 4, 5, 6, 42, 47
	Carcharodon carcharias (Linnaeus, 1758)	23	0.338	1, 2, 3, 4, 5, 6, 7, 8, 26, 29, 42, 45, 47, 50, 54, 55, 56, 58, 59, 60, 62, 63, 64
	Isurus oxyrinchus Rafinesque, 1810	15	0.221	3, 7, 8, 37, 39, 42, 45, 47, 54, 55, 56, 58, 60, 62, 63
	Isurus paucus Guitart, 1966	1	0.015	7
Myliobatidae	*Aetobatus narinari* (Euphrasen, 1790)	24	0.353	3, 8, 9, 26, 28, 29, 30, 31, 35, 39, 42, 47, 49, 50, 54, 55, 56, 58, 59, 60, 61, 62, 63, 64
	Myliobatis goodei Garman, 1885	5	0.074	54, 55, 56, 58, 59
	Rhinoptera bonasus (Mitchill, 1815)	10	0.147	7, 39, 54, 55, 56, 58, 59, 60, 62, 63
	Rhinoptera brasiliensis Müller, 1836	5	0.074	54, 59, 60, 61, 62
Odontaspididae	*Carcharias taurus* Rafinesque, 1810	26	0.382	1, 2, 3, 4, 5, 6, 7, 8, 35, 40, 41, 42, 43, 45, 47, 50, 53, 54, 55, 56, 57, 58, 59, 60, 62, 63
Sphyrnidae	*Sphyrna lewini* (Griffith & Smith, 1834)	2	0.029	3, 60
	Sphyrna mokarran (Rüppell, 1837)	2	0.029	3, 29
	Sphyrna tiburo (Linnaeus, 1758)	2	0.029	54, 59
	Sphyrna zygaena (Linnaeus, 1758)	4	0.059	3, 5, 47, 59

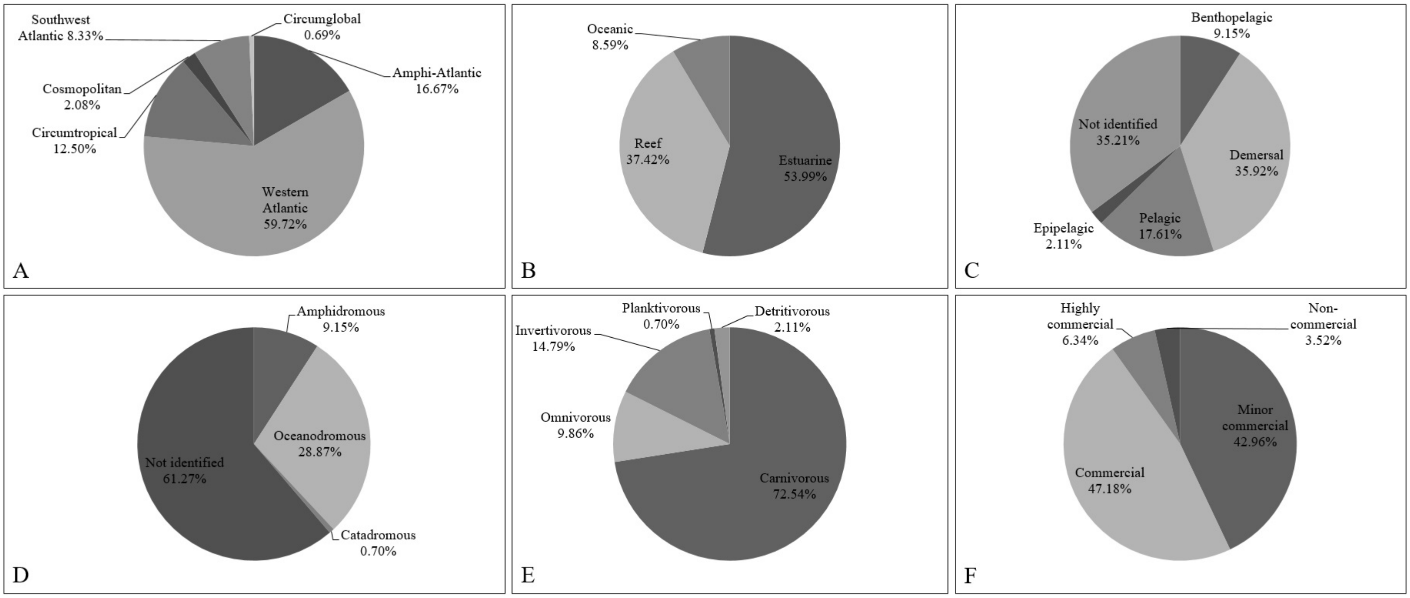

Figure 3. Biogeographical, ecological and economic data for the inventoried fish. A = Distribution; B = Environment; C = Habit; D = Behaviour; E = Feeding guild; F = Commercial value.

Table 4. Number of species from current fish inventories and middens from Rio de Janeiro (RJ), São Paulo (SP), Espírito Santo (ES) and the entire Southeast Region.

n spp.	RJ	SP	ES	Southeast Region
Overall Past	104	90	6	142
Overall Present	591	578	282	808
Commercial Past	101	87	6	137
Commercial Present	412	340	196	488
Past Exclusive	13	15	4	5

Discussion

Middens are artificial accumulations of wildlife and cultural remains that were built by groups of pre-colonial humans during the Holocene (Lima et al. 2003, Mendes et al. 2014). Therefore, the species compositions of the organisms found in middens are presumably non-random and biased samples of natural biological communities at those sites because they were influenced or determined by various cultural factors, including food preference, the technological level of the fishing gear, harvesting and hunting artefacts, food taboos, funerary or ritualistic practices and how the materials were discarded and/or used. Therefore, some researchers believe that the faunal data from middens have selectivity biases that complicate any related inferences about ecosystems and their biodiversity (Baisre 2010, Rodrigues et al. 2016a). However, the comparison between the inventory of the marine fish identified in middens and those from general surveys of ichthyological fauna showed no significant differences in the number of species either with or without commercial value, indicating that middens contain data that are not solely applicable to prehistoric fisheries. Such wildlife remains apparently represent the fauna existing at the time that the archaeological sites were created but are also repositories of broader biodiversity data, despite any bias associated with the composition of zoo-archaeological remains (Lindbladh et al. 2007, Froyd & Willis 2008).

In a recent study using taxonomic tests, Faria et al. (2014) showed that the malacological taxonomic diversity in the Tarioba midden (Rio de Janeiro, Brazil) was not significantly different from that of a comprehensive list of the mollusc species from the entire coast of the state of Rio de Janeiro. Those findings and the results of the present study suggest that middens may contain key indicators of past biodiversity, despite their limitations, and furthermore, some studies of middens have shown detectable changes in species composition over time (Dalzell 1998, Lotze & Milewski 2004, Rosenberg et al. 2005, Maschner et al. 2008, Souza et al. 2016).

Studies focused on inventorying the fish species in middens have usually involved a limited number of sites (sampling areas). For example, Kloker et al. (2010) recorded 17 fish species from two sites on the south coast of Brazil (Santa Catarina state). In contrast, as part of a greater sampling effort, Lopes et al. (2016) recorded 97 fish species in 13 sites located on the coast of Rio de Janeiro, and in this study, the study area corresponded to 68 middens distributed over 1,000 km of the southeast coast of Brazil.

In using even greater effort than that undertaken by Lopes et al. (2016), this study used data which came from different kinds of publications (scientific articles, books, thesis, dissertations, monographs, technical reports etc) encompassing 48 years (1968-2016) of studies in the field. Therefore, the data compiled here came from a myriad of objectives and methodologies, for example, primary data which came from excavations were originated since superficial sampling till total material recovery. Following excavation the material recovered were dry sieved or under current water using different mesh sizes (2 to 10mm). Identification of the zoo-archaeological vestiges was done by handling different manuals and reference collections. Due to that the list recovered was carefully scrutinized. Criteria such as using only the more inclusive taxa and species with biogeographic range defined were used. Furthermore, the Linean definition of the species was fully checked for ambiguities and classification changes. In using these criteria any inconvenience related to the heterogeneity of sources were surpassed and the methodological choice gave an extensive baseline of fishes biodiversity during the Holocene in the Brazilian Coast.

The species richness from the midden fish inventory in this study was lower than that of current fish checklists (Bizerril & Costa 2001, Menezes 2011, *species*Link), which likely resulted from the selected sampling methods. All current checklists were developed from extensive bibliographical surveys that included studies that employed diverse methods (fishing records, scientific collections, the testimony of scientific experts, museum collection documents and environmental monitoring) as well as data accumulated from several years of academic research. In contrast, the midden records were fundamentally related to prehistoric fisheries or related cultural activities (fishing gear, ritualistic symbols, ornaments and artefacts) as well as by-catch. In all cases, the midden fish records were always informed by an understanding of prehistoric culture; that is, the midden fish inventory was constructed with data from archaeological studies of fishing cultures and thus focused on a limited number of target species.

Regarding the target species, the occurrence frequencies of *M. furnieri* and *P. cromis* in middens indicate that they were preferentially fished species. Barbosa-Guimarães (2013) observed that *M. furnieri* was the main fish species in Saquarema middens (Rio de Janeiro, Brazil) and thus inferred that it was the primary food of the midden peoples of that region. In turn, Souza Cunha et al. (1981) notes the presence of *Pogonias* sp. in the midden Sambaqui de Camboinhas (Niterói, state of Rio de Janeiro, Brazil), and the remains of that genus are commonly found in coastal middens (Kneip et al. 1975). Lopes et al. (2016) recorded *M. furnieri* and *P. cromis* as two of the three most common species in their study of Rio de Janeiro middens, and these two species are currently considered key fishery resources in the Southeast Region of Brazil (Mulato et al. 2015, Santos et al. 2016). Furthermore, estuarine, demersal and carnivorous species typically have significant commercial value, thus composing key fishing stocks (Tacon 1994, Santos & Câmara 2002, Haimovici et al. 2014), and it can be deduced that such species were critical fishing resources for midden peoples in the past.

On the one hand, the presence of species such as *M. furnieri* and *P. cromis*, which occur at high relative frequencies in middens, indicates that midden fish inventories contain prehistoric fishery data, but the numbers of fish with and without commercial value in middens are not significantly different from those in checklists of ichthyological fauna for the same sites. Moreover, the high number of exclusive species with low frequencies of occurrence in middens corroborates the hypothesis that midden fish records contain key data on Holocene ichthyological fauna and that such species are most likely by-catch. Additionally, the occurrence frequencies show that it is unlikely that midden peoples from neighbouring regions consumed different or unique species. Thus, the results from this study indicate that middens contain data on midden fishing and culture as well as past biodiversity.

Biodiversity inventories are essential for establishing baselines that inform species management and conservation measures (Gordillo et al. 2014), especially those related to endangered species, including fish. Currently, the effects of overfishing, pollution, invasive species and other ecological impacts have reduced marine ichthyological diversity (Povey & Keough 1991, Brosnan & Crumrine 1994, Polunin & Roberts 1996, Costello et al. 2010). Therefore, the study of ichthyological midden remains is a key tool for understanding prehistoric biodiversity, enabling the establishment of a historical perspective and thus more complete baselines to inform more effective management measures and reduce the threat of extinction currently faced by marine fishes.

In summary, the midden inventory of the Holocene marine fish of the southeast coast of Brazil contains data on prehistoric fisheries, which is supported by the high number of species with low frequencies of occurrence (or unique species) as well as by chi-squared tests that failed to show significant differences between the midden fish inventory and current ichthyofauna checklists. Thus, the results from this study clearly indicate that zoo-archaeological fish remains are key evidence of Holocene biodiversity.

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Author Contributions

Augusto Barros Mendes: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intelectual content.

Michelle Rezende Duarte: Contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intelectual content

Edson Pereira da 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 and contribution to critical revision, adding intelectual content

Conflicts of interest

The authors declare that there is no conflict of interest related to the publication of the data in this article.

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Reef fishes of the Anchieta Island State Park, Southwestern Atlantic, Brazil

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Abstract: This paper presents a checklist of the reef fish fauna of the Anchieta Island State Park, a no-take zone in which no extractive activities are allowed, in Ubatuba, Southeastern Brazil. Data was obtained between 2011 and 2013, mainly through underwater observations with snorkelling and SCUBA, and secondarily using scientific fishing (trawling). Published and unpublished data were also verified and compiled. A total of 103 reef fish species, distributed in 78 genus and 50 families was recorded. Haemulidae was the richest family (n=7 species), followed by Epinephelidae (n=6), Pomacentridae, Carangidae and Tetraodontidae (n= 4 each). *Haemulon* was also the most speciose genera (n=5), followed by *Stegastes*, *Acanthurus* and *Mycteroperca* (n=3 each). Sixty-nine species (67%) are included in global and regional Red Lists. Twenty-five species (24.2%) are within the categories Critically Endangered (CR), Endangered (EN), Vulnerable (VU) and Near-Threathened (NT). The network of Marine Protected Areas in Southeastern Brazil is still incipient, and the considerable number of threatened species in the Anchieta Island State Park clearly indicates the importance of the study area for the conservation of coastal and reef fishes.

Key words: *rocky reefs; species richness; scientific diving; ichthyology; conservation.*

Peixes recifais do Parque Estadual da Ilha Anchieta, Sudoeste do Atlântico, Brasil

Resumo: Este artigo apresenta uma lista de espécies de peixes recifais do Parque Estadual da Ilha Anchieta, uma área na qual nenhuma atividade extrativista é permitida, em Ubatuba, Sudeste do Brasil. Os dados foram coletados entre 2011 e 2013, principalmente por observações subaquáticas com o uso de mergulhos livres e autônomos e, secundariamente, através de pescaria científica (arrasto). Dados publicados e não publicados também foram verificados e compilados. Foram registradas 103 espécies de peixes recifais distribuídas em 78 gêneros e 50 famílias. Haemulidae foi a família com maior número de espécies (n=7 espécies), seguida por Epinephelidae (n=6), Pomacentridae, Carangidae e Tetraodontidae (n=4 cada). Os gêneros com maior número de espécies foram *Haemulon* (n=5) e *Stegastes*¸ *Acanthurus* e *Mycteroperca* (n=3 cada). Sessenta e nove espécies (67%) estão inclusas em listas vermelhas de espécies ameaçadas globais e regionais. Vinte e cinco espécies (24,2%) são classificadas nas categorias Criticamente em Perigo (CR), Em Perigo (EN), Vulnerável (VU) e Quase Ameaçadas (NT). A rede de Áreas Marinhas Protegidas no Sudeste do Brasil ainda é incipiente e o considerável número de espécies ameaçadas no Parque Estadual da Ilha Anchieta indica claramente a importância da área de estudo para a conservação de peixes recifais e costeiros.

Palavras-chave: *recifes rochosos; riqueza de espécies; mergulho científico; ictiologia; conservação.*

Introduction

In Brazil, coralline and sandstone reefs occur in the Northern, Northeastern and Central coast from the Amazon River mouth south to the Doce River mouth, off Espírito Santo State (~19°50'S) (Moura et al. 2013, 2016, Pinheiro et al. 2015a), while rocky reefs predominate in the subtropical portion of the coast, southwards to Rio Grande do Sul State (~32°30'S), as well as in the three oceanic archipelagos (Ferreira et al. 2001, 2004, Krajewski & Floeter 2011, Magalhães et al. 2015). The physical complexity of coralline and rocky reefs is associated to a remarkably high diversity of fish and several other groups, which overcomes that of any other aquatic habitat (Reaka-Kudla 1997, Spalding et al. 2001).

Reefs increase shelter and provide feeding habitats for a broad taxonomic spectrum of fishes with specific morphological and functional adaptations, the so-called reef fishes (Luckhurst & Luckhurst 1978, Alvarez-Filip et al. 2009, Ménard et al. 2012, Nunes et al. 2015).

Because the Southeastern Brazilian coast is located in a tropical and subtropical transition zone (Floeter et al. 2001), the region's rocky reefs often present higher local (alpha) diversity of reef fishes than coralline reefs (e.g. Moura & Francini-Filho 2005, Gibran & Moura 2012), and may be considered biodiversity hotspots, akin to "marginal" areas of other biogeographic regions (Aburto-Oropeza & Balart 2001, Sala et al. 2012). The Brazilian coralline reef area represent 0.4% of global reef area, with 20% of reef fishes endemism, which represent a priority in conservation (Moura 2003, Moura & Francini-Filho 2005, Gibran & Moura 2012).

Information about fish assemblages associated to Brazilian subtropical rocky reefs steadily increased in the last two decades (e.g. Luiz Jr. & Floeter 2004, Bonaldo et al. 2005, Floeter et al. 2006, Luiz Jr. et al. 2008, Gibran & Moura 2012, Teixeira-Neves et al. 2015, Neves et al. 2016). However, major geographic gaps still remain along this broad (~2,000 km) and highly threatened (e.g. Gibran & Moura 2012) extension of the coast, where the country´s population is concentrated within less than 100 km from shoreline (IBGE 2016) with several multiple and interacting environmental stressors (pollution, harbors, tourism and fishing) are observed. Such information gaps are particularly relevant for the ongoing development of management plans for the large multiple-use Marine Protected Areas (MPAs) that have been established along most of coast of the State of São Paulo.

Fish faunal assessments in the north coast of São Paulo are concentrated on demersal species associated to unconsolidated substrates (e.g. Cunningham 1983, Nonato et al. 1983, Braga & Goitein 1984, Rocha et al. 2010). Only two reef fish assessments were carried out in the vicinity of the Anchieta Island State Park (AISP), the protected area focused by the present study. One of these studies tested the use of artificial reefs for habitat restoration (Cunningham & Saul, 2004) and the other was focused on cleaning symbiosis, involving 35 fish species (Sazima et al. 2000). Here, we provide a checklist of teleosts and elasmobranchs of the AISP and comment upon the known distribution and conservation status of the recorded species. The information provided herein fill a relevant gap about local reef fish communities and contribute to the future management plans of the AISP and MPAs around.

Material and Methods

1. Study area

Sampling was carried out between 2011 and 2013 in the AISP, municipality of Ubatuba, northern coast of São Paulo (23°31'-23°34'S, 45°02'-45°05'W) (Figure 1). The AISP was created in 1977 by the State Government (Decree 9,629, March 29 1977), covering the terrestrial part of the island. In 1983, a fishing ban perimeter with 17.37 km^2 was established around the AISP (Portaria SUDEPE 56, November 10 1983). This perimeter is relatively well enforced, due to the considerable movement of people who visit the park. In addition, the park's own security guards carry out frequent inspections around the island. Anchieta Island is separated from the mainland by a narrow (0.5 km) and relatively deep (33 m) channel. Its shoreline is predominantly granitic, with the exception of a 1.5 km length sandy beach in its northern, shallower and less exposed side. Depths reach up to 37 m around the island, with a benthic mosaic encompassing sandy, gravel and rocky bottom (Guillaumon et al. 1989).

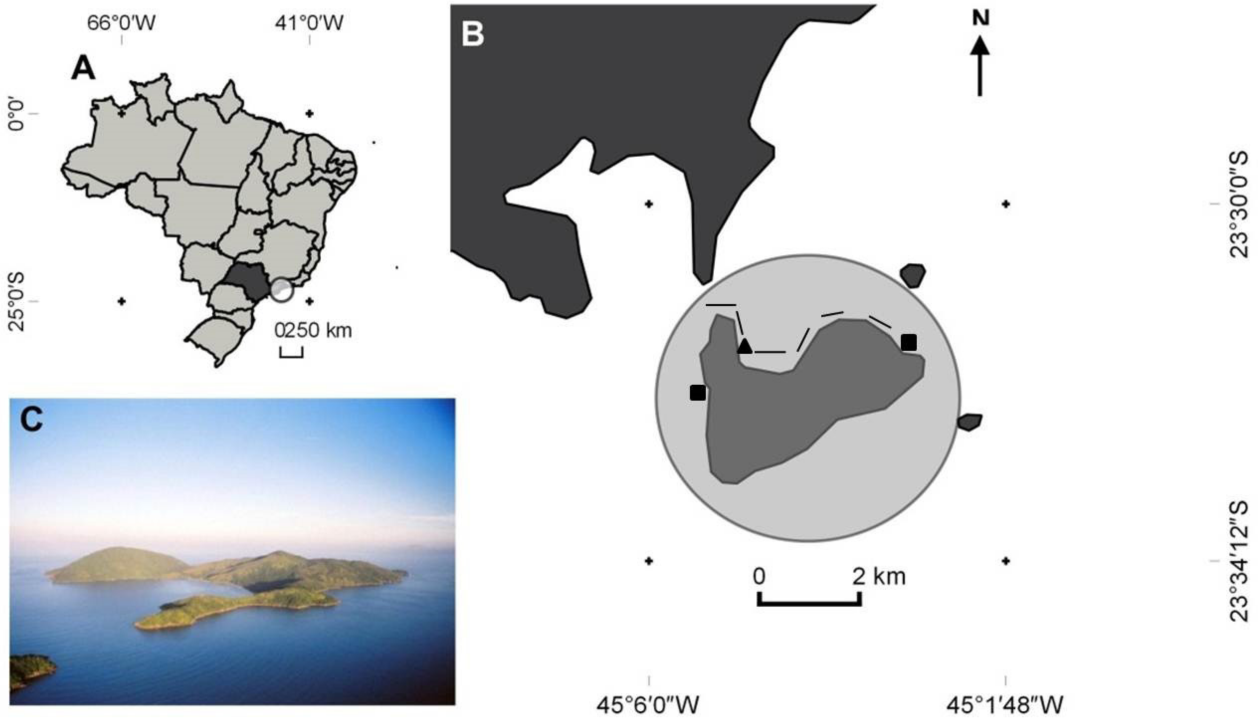

Figure 1. Study area, (A) Brazil in grey, São Paulo State in black and north coast of São Paulo inside the circle; (B) Anchieta Island inside the circle with the points of data collect: specific for this work (squares), Cunningham & Saul 2004 (triangle) and the area of bottom trawling (lines); (C) photograph of the Anchieta Island (credits: http://www.ambiente.sp.gov.br/parque-ilha-anchieta/galeria-de-fotos/).

2. Data collection

The checklist was compiled from snorkelling and SCUBA observations, scientific bottom trawling operations (research permit – SISBIO nº 32352-3), as well as from published (e.g. Sazima et al. 2000 and Cunningham & Saul 2004) and unpublished data (e.g. museum records – ZUEC). In total, 142 hours were spent on dives: (i) 20 hours with snorkelling and SCUBA for this work; (ii) 42 hours by Sazima et al. (2000); and (iii) 80 hours by Cunningham & Saul (2004). Dives were concentrated in the south and east sides of the island (i.e. South Beach and East Beach), and inside Palmas Bay, a cove in the west face of the island with depths of up to 12 m. All the reef fish observed during the dives were noted and, eventually, divers searched for cryptic species beneath crevices and rocks. Considering the different samplings, dives were carried over a depth range of 2-17 meters (for more details see Sazima et al. 2000 and Cunningham & Saul 2004). Twelve bottom trawls of 12 minutes each were carried out around the island, in the sand substrate adjacent to the rocky reefs, between 10 and 16 m depths. The trawl had 25mm of mesh size, three meters height and 7 meters length.

Identifications were based on Menezes & Figueiredo (1980, 1985), Figueiredo & Menezes (1978, 1980, 2000), and Humann & DeLoach (2002). The species were organized alphabetically within families, following Betancur-R et al. (2014) for teleosts, Rosa & Gadig (2014) for sharks and Last et al. (2016) for rays. Reef fish were considered as species that spend most of their time associated with consolidate substrates, and that commonly use these habitats for feeding, shelter or reproduction, including nektonic species (cf. Luiz Jr. et al. 2008). Geographical distributions followed Luiz Jr. et al. (2008), where: CT, Circumtropical; SE, Southeastern Brazil (endemic from between 20 - 27° S); TA, Trans-Atlantic (both sides of the Atlantic); WA, Western Atlantic (Northern and Southwestern Atlantic); BR, Brazilian Province endemic; SCa, Southern Caribbean (Venezuela, Trinidad and Tobago and other nearby islands); Pat, Patagonian (primarily occurring in temperate rocky reefs south to Argentina); EA, Eastern Atlantic. Conservation status were based on the International Red List of threatened species (IUCN – International Union for Conservation of Nature), the National Endangered Fauna – Fish and Aquatic Invertebrates (MMA – Ministério do Meio Ambiente) and on the São Paulo State List of threatened species (SMA – Secretaria do Meio Ambiente). Although the classification "Near Threatened (NT) is often not considered a threatened species, we have decided to include it because species in this category may be considered threatened in the near future.

As non-lethal methods were primarily adopted, "voucher-specimens" were stored as videos and photos in the ichthyological collection "Victor Sadowsky – UNESP Registro" (SADUNESP#0001). Specimens collected during past surveys (e.g. Sazima et al. 2000) were also deposited at the Museu de Zoologia/Universidade Estadual de Campinas - ZUEC Coleção de Peixes/Fish Collection.

Results

A total of 103 species belonging to 78 genera and 49 families were recorded (Table 1), comprising 16 elasmobranchs and 87 actinopterygians. Haemulidae (n=7 species; 6.7%) and Epinephelidae (n=6; 5.8%) were the richest families. *Haemulon* (n=5 species) was the most speciose genus, followed by *Stegastes*, *Acanthurus* and *Mycteroperca* (n=3 each). With almost 90% of the geographic range classifications, the species classified as Western Atlantic, Trans-Atlantic and Circumtropical were dominant (56%, 17% and 14% respectively) (Figure 2).

Sixty-nine species (67%) had their conservation status evaluated in at least one geographic scale, with 25 included within categories Critically Endangered (CR), Endangered (EN), Vulnerable (VU) and Near-Threathened (NT) (Table 1), eight of these species are represented in Figure 3. Highlighting some species that are highly targeted by fisheries, we had most from the Epinephelidae family, such as *Epinephelus marginatus*, *E. morio*, *Hyporthodus niveatus* and *Mycteroperca bonaci*, and some taxa of Lutjanidae like *Lujanus analis*.

Table 1 – Checklist of the reef fish species recorded at the Anchieta Island State Park. Geographic Range: CT, Circumtropical; SE, Southeastern Brazil; TA, Trans-Atlantic; WA, Western Atlantic; BR, Brazilian province; SCa, Southern Caribbean; Pat, Patagonian; EA, Eastern Atlantic. Main Threats: OF, Overfishing; BC, Bycatch; AT, Aquarium Trade. IUCN: CR, Critically Endangered; EN, Endangered; VU, Vulnerable; NT, Near Threatened; LC, Least Concern. BRAZIL: CR, Critically Endangered; VU, Vulnerable; SÃO PAULO STATE: NT, Near Threatened; NMA, Need Management Action; DD, Deficient Data. Record type: VIS, Visually (underwater observation); CTF, Scientific Fisheries; UND, Unpublished Data (Sazima et al.); REP, Reports (O.B.F. Gadig); PHO, Photographic Record; LIT, Literature (Sazima et al., 2000; Cunningham & Saul, 2004); MUS, Museum Vaucher (ZUEC).

FAMILY and *Species*	Geographic Range	Main Threat	IUCN	Brazil	São Paulo state	Record type
CARCHARHINIDAE						
Carcharhinus brevipinna (Müller & Henle, 1839)	CT	OF, BC	NT	-	NMA	REP
Galeocerdo cuvier (Péron & LeSueur, 1822)	CT	OF, BC	NT	-	DD	UND
SPHYRNIDAE						
Sphyrna lewini (Griffith & Smith, 1834)	CT	OF, BC	EN	CR	NMA	REP
RHINOBATIDAE						
Pseudobatos horkelii Müller & Henle, 1841	SE	BC	CR	CR	NMA	CTF
Pseudobatos percellens (Walbaum, 1792)	TA	BC	NT	-	NMA	CTF
Zapteryx brevirostris (Müller & Henle, 1841)	SE	BC	VU	VU	NMA	CTF
NARCINIDAE						
Narcine brasiliensis (von Olfers, 1831)	CT	BC	DD	-	DD	VIS, PHO
DASYATIDAE						
Dasyatis hypostigma Santos & Carvalho, 2004	SE	BC	DD	-	DD	VIS, CTF
Hypanus americanus (Hildebrand & Schroeder, 1928)	WA	BC	DD	-	DD	REP
Hypanus guttatus (Bloch & Scheider, 1801)	WA	BC	DD	-	DD	REP
GYMNURIDAE						
Gymnura altavela (Linnaeus, 1758)	TA	BC	VU	CR	DD	VIS, CTF
MYLIOBATIDAE						
Aetobatus narinari (Euphrasen, 1790)	CT	OF, BC	NT	-	DD	VIS, UND, REP

Table 1 – Continued...

FAMILY and *Species*	Geographic Range	Main Threat	IUCN	Brazil	São Paulo state	Record type
RHINOPTERIDAE						
Rhinoptera brasiliensis Müller, 1836	SE	OF, BC	EN	-	NMA	REP
Rhinoptera bonasus (Mitchill, 1815)	TA	OF, BC	NT	-	NMA	REP
MOBULIDAE						
Mobula hypostoma (Bancroft, 1831)	CT	OF, BC	DD	VU	NMA	REP
Mobula thurstoni (Lloyd, 1908)	CT	OF, BC	NT	VU	NT	REP
MURAENIDAE						
Gymnothorax funebris Ranzani, 1839	WA	-	LC	-	-	UND
Gymnothorax ocellatus Agassiz, 1831	WA	-	LC	-	-	VIS, CTF
CLUPEIDAE						
Harengula clupeola (Cuvier, 1829)	WA	-	-	-	DD	UND, MUS
SYNODONTIDAE						
Synodus foetens (Linnaeus, 1766)	WA	-	-	-	-	UND, LIT
HOLOCENTRIDAE						
Holocentrus adscensionis (Osbeck, 1765)	TA	-	-	-	-	UND, VIS
Myripristis jacobus Cuvier, 1829	TA	-	-	-	-	UND
APOGONIDAE						
Apogon americanus Castelnau, 1855	BR	-	-	-	-	UND, MUS
Apogon pseudomaculatus Longley, 1932	TA	-	LC	-	-	UND
Astrapogon puncticulatus (Poey, 1867)	WA	-	LC	-	-	UND, MUS
ELEOTRIDAE						
Eleotris pisonis (Gmelin, 1789)	WA	-	LC	-	-	UND, MUS
GOBIIDAE						
Awaous tajasica (Lichtenstein, 1822)	BR	-	-	-	-	UND, MUS
Coryphopterus glaucofraenum Gill, 1863	WA	-	LC	-	-	UND, VIS
Elacatinus figaro Sazima, Moura & Rosa, 1997	BR	AT	-	VU	NMA	LIT, VIS, MUS
AULOSTOMIDAE						
Aulostomus maculatus Valenciennes, 1841	WA	-	-	-	-	VIS
FISTULARIDAE						
Fistularia tabacaria Linnaeus, 1758	TA	-	-	-	-	LIT
DACTYLOPTERIDAE						
Dactylopterus volitans (Linnaeus, 1758)	TA	-	-	-	-	VIS
MULLIDAE						
Pseudupeneus maculatus (Bloch, 1793)	WA	-	-	-	DD	UND, VIS, LIT
CENTROPOMIDAE						
Centropomus parallelus Poey, 1860	WA	OF	LC	-	NT	VIS, MUS
Centropomus undecimalis (Bloch, 1792)	WA	OF	LC	-	NT	VIS
CARANGIDAE						
Carangoides crysos (Mitchill, 1815)	TA	OF	LC	-	-	UND, MUS
Caranx latus Agassiz, 1831	TA	OF	-	-	-	UND, MUS
Pseudocaranx dentex (Bloch & Schneider, 1801)	CT	-	-	-	-	VIS, PHO
Trachinotus goodei Jordan & Evermann, 1896	WA	-	LC	-	-	UND
PARALICHTHYIDAE						
Syacium sp.	-	-	-	-	-	VIS
POMACENTRIDAE						
Abudefduf saxatilis (Linnaeus, 1758)	CT	-	-	-	-	UND, VIS, LIT
Chromis multilineata (Guichenot, 1853)	TA	-	-	-	-	UND, VIS, LIT
Stegastes fuscus (Cuvier, 1830)	BR	-	LC	-	-	UND, VIS, LIT
Stegastes pictus (Castelnau, 1855)	BR+SCa	-	-	-	-	UND, VIS
Stegastes variabilis (Castelnau, 1855)	WA	-	-	-	-	UND, VIS, PHO
HEMIRAMPHIDAE						
Hyporhamphus sp.	-	-	-	-		UND, MUS
MUGILIDAE						
Mugil curema Valenciennes, 1836	CT	OF	LC	-	DD	UND, LIT, MUS
Mugil liza Valenciennes 1836	WA	OF	DD	-	NMA	VIS
BLENIIDAE						
Parablennius marmoreus (Poey, 1876)	WA	-	LC	-	-	VIS, PHO
Parablennius pilicornis (Cuvier, 1829)	TA	-	LC	-	-	UND
Scartella cristata (Linnaeus, 1758)	CT	-	LC	-	-	UND

Table 1 – Continued**...**

FAMILY and *Species*	**Geographic Range**	**Main Threat**	**IUCN**	**Brazil**	**São Paulo state**	**Record type**
LABRISOMIDAE						
Labrisomus nuchipinnis (Quoy & Gaimardi, 1824)	TA	-	LC	-	-	UND
Malacoctenus delalandii (Valenciennes, 1836)	WA	-	LC	-	-	UND
GERREIDAE						
Diapterus rhombeus (Cuvier, 1829)	WA	-	LC	-	-	LIT, MUS
Eucinostomus melanopterus (Bleeker, 1863)	TA	-	LC	-	-	UND, MUS
HAEMULIDAE						
Anisotremus surinamensis (Bloch, 1791)	WA	OF	-	-	-	UND, VIS, LIT
Anisotremus virginicus (Linnaeus, 1758)	WA	-	-	-	-	UND, VIS, PHO, LIT
Haemulon aurolineatum Cuvier, 1830	WA	-	-	-	-	UND, LIT, MUS
Haemulon chrysargyreum Günther, 1859	WA	-	-	-	-	VIS
Haemulon parra (Desmarest, 1823)	WA	-	-	-	-	VIS, PHO
Haemulon plumierii (Lacepède, 1801)	WA	-	-	-	-	UND, LIT
Haemulon steindachneri (Jordan & Gilbert, 1882)	WA	-	LC	-	-	UND, VIS, PHO, LIT
LUTJANIDAE						
Lutjanus analis (Cuvier, 1828)	WA	OF	VU		NMA	VIS, PHO, LIT
Lutjanus synagris (Linnaeus, 1758)	WA	OF	-	-	-	LIT, MUS
Ocyurus chrysurus (Bloch, 1791)	WA	-	-		NMA	UND
POMACANTHIDAE						
Holacanthus tricolor (Bloch, 1795)	WA	AT	LC	-	NT	UND, VIS, LIT
Pomacanthus paru (Bloch, 1787)	WA	AT	LC	-	NT	UND, VIS, LIT
PRIACANTHIDAE						
Heteropriacanthus cruentatus (Lacepède, 1801)	CT	-	-	-	-	VIS, PHO
Priacanthus arenatus Cuvier, 1829	TA	-	-	-	-	VIS
SCIAENIDAE						
Menticirrhus littoralis (Holbrook, 1847)	WA	OF	LC	-	NT	UND
Odontoscion dentex (Cuvier, 1830)	WA	-	LC	-	DD	UND
Pareques acuminatus (Bloch & Schneider, 1801)	WA	-	LC	-	-	UND, VIS
LABRIDAE						
Bodianus rufus (Linnaeus, 1758)	WA	-	LC	-	-	UND, LIT
Cryptotomus roseus Cope, 1871	WA	-	LC	-	-	LIT
Halichoeres poeyi (Steindachner, 1867)	WA	-	LC	-	-	UND, VIS, PHO, LIT
Scarus taeniopterus Lesson, 1829	WA	OF	LC	-	-	LIT
Sparisoma axillare (Steindachner, 1878)	BR	OF	DD	VU	NMA	VIS
LOBOTIDAE						
Lobotes surinamensis (Bloch, 1790)	CT	-	-	-	DD	UND, MUS
EPHIPPIDAE						
Chaetodipterus faber (Broussonet, 1782)	WA	-	-	-	-	VIS, PHO
SPARIDAE						
Diplodus argenteus (Valenciennes, 1830)	WA	-	LC	-	-	UND, LIT
CHAETODONTIDAE						
Chaetodon striatus Linnaeus, 1758	WA	AT	LC	-	-	UND, VIS, LIT
OGCOCEPHALIDAE						
Ogcocephalus vespertilio (Linnaeus, 1758)	WA	-	-	-	-	UND, VIS
DIODONTIDAE						
Chilomycterus spinosus spinosus (Linnaeus, 1758)	WA	-	-	-	DD	VIS
TETRAODONTIDAE						
Canthigaster figueiredoi Moura & Castro, 2002	BR+SCa	AT	LC	-	-	VIS
Canthigaster rostrata (Bloch, 1786)	WA	AT	LC	-	-	UND, LIT
Sphoeroides greeleyi Gilbert, 1900	WA	-	LC	-	DD	UND
Sphoeroides spengleri (Bloch, 1785)	WA	-	LC	-	DD	UND
MONACANTHIDAE						
Aluterus scriptus (Osbeck, 1765)	CT	-	-	-	-	UND
Stephanolepis setifer (Bennett, 1831)	WA	-	-	-	-	UND
ACANTHURIDAE						
Acanthurus bahianus Castelnau, 1855	WA	AT	LC	-	-	UND, VIS, LIT
Acanthurus coeruleus Bloch & Schneider, 1801	WA	AT	LC	-	-	UND, VIS, LIT
Acanthurus chirurgus (Bloch, 1787)	TA	AT	LC	-	-	UND, VIS, LIT

Table 1 – Continued**...**

FAMILY and *Species*	Geographic Range	Main Threat	IUCN	Brazil	São Paulo state	Record type
PEMPHERIDIDAE						
Pempheris schomburgkii Muller & Troschel, 1848	WA	-	-	-	-	UND
KYPHOSIDAE						
Kyphosus sp.	TA	-	-	-	-	UND, MUS
SERRANIDAE						
Serranus baldwini (Evermann & Marsh, 1899)	WA	-	LC	-	-	UND
Serranus flaviventris (Cuvier, 1829)	WA	-	LC	-	-	UND
EPINEPHELIDAE						
Epinephelus marginatus (Lowe, 1834)	SE+Pat+EA	OF	EN	VU	NMA	UND, VIS, LIT
Epinephelus morio (Valenciennes, 1828)	WA	OF	NT	VU	NMA	UND, VIS, LIT
Hyporthodus niveatus (Valenciennes, 1828)	WA	OF	VU	VU	NMA	UND, PHO
Mycteroperca acutirostris (Velenciennes, 1828)	WA	OF	LC	-	-	VIS, PHO, LIT
Mycteroperca bonaci (Poey, 1860)	WA	OF	NT	VU	NMA	UND
Mycteroperca interstitialis (Poey, 1860)	WA	OF	VU	VU	NMA	UND, VIS, PHO
SCORPAENIDAE						
Scorpaena plumieri Bloch, 1789	WA+CA	-	-	-	-	VIS

*Voucher used as reference: *Harengula clupeola* ZUEC-PIS 3019, 3035; *Apogon americanus* ZUEC-PIS 2709; *Astrapogon puncticulatus* ZUEC-PIS 2710, 2711; *Eleotronis pisonis* ZUEC-PIS 2728, 3009, 3127; *Awaous tajasica* ZUEC-PIS 2731, 3008; *Elacatinus figaro* ZUEC-PIS 2703, 2724, 2725, 2726, 2727, 2816, 2817, 3012; *Centropomus parallelus* ZUEC-PIS 3017, 4334; *Carangoides crysos* ZUEC-PIS 2754, 2900, 2901; *Caranx latus* ZUEC-PIS 2902, 2903; *Hyporhamphus* sp. ZUEC-PIS 2911, 2912; *Mugil curema* ZUEC-PIS 2729, 3001, 3002, 3016; *Diapterus rhombeus* ZUEC-PIS 2904; *Eucinostomus melanopterus* ZUEC-PIS 2913, 3000, 3010, 8012; *Haemulon aurolineatum* ZUEC-PIS 2914, 2915, 2916, 2917, 3022; *Lutjanus synagris* ZUEC-PIS 2929, 2930, 2931; *Lobotes surinamensis* ZUEC-PIS 2715; *Kyphosus* sp. ZUEC-PIS 2896;

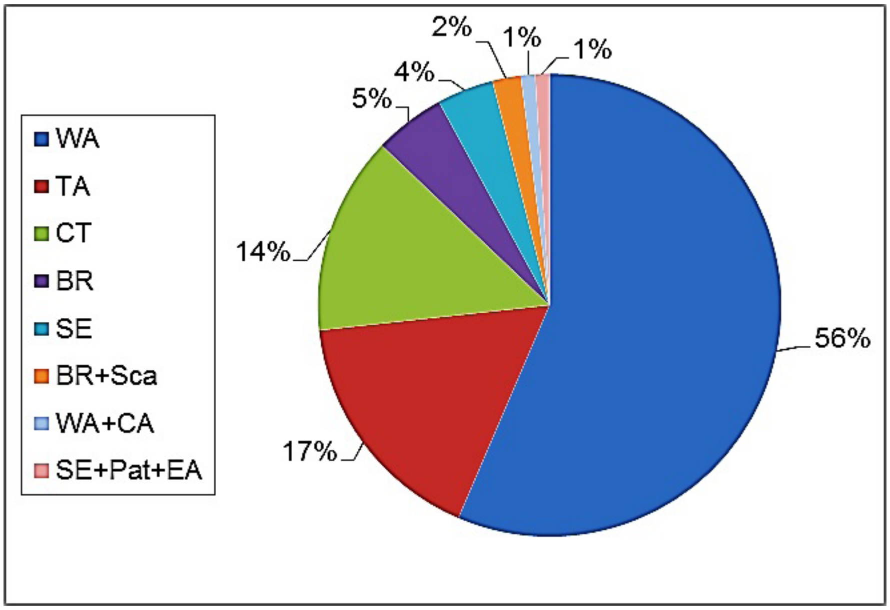

Figure 2. Geographic distribution of the species recorded at Anchieta Island State Park: WA, Western Atlantic (Northern and Southern West Atlantic); TA, Trans-Atlantic (both sides of the Atlantic); CT, Circumtropical; BR, Brazilian Province; SE, Southeastern Brazil (20- 27° S); ; SCa, Southern Caribbean (Venezuela, Trinidad and Tobago and other neighboring islands); CA, Central Atlantic; Pat, Patagonian (primarily in temperate rocky reefs south to Argentina); EA, Eastern Atlantic.

Discussion

We provide a checklist of 103 reef fishes that occur in the AISP, 77 of which appearing for the first time in the literature for this area. Seventeen (22%) species refer to elasmobranchs which, due to specific habits and extremely reduced abundance, are only rarely recorded in faunal surveys, especially using visual census. The fact that several elasmobranchs were recorded by consulting experts, highlight the importance of secondary unpublished data. The use of BRUVS (Baited Remoted Underwater Video Stations) was also particularly relevant, once this tool allows recording species that often remain unseen by divers, such as elasmobranchs. Its use along the Brazilian coast is recent, but preliminary results have shown great potential to record sharks and rays in reef environments (Rolim et al. 2017). For instance, we recorded comparatively more reef fish species than other surveys conducted in nearby areas, i.e. 106 species in Gibran & Moura (2012) and 67 species in Teixeira-Neves et al. (2015).

Biogeographic affinities of the AISP reef fish fauna are similar to those recorded in other similar assessments carried out in Southeastern Brazil (Luiz Jr. et al. 2008, Daros et al. 2012, Pinheiro et al. 2015b), with most species being widely ranging across the Western Atlantic (WA). Anchieta

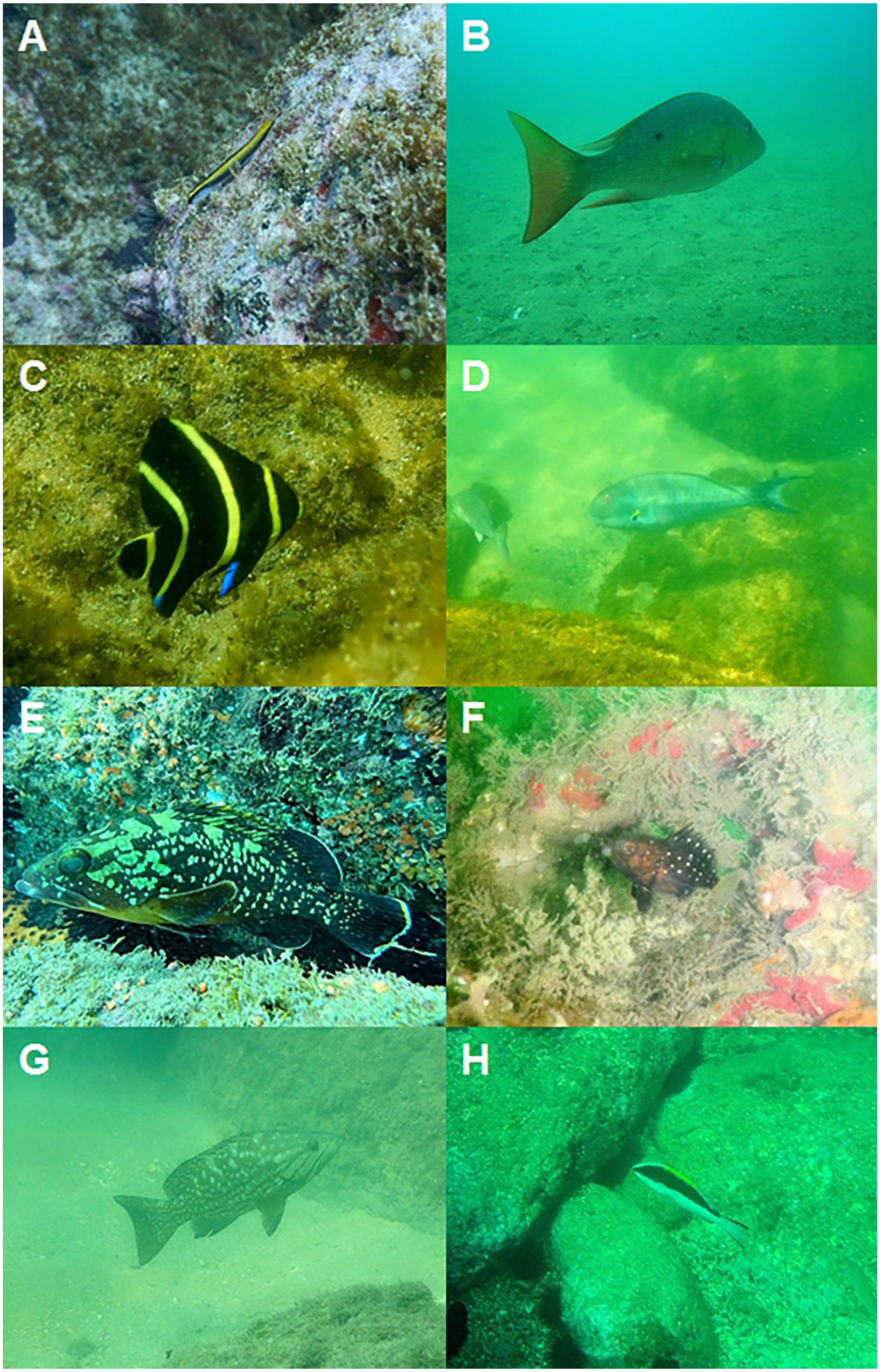

Figure 3. Some species included within threatened categories that were recovered on the AISP. A, *Elacatinus figaro* Sazima, Moura & Rosa, 1997 (<2cm); B, *Lutjanus analis* (Cuvier, 1828); C, *Pomacanthus paru* (Bloch, 1787) (juvenile) (2-10cm); D, *Sparisoma axillare* (Steindachner, 1878) (terminal phase) (>30cm); E, *Epinephelus marginatus* (Lowe, 1834); F, *Hyporthodus niveatus* (Valenciennes, 1828) (juvenile) (2-10cm); G, *Mycteroperca acutirostris* (Valenciennes, 1828); H, *Mycteroperca interstitialis* (Poey, 1860) (juvenile) (2-10cm).

Island is a coastal site largely dominated by the warm and less saline (due to riverine inflow) Coastal Water (CW), as well as by the warm (>18°C) and saline water from the south-flowing Brazil Current (BC) (Matsuura 1986). Luiz Jr. et al. (2008) pointed out the importance of the BC to provide a proper water temperature and larval input to the subtropical rocky reefs, explaining the large amount of tropical reef fishes in this subtropical area. In addition, the region is affected by upwellings of the cold (8-18°C) South Atlantic Central Water (SACW) during the austral summer and spring (Matsuura 1986). Such frequent cold water intrusions, as well as the relatively wide depth range of the AISP, account for the presence of several reef fishes with subtropical and temperate affinities (Floeter et al. 2001, Luiz Jr. et al. 2008, Gibran & Moura 2012, Pinheiro et al. 2015b). Furthermore, the high structural complexity of rocky shores (Ferreira et al. 2001, Dominici-Arosema & Wolf 2006, Gibran & Moura 2012, Teixeira-Neves et al. 2015) and the broad depth range of coastal islands (Pereira-Filho et al. 2011, Gibran & Moura 2012, Teixeira-Neves et al. 2015), as well as the proximity to large estuaries and the protected status of the AISP, are responsible for the relatively high richness of fishes recorded herein.

Overfishing and bycatch are the main stressors operating over 85% of the endangered species recorded at AISP, which mostly include mesopredators (i.e. medium to large size carnivores) such as groupers (*E. marginatus*, *E. morio*, *M. acutirostris* and *M. bonaci*), and large herbivorous like *S. axillare* (Floeter et al. 2006, Francini-Filho & Moura 2008, Teixeira-Neves et al. 2015). These species are targeted by both professional and recreational fisheries, including spearfishing, which is poorly regulated and monitored in Brazil (Nunes et al. 2012; Freire et al. 2016). Most of the species of elasmobranchs recorded (64.7%) are either CR, EN, VU or NT, e.g. *Sphyrna lewini*, *Pseudobatos horkelii* and *Rhinoptera brasiliensis*, and its population status is more critical than that of actinopterygians. With extremely reduced populations, elasmobranchs are curently captured mostly as bycatch (Rosa & Gadig 2014), and therefore deserve specific mitigation measures directed to fisheries targeting other resources (especially longlines, gillnets and trawls). Among actinopterygians, it is remarkable that 83.3% of the recorded epinephelids (groupers) are either CR, EN, VU or NT. Besides being protogynous hermaphrodites, epinephelids are slow-growing and long-living species with late maturity (Andrade et al. 2003, Mitcheson et al. 2008, Reñones et al. 2010), being thus highly vulnerable to fishery.

The protected status of the marine perimeter of AISP is well justified by its relatively high fish richness and concentration of threathened species. However, the population status of these species and the reef fish community structure are still poorly known (e.g. Sazima et al. 2000), impeding a thorough assessment of long-term of population viability and the potential of AISP to outsource unprotected areas in the vicinities (e.g. Floeter et al. 2006, Francini-Filho & Moura 2008). For instance, it is unclear whether the current no-take zoning at the regional level is sufficient to ensure long term biodiversity persistence and fisheries sustainability, as stated in the objectives of the State MPA network under development along the São Paulo coast.

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Authors Contributions

Gabriel Raposo Silva de Souza – Substantial contribution to the idea and design of the work, contribution to data collection, contribution in the analysis and interpretation of data and contribution in the writing of the work.

Otto B. F. Gadig – contribution to data collection, contribution in the analysis and interpretation of data and contribution in the writing of the work.

Fábio dos Santos Motta – Contribution to data collection, contribution in the analysis and interpretation of data, contribution in the writing of the work and contribution in critical review adding intellectual content.

Rodrigo Leão de Moura – Contribution to data collection, contribution in the analysis and interpretation of data, contribution in the writing of the work and contribution in critical review adding intellectual content.

Ronaldo Bastos Francini-Filho - Contribution to data collection, contribution in the analysis and interpretation of data, contribution in the writing of the work and contribution in critical review adding intellectual content..

Domingos Garrone Neto – Substantial contribution to the idea and design of the work, contribution to data collection, contribution in the analysis and interpretation of data and contribution in the writing of the work.

Conflicts of interest

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

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Leptophlebiidae (Insecta: Ephemeroptera) from state of Bahia, Brazil

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Abstract: Leptophlebiidae is the family with the most diversity of species and wide distribution in the Southern Hemisphere. There are 95 species of Leptophlebiidae recorded in Brazil, but for the state of Bahia there are only 21 species. A check list and new records of Leptophlebiidae (Ephemeroptera) are presented to the State of Bahia. A total of 16 genera and 40 species of Leptophlebiidae were identified on this study. The species *Fittkaulus cururuensis, Hylister obliquus, Miroculis* (*atroari*) *duckensis* and *Miroculis* (*Ommaethus*) *froehlichi* are reported for the first time to the State of Bahia, increasing the number of Leptophlebiidae species from 21 to 25. Now, the Bahia is one of the states with the largest number of Leptophlebiidae species records, behind only of the state of Espírito Santo which has 26 species of this family.

Keywords: *Neotropical, Northeastern, Check List.*

Leptophlebiidae (Insecta: Ephemeroptera) do Estado da Bahia, Brasil

Resumo: Leptophlebiidae é a família com maior diversidade de espécies e com maior distribuição no Hemisfério Sul. Existem 95 espécies de Leptophlebiidae registradas no Brasil, mas no estado da Bahia existem apenas 21 espécies. Uma lista e os novos registros de Leptophlebiidae (Ephemeroptera) são apresentados ao Estado da Bahia. Um total de 16 gêneros e 40 espécies de Leptophlebiidae foram identificadas neste trabalho. As espécies *Fittkaulus cururuensis*, *Hylister obliquus, Miroculis (atroari) duckensis* e *Miroculis (Ommaethus) froehlichi* são relatadas pela primeira vez no Estado da Bahia, aumentando o número de espécies de Leptophlebiidae de 21 para 25. Agora, a Bahia é um dos estados com o maior número de registros de espécies de Leptophlebiidae, atrás apenas do estado do Espírito Santo com 26 espécies desta família.

Palavras-chave: *Neotropical, Nordeste, Check List.*

Introduction

The family Leptophlebiidae composes the most diverse group of Ephemeroptera on Neotropical region, with wide distribution and greater diversity in the Southern Hemisphere (Domínguez et al. 2006). In South America, the family is represented by 43 genera and 168 species, all belonging to the subfamily Atalophlebiinae and Terpidinae (Domínguez et al. 2013, Salles et al. 2017). Among the genera described for South America, 26 of them are recorded in Brazil, being *Miroculis* Edmunds and *Thraulodes* Ulmer, the most representative genera in the country in number of described species (Lopes et al. 2007, Salles et al. 2017).

There are 95 species of Leptophlebiidae recorded in Brazil, but in state of Bahia there is only 21 species (Campos et al. 2016, Campos et al. 2017, Salles et al. 2017): *Askola emmerichi* Domínguez, Molineri & Mariano, 2009; *Farrodes carioca* Domínguez, Molineri & Peters, 1996; *F. tepui* Domínguez Molineri & Peters, 1996; *Fittkaulus maculatus* Savage & Peters, 1978; *Hagenulopsis minuta* Spieth, 1943; *Hermanella angeli* Almeida, Costa & Mariano, 2016; *Hermanella mazama* (Nascimento, Mariano & Salles, 2012); *Hydrosmilodon gilliesae* Thomas & Péru, 2004; *Hylister plaumanni* Domínguez & Flowers, 1989; *Massartella brieni* (Lestage, 1924); *Miroculis* (*Miroculis*) *fittkaui* Savage & Peters, 1983*; Needhamella ehrhardti* (Ulmer, 1920); *Paramaka convexa* (Spieth, 1943); *Perissophlebiodes flinti* (Savage, 1982); *Simothraulopsis demerara* (Traver, 1947); *S. diamantinensis* Mariano, 2010; *S. janae* Mariano, 2010; *Thraulodes luizgonzagai* Lima et al., 2013; *Ulmeritoides angelus* Souto et al., 2016; *Ulmeritoides flavopedes* (Spieth, 1943) and *Ulmeritus saopaulensis* (Traver, 1946).

Due to the importance of the family in relation to ecology, conservation of aquatic environments and considering the limited knowledge of the taxonomy of the group in Bahia, this study aims to present an inventory of the fauna the family Leptophlebiidae (Ephemeroptera: Insecta) in state of Bahia.

Studies involving the diversity of aquatic insects are important for biodiversity. The Ephemeroptera distribution and diversity knowledge in Brazil are still incipient as well discussed by some authors (Mariano and Polegatto 2011, Lima et al. 2010, Salles et al. 2014, Campos et al. 2016). This occurs not just in mayflies but also in others groups of aquatic insects such as Trichoptera (Calor 2011) and Plecoptera (Froehlich

2011). Most of this gap probably is due the few projects developed in the Northeast region, as well as the lack of specific groups of researchers in this region. This work shows that there is a lot to be done since of all the previously registered Leptophlebiidae species for the State of Bahia, only four of them were described with material collected from the state (*H. angeli*, *T. luizgonzagai*, *S. diamantinensis* and *S. janae*).

Material and Methods

The material examined was obtained between 2008 and 2016 in 23 municipalities from state of Bahia, Brazil. All material is deposited in the collection of the Laboratório de Organismos Aquáticos from Universidade Estadual de Santa Cruz, Ilhéus, State of Bahia, Brazil (LOA/UESC). The nymphs were collected using D-shaped nets and the adults were collected with light trap and light pan trap (Calor & Mariano 2012), then all material was preserved in ethyl alcohol 80%. Male legs, genitalia and nymphal parts were dissected and mounted in Euparal; wings were mounted dry. The identification of the species were based according to Domínguez et al. 2006 and other specific papers.

The samples were made in lentic and lotic environments, in different substrates (litter deposited in the bottom of the bed, litter retained in areas of stream, sand, stone and roots). When possible, some nymphs were reared in the field with the aim to obtain the subimago stage and later the imago for possible associations between it. The collections had SISBIO Authorization number 24195-1.

1. Study area

The State of Bahia (Figure 1) is located in the northeastern region of Brazil, covering an area of 564,692,669 km^2, thus the largest State in the territorial extension of the Northeast region and approximately 7% of the national territory. The state presents three biomes: Atlantic Forest and Savanna (Cerrado and Caatinga), as well as the Coastal Zone, with its ecosystems that are repeated all along the coast. The vegetation is distinguished by a great diversity of ecosystems, rupestrian fields, mangrove, transition areas, as well as seasonal forests and ecosystems such as: beaches and mangroves. The study was carried out in the State of Bahia in the municipalities listed in Table 1. There were 43 collection points between varied water bodies, i.e. streams, rivers and waterfalls (Figure 2). (SEI 2017).

Results and Discussion

After the compilation of species records for the State (Da-Silva 1992; Da-Silva, Salles & Polegatto 2008; Lima et al. 2012; Mariano & Costa 2014; Almeida et al. 2016; Campos et al. 2016; Lima et al. 2016; Campos et al. 2017, Salles et al. 2017) and the material deposited at MZUESC, a table with all the occurrence and new records of mayfly species from state Bahia is provided below (Table 2). For each species we list the data of geographical distribution and the reference.

A total of 5.746 specimens from the 43 sites were collected, distributed in 16 genera and 40 species of Leptophlebiidae were founded on this work (Table 2.). From these 40 morphotypes, 25 were identified in species and 10 identified in morphotypes because we are not able to determine the species, mostly because of the undescribed stage of some Leptophlebiidae species. Besides there are four new species in process of description. The new records are: *Fittkaulus cururuensis*, *Hylister obliquus, Miroculis* (*Atroari*) *duckensis*, and *Miroculis* (*Ommaethus*) *froehlichi*. Four new species are in description process, i.e. *Miroculis* (*Miroculis*) sp. nov., *Needhamella* sp. nov., *Paramaka* sp. nov. and *Ulmeritoides* sp. nov.. Now, Bahia has 25 species of Leptohlebiidae and is one of the states with the largest number of Leptophlebiidae species records, together with the state of Espírito Santos which has 26 species of Leptophlebiidae (Salles et al. 2017).

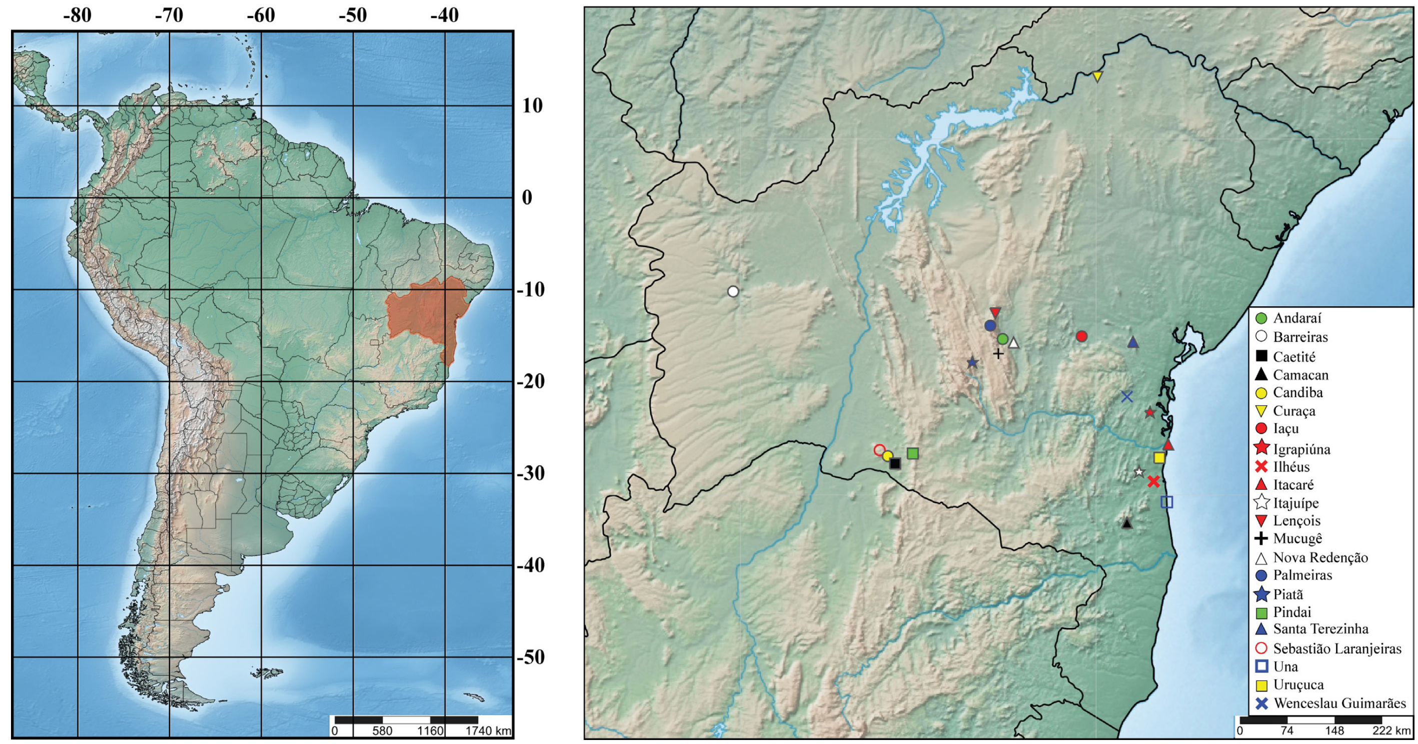

Figure 1. Sampling sites in the State of Bahia region where Leptophlebiidae (Insecta: Ephemeroptera) species were collected.

Table 1. Sample sites (P) at state of Bahia, Brazil, followed by county, location, date, coordinates and collectors. (PESC= Parque Estadual da Serra do Conduru; PNCD= Parque Nacional da Chapada Diamantina; RPPN= Reserva Particular do Patrimônio Natural; REM= Reserva Ecológica da Michelin; LEAq: Laboratório de Entomologia Aquática; LOA: Laboratório de Organismos Aquáticos).

COUNTY	SITES	LOCATION	DATES	COORDENATES	COLLECTORS
Abaíra	**P1**	Catolés, Cachoeira do Guarda Mó	x.2013	13º49’29.2”S, 39º10’27.2”W, 1266m	Calor, Dias & Campos
	P2	Catolés, Rio Catolés de cima, Entrada para Inúbia (Abaixo da Ponte)	x.2013	-	Calor, Dias & Campos
	P3	Catolés, Rio da Grota	x.2013	13º17’10”S, 41º52’8.3”W, 1119m	Calor, Dias & Campos
Andaraí	**P4**	Rio Água Boa, Igatu district	ii.2011; iii.2011	11°53’S,45°25’W, 573m	Calor, Camalier & Zanata; Bravo, Menezes, Silva & Neto
	P5	Rio Coisa Boa, Igatu district	iii.2011	-	-
Barreiras	**P6**	Rio das Ondas	x.2008	12°08’334”S, 45°06’140”W	Calor, Mariano & Mateus
	P7	Rio de Janeiro, Cachoeira Acaba Vidas	x.2008	11°53’673”S, 45°36’096”W	Calor, Mariano & Mateus
Caetité	**P8**	Rio Cachoeira Alta	xi.2012; ii.2013; v.2013; viii.2013	14º33’41,5”S,42º49’35,3”W, 823m	Nogueira, Santos & Silva
	P9	Rio Moita dos Porcos	viii.2012; xi.2012; ii.2013; v.2013	14º10’09,1”S,42º31’16,3”W, 800m	Nogueira, Santos & Silva
Camacan	**P10**	Córrego Ponte	viii.2008	352m	Calor, Lecci, Pinho & Moretto
	P11	Fazenda Altamira	x.2008	15°25’16”S, 39°33’57”W, 300m	Calor, Mariano & Mateus
	P12	Fazenda Paris, Rio Braço do Sul	iv.2011	15°25’17”S, 39°34’0,2”W	França & Barreto
	P13	Fazenda Waldemar da Farmácia	iii.2011; viii.2008	15°25’17”S, 39°34’011”W, 310 m	Calor, Quinteiro, Lecci & Barreto; Calor, Lecci, Pinho & Moretto
	P14	RPPN Serra Bonita, Riacho 1 MCAM 3	iv.2011	15°23’31”S, 39°33’53”W	Quinteiro, França & Barreto
	P15	RPPN Serra Bonita, Segundo Riacho Trilha da Cachoeira	iii.2011	15°23’31”S, 39°33’53”W	França
Candiba	**P16**	Rio Brejo da Taboa	viii.2012; xi.2012; v.2013	42°55’37”S, 14°27’22”W, 1054m	Nogueira, Santos & Silva
Curaçá	**P17**	Recanto Campestre, Rio São Francisco	v.2011; v.2011	-	França
Iaçu	**P18**	Rio Paraguaçu	v.2010	-	-
Igrapiúna	**P19**	REM	ii.2010; ix.2012	-	Equipe LEAq
	P20	REM, Córrego das Matas, trilha do Guigó	ix.2012	13º49’24.6”S, 39º10’9.0”W	Equipe LEAq
	P21	REM, Pacangê – 2ª ponte	ix.2012	13º50’17.1” S, 39º14’27.7” W	Equipe LEAq
Ilhéus	**P22**	Ilhéus, UESC, Rio Cabruca	ix.2014	14°47’42.4”S, 39°10’19.2”W	Equipe LOA
Itacaré	**P23**	Cachoeira Bom Sossego	vii.2012; ix.2012	14°20’05.2”S, 39°01’27.4”W	Mariano, Almeida & Costa
	P24	Fazenda Me Adora, Afluente Rio de Contas	viii.2012	-	Mariano, Almeida & Costa
	P25	Riacho da Prainha	viii.2012	14°17’27.6”S 38°59’09.2”W	Mariano, Almeida & Costa
	P26	Taboquinhas, Cachoeira (entrada a direita antes da cidade)	x.2008	14°21’21.2”S, 39°09’56.4”W	Mariano, Silva & Craveiro
Itajuípe	**P27**	Rio Almada	xii.2011; iii.2012; vii 2012; ix.2012	14º40’26,9”S, 39º23’36,8”W	Souza; Souza & Silva
Lençóis	**P28**	PNCD, Córrego Ribeirão	x.2008	04°85’021”S, 83°98’176”W	Calor, Mariano & Mateus
	P29	PNCD, Rio Capivara	x.2008	-	Calor, Mariano & Mateus
	P30	PNCD, Rio Mucugezinho	viii.2010; x.2008	12°27’638”S, 41°25’205”W	Calor, Lecci, Brantes, Quinteiro, França & Camalier; Calor, Mariano & Mateus
Mucugê	**P31**	Rio Cumbuca	vii.2010	-	Calor, Lecci, Camelier, Arantes, Quinteiros & França
Nova Redenção	**P32**	Rio Paraguaçú	vii.2010	-	Equipe LEAq.
Palmeiras	**P33**	Riacho Cachoeira Batista	vii.2011	12°37’21,7”S, 41°29’11,7”W	Calor, Camalier & Burguer
Piatã	**P34**	Rio Machado, Cachoeira Caxibarí	vii.2010	12°08’334”S, 45°06’140”W, 492m	Equipe LEAq.
Pindaí	**P35**	Rio Umburanas	viii.2012; xi.2012; ii.2013; v.2013	14º25’08,5”S, 42º34’38,1”W 877m	Nogueira, Santos & Silva
	P36	Rio Pires		42°49’27”S, 14°33’43”W, 856m	Nogueira, Santos & Silva
Santa Terezinha	**P37**	Reserva Gambá, Pedra Branca, Serra da Jibóia, Cachoeira, Lajedo	xi.2010	12°51’00”S, 39°28’48”W, 678m	Calor, Mariano & Quinteiros
Sebastião Laranjeiras	**P38**	Riacho da Mandiroba	viii.2012; xi.2012; ii.2013; v.2013	14°22’09”S, 43°02’34”W, 950m	Nogueira, Santos & Silva
	P39	Rio da Mata	viii.2012; xi.2012; ii.2013; v.2013	14°15”50”S, 43°09’59”W, 1150m	Nogueira, Santos & Silva
Una	**P40**	RPPN Fazenda Ararauna	2014	15°18’12.8”S, 39°09’41.8”W	Pereira, T.P.L.
Uruçuca	**P41**	PESC, Córrego da Samambaia, Lago da Sede, Córrego dos Pinga	iv.2011; vi.2011; iii.2011; x.2010; vi.2011	14°29’36.5”S, 39°08’09.3”W 14°29’32.2”S, 39°08’09.9”W	Costa & Mariano; Mariano, Costa & Soledade; Mariano, Costa & Pereira; Mariano, Costa, Pereira & Silva; Mariano, Costa & Soledade
	P42	PESC	-	**-**	Equipe LOA
Wenceslau Guimarães	**P43**	Cachoeira Serra Grande, Sede	x.2010; x.2010	-	-

Table 2. List of species of Leptophlebiidae from in state of Bahia, Northeast region, Brazil, followed by sites, previously records and references. * *Askola* sp. 1 was recorded in three differents localities, Santa Terezinha (Costa & Mariano 2014), Igrapiúna (Campos et al. 2016) and Catolés (Campos et al. 2017).

Genus	Species	Sites	Previously records	References (Records for State of Bahia)
***Askola* Peters, 1969**	*A. emmerichi* Domínguez, Molineri & Mariano, 2009	-	AM, RR, BA (Barreiras)	Lima et al. 2016
	Askola sp1.*	P41	BA (Santa Teresinha; Igrapiúna; Catolés)	Mariano & Costa 2014; Campos et al. 2016; Campos et al. 2017
	Askola sp2.	-	BA (Igrapiúna; Santa Terezinha)	Campos et al. 2017
***Farrodes* Peters, 1971**	*F. carioca* Domínguez, Molineri & Peters, 1996	P4, P5, P6, P7, P10, P12, P13, P14,P19, P28, P30, P31, P32, P34, P43	ES, RJ, BA (Barreiras; Igrapiúna; Catolés; Lençois; Santa Terezinha)	Lima et al. 2016; Campos et al. 2016; Campos et al. 2017
	F. tepui Domínguez, Molineri & Peters, 1996	-	PE, BA (Barreiras)	Lima et al. 2016
	Farrodes sp1.	P9, P16, P27, P35, P38, P39, P42	-	-
***Fittkaulus* Savage & Peters, 1978**	*Fi. cururuensis* Savage, 1986	P42	ES, MT, PA, PE, **NEW RECORD FOR BAHIA**	-
	Fi. maculatus Savage & Peters, 1978	-	PA, BA (Nova Viçosa)	Da-Silva 1992
***Hagenulopsis* Ulmer, 1920**	*H. minuta* Spieth, 1943	-	AM, PA, RR, BA (Barreiras)	Lima et al. 2016
	Hagenulopsis sp1.	P27	-	-
***Hermanella* Needham & Murphy, 1924**	*Hermanella angeli* Almeida, Costa & Mariano, 2016	-	BA (Itacaré; Igrapiúna)	Almeida et al. 2016; Campos et al. 2016
	He. mazama (Nascimento, Mariano & Salles, 2012)	-	ES, BA (Igrapiúna)	Campos et al. 2016
	Hermanella sp1.	P38	-	-
***Hydrosmilodon* Flowers & Domínguez, 1992**	*Hy. gilliesae* Thomas & Péru, 2004	P19, P27	AM, ES, MT, PE, BA (Ituberá, Igrapiúna; Lençois; Barreiras)	Lima et al. 2012; Campos et al. 2016; Salles et al. 2016
***Hylister* Domínguez & Flowers, 1989**	*Hyl. obliquus* Nascimento & Salles, 2013	P40	ES, **NEW RECORD FOR BAHIA**	-
	Hyl. plaumanni Domínguez & Flowers, 1989	-	ES, MG, PR, RJ, SC, BA (Ituberá, Igrapiúna)	Lima et al. 2012; Campos et al. 2016
***Massartella* Lestage 1930**	*M. brieni* (Lestage, 1924)	P8, P15	ES, MG, PR, RJ, RS, SP, ba (Santa Teresinha; Catolés; Elísio Medrado)	Mariano & Costa 2014; Campos et al. 2017
***Miroculis* Edmunds, 1963**	*Mi.* (*atroari*) *duckensis* Savage & Peters, 1983	P41	AM, **NEW RECORD FOR BAHIA**	-
	Mi.(*Miroculis*) *fittkaui* Savage & Peters, 1983	P22	ES, PA, PE BA (Igrapiúna)	Campos et al. 2016
	Mi. (*Ommaethus*) *froehlichi* Savage & Peters, 1983	P43	RJ, SP, **NEW RECORD FOR BAHIA**	-
	Miroculis sp.	P40, P42	-	-
	Miroculis (*Miroculis*) sp. nov.	P9, P35, P38, P39	-	-
***Needhamella* Domínguez & Flowers, 1989**	*N. ehrhardti* (Ulmer, 1920)	-	AM, ES, GO, PR, PE, RJ, RS, SC, BA (Amargosa; Lençois)	Lima et al. 2012
	Needhamella sp. nov.	P26	-	-
***Paramaka* Savage & Domínguez, 1992**	*P. convexa* (Spieth, 1943)	P7	MT, PA, BA (Barreiras)	Mariano 2011
	Paramaka sp.	P27	BA (Barreiras)	Lima et al. 2016
	Paramaka sp. nov.	P42	-	-
***Perissophlebiodes* Savage, 1983**	*Pe. flinti* (Savage, 1982)	-	ES, MG, RJ, SP, BA (Lençois)	Da-Silva, Salles & Polegatto 2008
***Simothraulopsis* Demoulin, 1966**	*S. demerara* (Traver, 1947)	P19, P20, P43	AM, AP, ES, MT, PA, PE, PI, RO, RR, BA (Barreiras; Côcos; Igrapiúna)	Lima et al. 2016; Campos et al. 2016; Nascimento et al. 2017
	S. diamantinensis Mariano, 2010	P5, P12, P13, P18, P30, P31, P33, P42	ES, BA (Abaíra; Andaraí; Lençois; Palmeira; Catolés)	Mariano 2010; Campos et al. 2017; Nascimento et al. 2017
	S. janae Mariano, 2010	P7	MT, MG, PA, PE, RO, RR, BA (Barreiras; Rio de Contas)	Mariano 2010; Nascimento et al. 2017

Table 2. Continued...

Genus	Species	Sites	Previously records	References (Records for State of Bahia)
Thraulodes **Ulmer, 1920**	*T. luizgonzagai* Lima, Mariano & Pinheiro 2013	-	PE, BA (Juazeiro)	Lima, Mariano & Pinheiro 2013; Campos et al. 2017
	Thraulodes sp1.	-	BA (Curaça)	Mariano & Costa 2014; Campos et al. 2017
	Thraulodes sp2.	-	BA (Catolés)	Campos et al. 2017
	Thraulodes sp3.	-	BA (Rio de Contas)	Campos et al. 2017
Ulmeritoides **Traver, 1959**	*U. angelus* Souto, Da-Silva, Nessimian & Gonçalves	-	MG, BA (Catolés; Piatã)	Campos et al. 2017
	*U. flavopedes (*Spieth, 1943)	P8, P9, P35, P38, P39	MT, PE, RR, BA (Barreiras)	Lima et al. 2016
	Ulmeritoides sp. nov.	P1, P2, P3	-	-
Ulmeritus **Traver, 1956**	*Ul. saopaulensis* (Traver, 1946)	-	MG, SP, BA (Maracás)	Campos et al. 2017

Figure 2. A - Parque Estadual da Serra do Conduru (P42); B - Cachoeira Acaba Vidas (P7); C - Riacho da Mandiroba (P38); D - Rio Mucugezinho (P30).

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Author Contributions

Sabrina Santos Costa: the author contributed in field collection, in the identifications and preparation of the manuscript.

Maria Adonay Melo Nogueira: the author contributed in field collection, in the identifications and preparation of the manuscript.

Edineusa Pereira dos Santos: the author contributed in field collection, in the identifications and preparation of the manuscript.

Everlin Almeida: the author contributed in field collection, in the identifications and preparation of the manuscript.

Francine Novais Souza: the author contributed in field collection, in the identifications and preparation of the manuscript.

Michelle Maria Lima de Sousa: the author contributed in field collection, in the identifications and preparation of the manuscript.

Vinicius de Assis Silva: the author contributed in field collection, in the identifications and preparation of the manuscript.

Rodolfo Mariano: the author contributed in field collection, in the identifications and preparation of the manuscript.

Conflicts of interest

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

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Mammals of a restinga forest in Mataraca, Paraíba, northeastern Brazil, and its affinities to restinga areas in Brazil

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Abstract: The Restinga forests are plant formations associated to the Atlantic Forest which still have not been much studied in terms of their mastofauna. The objective of the present work was to list for the first time the mammal species found in a Restinga on northeastern Brazil and show the similarities on species composition to other areas of the same environment and of Atlantic Forest. Our records were based on several complementary approaches: field survey, interviews, and museum collections. Subsequently, we performed similarity analysis between Restinga areas along Brazil and Atlantic Forest areas. We found a total of 30 species of mammals distributed in 28 genera, 16 families and 7 orders for Restinga of Mataraca. Species richness was similar to other Restinga areas and similarity analyzes pointed out that Restingas are more closely related to adjacent Atlantic Forest areas than to other Restingas in the country. This suggests that Restingas do not exhibit an autochthone fauna, but rather a faunal subsample of neighbor Atlantic Forest. Therefore, conservations policies for the Restinga habitat depends not only on actions focused on this habitat, but on initiatives that encompass nearby Atlantic Forest remnants, allowing the connectivity between these habitats.

Keywords: *Atlantic Forest, Mammals, Restinga, Mataraca, Paraíba.*

Mamíferos de uma floresta de Restinga em Mataraca, Paraíba, Nordeste do Brasil e suas afinidades com áreas de Restinga no Brasil

Resumo: As restingas são formações vegetais associadas à Floresta Atlântica e ainda pouco exploradas em relação a sua mastofauna. O objetivo desse trabalho foi listar pela primeira vez as espécies de mamíferos presentes em uma restinga no nordeste do Brasil e mostrar sua similaridade com outras áreas do mesmo ambiente e de Mata Atlântica. A listagem foi baseada em diversas abordagens complementares: coletas, entrevistas, material em coleção. Posteriormente realizamos análises de similaridade entre áreas de restinga ao longo do Brasil e áreas de Mata Atlântica. Encontramos um total de 30 espécies de mamíferos distribuídas em 28 gêneros, 16 famílias e sete ordens para restinga de Mataraca. A riqueza ficou próxima a de outras áreas de restinga e as análises de similaridade apontaram que as restingas são mais proximamente relacionadas com as áreas adjacentes de Floresta Atlântica do que com as outras restingas no país. Isso sugere que as Restingas não exibem uma fauna autóctone ou endêmica, mas que sua fauna representa um subconjunto da fauna da Floresta Atlântica mais próxima. Dessa forma, estratégias para a conservação das Restingas não dependem apenas de ações nesse ambiente, mas de iniciativas que atinjam áreas de Mata Atlântica próximas, que garantam a conectividade entre estas.

Palavras-chave: *Floresta Atlântica, Mamíferos*, *Restinga, Mataraca, Paraíba.*

Introduction

The Restinga forests of northeastern Brazil grow on the coastal sandy lowland areas that were formed from sand deposits in regions that were below sea level during the Quaternary period (Thomas & Barbosa 2008). The Restinga forests form a vegetation complex typical of the coastal zone, very common along beaches, sandy cords and coastal surfaces, where the soil is sandy and poor in nutrients (Thomas & Barbosa 2008). This vegetation, contiguous to the Atlantic Forest, constitutes a mosaic of phytophysiognomies, ranging from open grassland savannas to forests (Pereira 2001, Scarano 2002). This diversity of environments favors the presence of plant species with different ecological requirements, which could suggest the presence of a great biological diversity in these areas (Moreira & Mendes 2010).

Restingas are under great anthropic pressure, mainly from habitat loss and fragmentation, due to real estate activities (Zamith & Scarano 2006, Rocha et al. 2007) and industrial activities, leading to decline of populations of mammals (Fonseca et al. 1996). The composition of the mammal fauna in this coastal habitat is poorly known and the few studies are restricted to the southern (e.g. Fabián et al. 2010, Quintela et al. 2012) and southeastern Brazil (e.g. Cerqueira et al. 1990, Cerqueira 2000, Luz et al. 2009, 2011, Oprea et al. 2009, Nogueira et al. 2010, Pêssoa et al. 2010). The mammal fauna of Restinga has been considered as a subset of the fauna of the Atlantic Forest with no particular identity (Cerqueira et al. 1990, Cerqueira 2000), although three rodent species are endemic of these areas: *Cerradomys goytaca*, *Ctenomys lami* and *Ctenomys flamarioni* (Pêssoa et al. 2010, Tavares et al 2011, Lopes et al. 2010).

Until now, no consistent sampling effort, either short-term collecting or exhaustive inventory (Voss & Emmons 1996), has documented the diversity of mammals in the Restingas of northeastern Brazil. Therefore, in this contribution we aim to provide the first survey of mammals of a Restinga area on northern Paraíba state on northeastern Brazil, based on short-term collecting and to compare the similarity on the species richness with other Restinga and Atlantic Forest areas.

Material and Methods

1. Studied Area

The present work was carried out in the Mataraca municipality, on the northern portion of the coast of the state of Paraíba (6º29’S, 34º56’W) (Figure 1), at the hydrographic basin of Guaju river, located mainly on coastal dunes (Figure 2). The geological constitution is essentially sedimentary, represented by sandy-clayey sediments of Neogeno-Quaternary age, belonging to the Barreiras Group and sandy sediments of recent age (Mabesone & Castro 1975). The region’s climate is of the Am type, following Köppen’s classification, the wet season occurs from April to August and the average annual precipitation and temperature is 1.745 mm and 25.5°C respectively (Cunha et al. 2003).

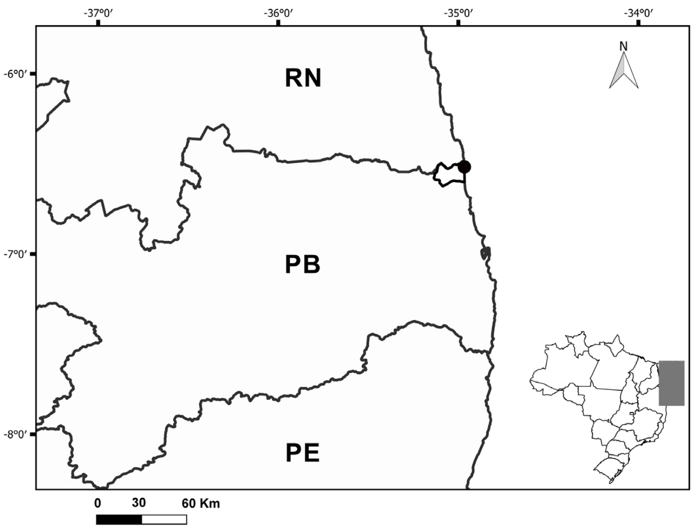

Figure 1. Sampling site on the municipality of Mataraca, Brazil. RN= Rio Grande do Norte state; PB= Paraíba state; PE= Pernambuco state.

The area belonged to Lyondell Chemical Company, which mined the studied area for 33 years. The company carried out a habitat restoration project to compensate the damage of mining activities, and several patches of different ages of restoration exist in the area, as well as fully protected Restinga areas. The field work occurred in these protected areas of Open Restinga and Restinga Forest habitats (Figure 2) to avoid the impact of restoration on our survey. The first habitat is located in the regions closest to the sea and with sandy soils; the vegetation is mainly shrub with shallow litter. The most abundant plant species are: *Anacardium occidentale*, *Tocoyena selloana*, and *Mimosa caesalpiniaefolea.* The Restinga forests are composed by emergent trees of up to 10m in height, and abundance of lianas. The canopy is closed, the understory in moderately open, and the forest floor is covered by dense litter and decaying trunks. The most abundant plant species are: *Sacoglottis mattogrossensis*, *Chamaecrista ensiformis*, *Protium heptaphyllum* (Duré 2013).

2. Data collection

The species list was established through three methodologies: field inventory, interviews and study of specimens at the Mammal Collection of the Universidade Federal da Paraíba (UFPB). We follow the nomenclature proposed by Wilson & Reeder (2005) for orders Carnivora and Primates, Gardner (2008) for Didelphimorphia, Pilosa, Cingulata and Chiroptera, Feijó & Langguth (2013) for the genus *Dasyprocta,* Gurgel-Filho et al. (2015) for the genus *Didelphis* and Patton et al. (2015) for the order Rodentia.

3. Field Inventory

The field inventory was carried out between October 2005 and July 2008 with two campaigns per year (dry and rainy seasons), where small non-volant mammals were sampled through transects with conventional (live traps: Sherman’s and Tomahawk’s) and pitfall traps (see Voss & Emmons, 1996). Conventional traps were placed on and above the ground (up to a height of 2 m) and baited with bananas, peanut and oatmeal mixed. The material collected was deposited at the Mammal collection of the Universidade Federal da Paraíba (UFPB). All specimens were collected and prepared according to protocols established and approved by the “Animal Care and Use Committee” of the American Society of Mammalogists (Sikes et al. 2016).

Medium and large mammals were sampled through daily censuses on previous known transects (10 km) in order to record direct and indirect vestiges. Transects were surveyed by car at low speed and walked twice a day (whenever it is possible, early morning and evening), throughout the campaigns. Transects made by car corresponded to access ways between small mammals surveyed areas. The routes carried out by foot correspond to trails in the vicinities of the trapping points. This methodology was complementary and not standardized, thus we have no available information in effort and success for this approach.

4. Interviews

The interviews were conducted during field inventories with residents and workers of the mining company, that known the area and its fauna in detail. We asked them about medium and large mammals, using colored

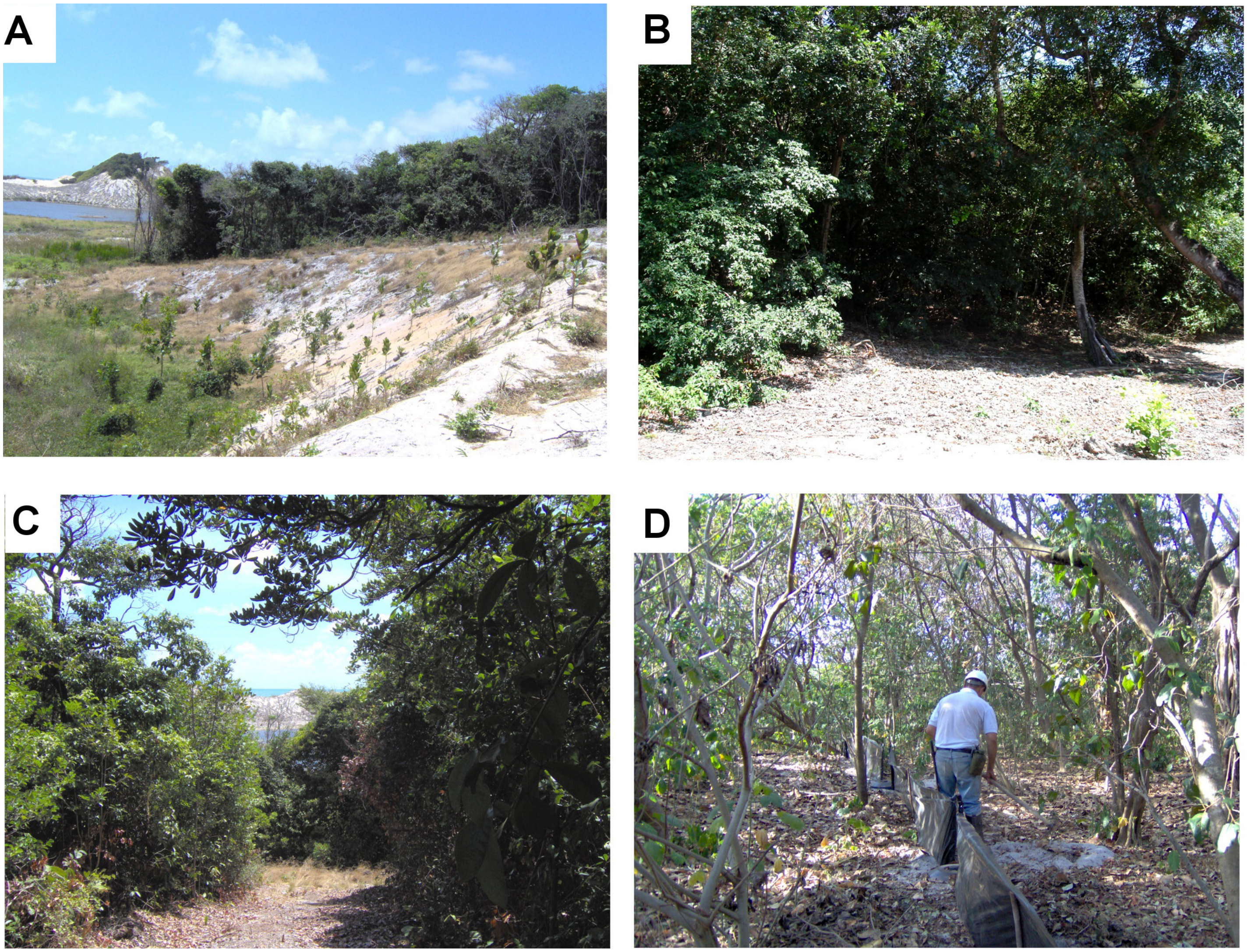

Figure 2. Restinga landscape in the sand dunes in Mataraca, Paraíba. (A) Note the forest on the upper parts of the dunes, the grassy and bush habitats on the slopes and on the lower portions; the lagoon on the background is surrounded by grassland. (B) Detail of the border of a fragment of Restinga Forest. (C) Trail on the Restinga habitat, with sand dunes and the ocean in the background. (D) Detail of open forest Restinga habitat showing one pitfall trap line (Photos: Bruno Campos).

plates as templates from Linares (1998), Emmons & Feer (1990) and Eisenberg & Redford (1999), as suggested by Voss & Emmons (1996).

5. Mammal collection of UFPB

Records for the small-volant mammals from Restinga of Mataraca were obtained at the UFPB collection. Additionally, we also included records of medium mammals, rodents and marsupials.

6. Analyses

We calculate the sampling effort (number of traps * sampling days) and the sampling success ([number of records / sampling effort] * 100) only for the live-trap sampling (conventional and pitfall); these data are also employed to obtain rarefaction curve. The diversity index (Jackknife 1 based on 1,000 runs) was calculated through EstimateS 9.0 (Colwell 2013).

Analyzes of similarities were performed using the PAST program (Hammer et al. 2009) through an array of localities * species and the dendograms were generated using the coefficient of Jaccard. Two levels of analyses were performed, one for all mammals and one for non-volant small mammals. These two approaches were used because the knowledge about Restinga mammals is mainly focused on small non-volant mammals. We included in the analyses only areas of Restinga that had been inventoried with at least two methodological approaches (e.g., pitfall and livetraps, or trapping and museum database), and with a sampling effort greater than 500 traps per night for the small mammals (Table 1; Supplementary Material 1 for data matrix). In order to test similarity among ecosystems, close Atlantic Forest areas of the Restinga were included for comparisons (Table 1). Although the efforts are different, the similarity analysis will provide an initial picture of the similarities between the Restingas and adjacent Atlantic Forest areas and may respond whether the Restinga forest forms an independent unit or represent a subgroup of the Atlantic Forest.

Results

A total of 30 species, distributed in 28 genera, 16 families and seven orders were registered for the Restinga of Mataraca (Table 2). The total sampling effort was 3.758 traps-night for the conventional live

Table 1. Mammal inventories in Restinga and neighboring Atlantic Forest areas in Brazil employed in the analyses of similarity, indicating the mammal groups included in the analyses.

Localities	Vegetation	Group of Mammals	Reference
Barra de Maricá, Maricá, Rio de Janeiro	Restinga	All	Cerqueira et al. 1990, Cerqueira 2010
REBIO Guaribas, Mamanguape, Paraíba	Atlantic Forest with savanna's patches	All	Feijó et al. 2016
Mata do Buraquinho, João Pessoa, Paraíba	Atlantic Forest	All	Percequillo et al. 2007
Parque Nacional Restinga de Jurubatiba, Rio de Janeiro	Restinga	All	Pêssoa et al. 2010
Parque Estadual Paulo César Vinha, Guarapari, Espirito Santo	Restinga	All	Moreira & Mendes 2010
Reserva Biológica Poço das Antas, Rio de Janeiro	Atlantic Forest	All	Pêssoa et al. 2010
Sítio Santana, Quissamã, Rio de Janeiro	Restinga	Small non-volant	Pêssoa et al. 2010
Parque Natural Municipal Fazenda Atalaia, Macaé, Rio de Janeiro	Atlantic Forest	Small non-volant	Pêssoa et al. 2010
Cabiúnas, Macaé, Rio de Janeiro	Atlantic Forest	Small non-volant	Pêssoa et al. 2010
Mata da Estrada Velha, Rio Grande, Rio Grande do Sul	Restinga	Small non-volant	Quintela et al. 2012
Arroio Bolaxa, Rio Grande, Rio Grande do Sul	Restinga	Small non-volant	Quintela et al. 2012

Table 2. Species of mammals recorded in Mataraca Restinga, Paraíba, and the sampling method. LT – live trap; CO– UFPB collection; IN –interview; VE- Vestiges; DO – Direct observation. *According to Gurgel-Filho et al. (2015).

Taxa	Sampling method
ORDER DIDELPHIMORPHIA	
Didelphidae	
Caluromys philander (Linnaeus, 1758)	CO
**Didelphis marsupialis* Linnaeus, 1758	LT/VE/DO
Marmosa murina (Linnaeus, 1758)	LT
Monodelphis domestica (Wagner, 1842)	LT/DO
Gracilinanus agilis (Burmeister, 1854)	LT
Marmosa demerarae (Thomas 1920)	LT/DO
ORDER CINGULATA	
Dasypodidae	
Dasypus novemcinctus Linnaeus, 1758	IN
Chlamyphoridae	
Euphractus sexcinctus (Linnaeus, 1758)	LT/VE/IN/CO/DO
ORDER PILOSA	
Bradypodidae	
Bradypus variegatus (Schinz, 1825)	IN
Myrmecophagidae	
Tamandua tetradactyla (Linnaeus, 1758)	IN
Cyclopes didactylus (Linnaeus, 1758)	IN
ORDER CHIROPTERA	
Emballonuridae	
Rhynchonycteris naso (Wied-Neuwied, 1820)	CO
Phyllostomidae	
Phyllostomus discolor (Wagner, 1843):	CO
Glossophaga soricina (Pallas, 1766)	CO
Carollia perspicillata (Linnaeus, 1758)	CO
Artibeus planirostris (Spix, 1823)	CO
Artibeus cinereus (Gervais, 1856)	CO
Platyrrhinus lineatus (E. Geoffroy 1810)	CO
ORDER PRIMATES	
Cebidae	
Callithrix jacchus (Linnaeus, 1758)	VE/IN/DO
Cebus (Sapajus) flavius (Schreber, 1774)	VE/IN/DO
Atelidae	
Alouatta belzebul (Linnaeus 1766)	VE/IN/DO
ORDER RODENTIA	
Cricetidae	
Oligoryzomys nigripes (Olfers, 1818)	LT
Dasyproctidae	
Dasyprocta iacki Feijó and Langguth, 2013	VE/IN/DO
Erethizontidae	
Coendou prehensilis (Linnaeus, 1758)	VE/IN/DO
Echimyidae	
Thrichomys laurentius (Thomas, 1904)	LT
Phyllomys blainvilii (Jourdan, 1837)	LT
ORDER CARNIVORA	
Felidae	
Leopardus sp.	VE/IN
Canidae	
Cerdocyon thous (Linnaeus, 1766)	VE/IN/DO
Mustelidae	
Lontra longicaudis (Olfers, 1818)	IN
Procyonidae	
Procyon cancrivorus (Cuvier, 1793)	VE/IN

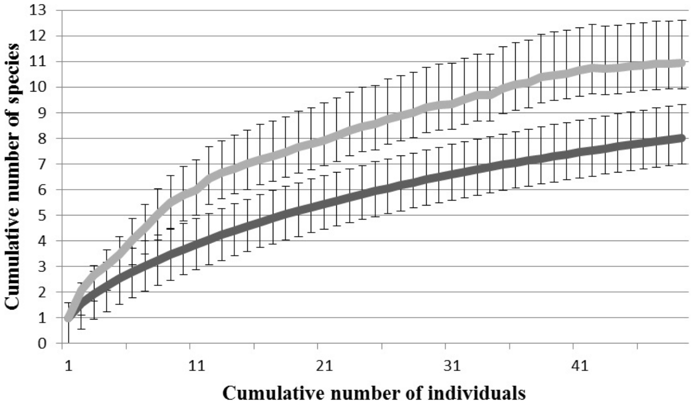

Figure 3. Curve of species accumulation for the non volant small mammals recorded in Restinga in Mataraca, Northeastern Brazil. Gray curve= jacknife 1; Dark curve=observed data. The vertical bars correspond to standard deviation.

trap and 3.090 traps-night for the pitfall. The small mammal sampling during field inventories resulted in the capture of 49 individuals classified in eight species of the orders Rodentia and Didelphimorphia, with 3 and 5 species, respectively. We also obtained 2 individuals of 1 species of the order Cingulata (*Euphractus sexcinctus*) during the surveys; the specimens were captured in pitfall traps.

The censuses resulted in the record of six species, being five through indirect evidence such as tracks, two through scats, and nine by direct observations (Table 2). Four other species were recorded exclusively through interviews: *Dasypus novemcinctus*, *Bradypus variegatus*, *Tamandua tetradactyla* and *Cyclopes didactylus*. The specimens of UFPB represent eight species, seven bats and the marsupial *Caluromys philander* (Table 2).

The capture success for small mammals with the conventional traps was 1.27% and 0.32% with the pitfall traps; considering both methods, we have a capture success rate of 0.71%. The rarefaction curves show a small tendency to the increase of small mammal species (Figure 3). The Jackknife 1 suggests a higher richness of small mammal species in the area than we recorded with significant difference (observed = 8, expected = 11; Figure 3). The most abundant species was the white-eared opossum *Didelphis marsupialis*, followed by *Monodelphis domestica* representing 80% of total captures (Figure 4).

The analyses of similarity for all terrestrial mammal groups (Figure 5) showed that localities of Restinga (Mataraca) and Atlantic Forest (Reserva Biológica Guaribas and Mata do Buraquinho) from

Northeastern Brazil are more similar among themselves, as the areas of Restinga and Atlantic Forest from Southeastern Brazil. Considering only the small non-volant mammals (Figure 6), our results recovered that localities of northeastern Brazil share faunal elements, regardless of the habitat, as areas from other regions (Southern and Southeastern); in this analyses, the Restinga of Rio Grande do Sul is more similar with the areas of northeastern Brazil than those with southeastern.

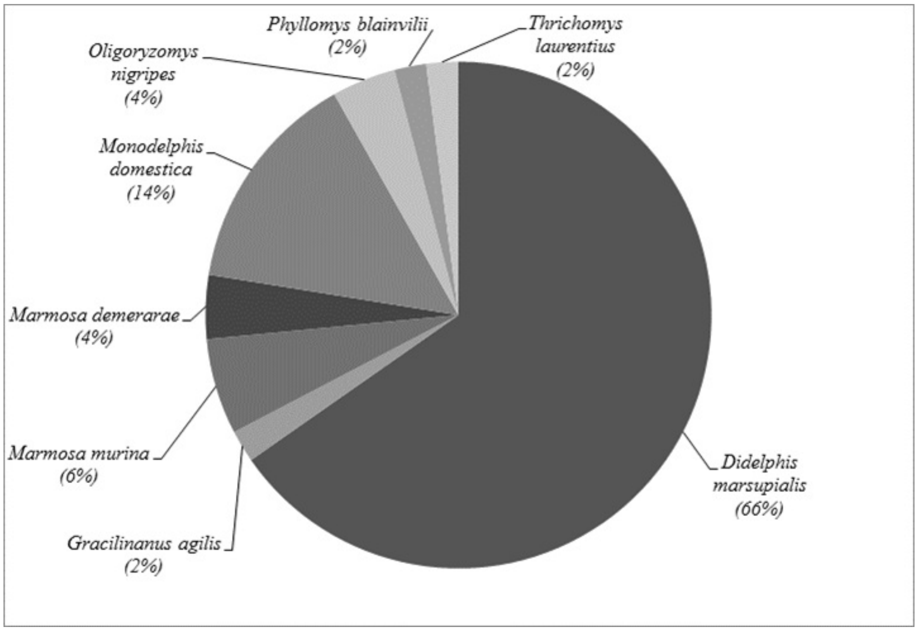

Figure 4. Pie-chart graphic illustrating the abundance of the small mammal species recorded in Mataraca, Paraíba. The absulute values were: *Thrichomys laurentius, Phyllomys blainvilii and Gracilinanus agilis* **(**n=1); *Oligoryzomys nigripes* and *Marmosa dermerarae* (n=2); *Marmosa murina* (n=3); *Monodelphis domestica* (n=7); *Didelphis marsupialis* (n=32).

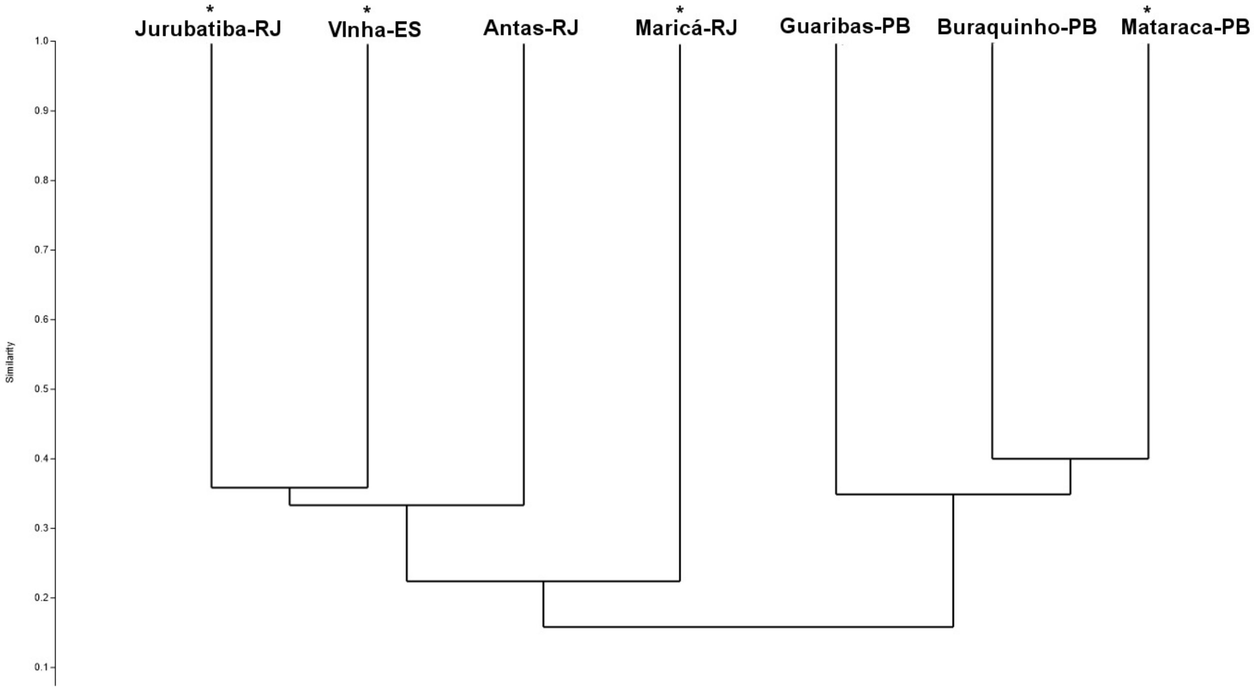

Figure 5. Similarity dendrogram between Atlantic Forest and Restinga localities for small, medium and large species of mammals. The asterisks identify areas of Restinga. The data was compiled according the works listed in Table 1. Acronyms of the areas as follow: Parque Nacional de Jurubatiba, Rio de Janeiro state (Jurubatiba-RJ); Reserva Biológica Poço das Antas, Rio de Janeiro state (Antas-RJ); Parque Estadual Paulo Cesar Vinhas, Espírito Santo state (Vinha-ES); Barra de Maricá, Rio de Janeiro state (Maricá-RJ); Reserva Biológica Guaribas, Paraíba state (Guaribas-PB); Refúgio de Vida Silvestre Mata do Buraquinho, Paraíba state (Buraquinho-PB).

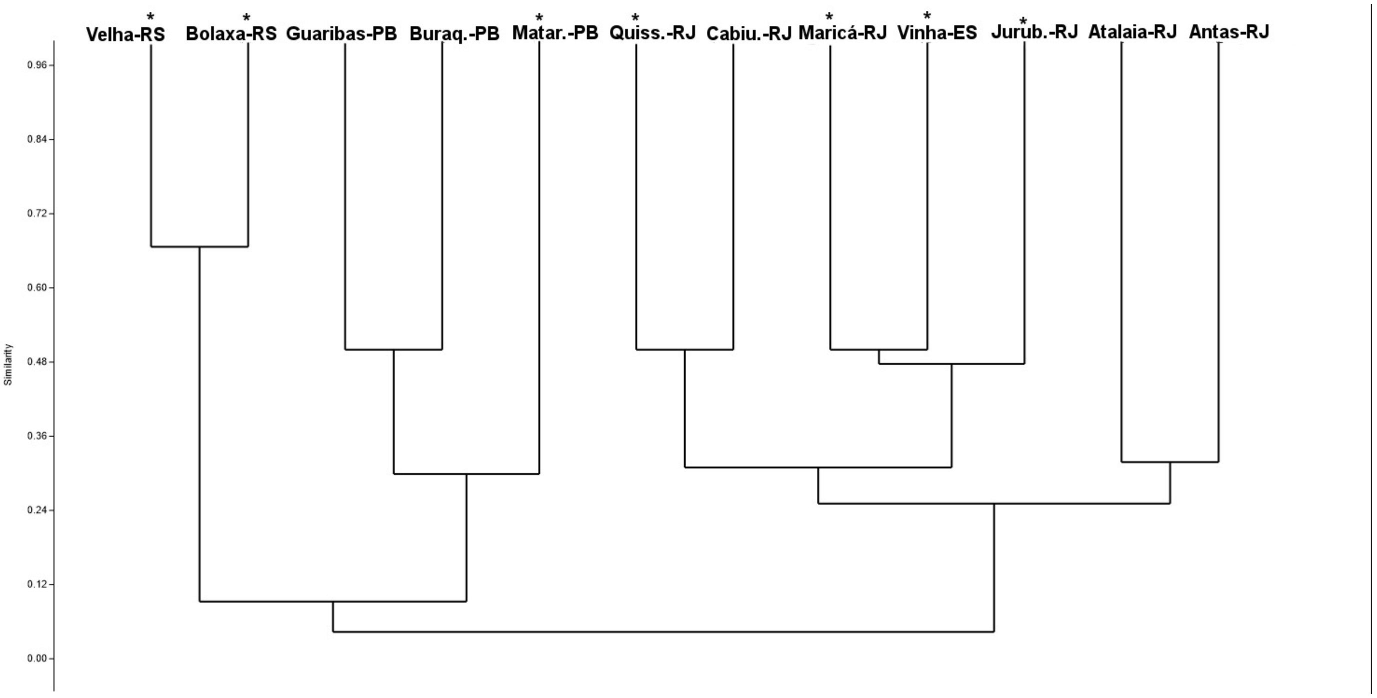

Figure 6. Similarity dendrogram between Atlantic Forest and Restinga localities for small non volant mammals. The asterisks identify areas of Restinga. The data was compiled according the works listed in Table 1. Acronyms of the areas as follows: Parque Nacional de Jurubatiba, Rio de Janeiro state (Jurub.-RJ); Reserva Biológica Poço das Antas, Rio de Janeiro state (Antas-RJ); Quissamã,, Rio de Janeiro state (Quiss.-RJ); Cabiunas,, Rio de Janeiro state (Cabiu.-RJ); Parque Natural Municipal de Atalaia,, Rio de Janeiro state (Atalaia-RJ); Parque Estadual Paulo Cesar Vinhas, Espírito Santo state (Vinhas-ES); Barra de Maricá,, Rio de Janeiro state (Maricá-RJ); Reserva Biológica Guaribas, Paraíba state (Guaribas-PB); Mata do Buraquinho, Paraíba state (Buraq.-PB); Estrada Velha, Rio Grande do Sul state (Velha-RS); Arroio Bolaxa, Rio Grande do Sul state (Bolaxa-RS).

Discussion

We recovered 30 species of mammals in Mataraca during 3 years of standardized sampling effort, interviews and sorting of museum specimens. Six species of marsupials, five xenarthrans, seven bats, three monkeys, five rodents and four carnivores compose the list, and half of them are medium and large mammals (n=15). Apart from the three species of monkeys, which are typical from northeastern region (and eastern Amazon, in the case of *Alouatta belzebul*), all medium and large mammals are widespread throughout South America. *Callithrix jacchus* is also widespread, but it was originally from this region (Hershkovitz 1977).The same is valid for the species of bats sampled and for some species of rodents (*Oligoryzomys nigripes* and *Coendou prehensilis* are widespread in Brazil); the remaining species of rodents, *Thrichomys laurentius*, *Phyllomys blainvilii* and *Dasyprocta iacki*, are more restricted to northeastern region (Pêssoa et al. 2015, Campos & Percequillo 2007, Feijó & Langguth 2013, Leite & Loss 2015).

The number of species surveyed on Mataraca is similar to the one observed in other areas of the northeastern portion of the Atlantic Forest. Percequillo et al. (2007) recorded 37 species for João Pessoa, Paraíba and Vieira (1953) registered 28 species in the state of Alagoas. In addition to the similarity in richness, these faunas are similar in terms of their composition, being mostly composed by elements of wide geographic distribution, generalists in habitat and dietary, and tolerant to environmental disturbances, as *Didelphis marsupialis*, *Monodelphis domestica*, *Euphractus sexcinctus* and *Cerdocyon thous*. Feijó et al. (2016) listed 70 species of mammals to the Reserva Biológica Guaribas, distant about 25 km from the Restinga of Mataraca which corresponds to the closest site with a long-term inventory. This great diversity reported by these authors can be explained by the nearly inventory (30 years) of mammals in this area. In addition, Reserva Biológica Guaribas shows different phytophysiognomies, which should favors this higher diversity compared to other areas of the Atlantic Forest. On the other hand, Mataraca presents species as *Gracilinanus agilis*, *Rhynchonycteris naso* and *Phyllomys blainvilii* that were not recovered from Reserva Biológica Guaribas. In comparison to other Restinga areas, where the richness of all mammals range from 18 species in Barra de Maricá, Rio de Janeiro (Cerqueira 1990, 2000) to 39 species in the Parque Estadual Paulo César Vinha, Espírito Santo (Moreira & Mendes 2010), Mataraca stands out as one of the most diverse.

The six species of marsupials recorded in the Restinga of Mataraca represent 60% of the known diversity of this group for the Atlantic Forest on the northern bank of Rio São Francisco (Oliveira & Langguth 2004, Bovendorp et al. 2017). This number also corresponds to 27% of marsupial species recorded for the Brazilian Atlantic Forest (Paglia et al. 2012). According to Oliveira & Langguth (2004), the two species from Paraíba not sampled in this study are *Thylamys karimii* and *Monodelphis americana*, species usually uncommon in field surveys in this state. The first species exhibits a distribution throughout open formations (Carmignotto & Monfort 2006), as the phytophysiognomies of Cerrado inserted in the Atlantic Forest of Paraíba (Thomas & Barbosa 2008), and the second shows preference for more humid forests (Pardini et al. 2010). As well as Mataraca, Mata do Buraquinho (Percequillo et al. 2007) and Reserva Biológica Guaribas (Feijó et al. 2016) also count six species of marsupials. The composition differs regarding the presence of *Gracilinanus agilis* at Mataraca, *Thylamys karimii* at REBIO Guaribas and *Monodelphis americana* at Mata do Buraquinho.

Another noteworthy aspect of the mammalian fauna of Mataraca is the absence of rodents of the family Cricetidae, subfamily Sigmodontinae, with the exception of *Oligoryzomys nigripes*. This is the most diverse group of Neotropical mammals (Musser & Carleton 2005, Patton et al.

2015), being represented in the Atlantic Forest of Northeastern Brazil by 16 species (Bovendorp et al. 2017). In general, this group is representative in terms of diversity and abundance in communities of small mammals (e.g., Bergallo 1994, Vivo & Gregorin 2000, Pardini 2004). A possible explanation for the absence of species of sigmodontine in Mataraca is the dominance in the area of the white-eared opossum, *D. marsupialis*, with 66% of abundance (32 specimens were trapped), (Figure 4). The great abundance of marsupials of the genus *Didelphis* has been associated with the decrease on the diversity of the small mammals' communities in areas of secondary forests (Fonseca & Robinson 1990), as in Mataraca. In addition, Moura et al. (2008) suggest a greater sensitivity of rodents to drier environments, such as semideciduous forest, than marsupials. The sandy soil of Mataraca is very well drained and although a thick leaf litter is present, this stratum of the forest is quite dry (Figure 2 -D). Unlike other restinga areas from Southern Brazil (Cerqueira 2000, Quintela et al. 2012), we did not register the exotic murids *Mus musculus* and *Rattus sp*., which suggests that the area is free of invasive species that can present a deleterious effect on the community of small mammals. It is also noteworthy the presence of the echimyid rodent *Phyllomys blainvilii*. This record extended the distribution of the genus to the north (Campos & Percequillo 2007). Recently, Loss & Leite (2011) and Leite & Loss (2015) pointed out that these specimens may represent a species not yet described, increasing the importance of future studies in the region.

Seven species of bats represent a small diversity compared to other nearby areas of the Atlantic Forest. Leal et al. (2013) registered nine species for the *campus* of the Universidade Federal de Pernambuco in the city of Recife. Percequillo et al. (2007) recorded 14 species for an urban fragment in João Pessoa, Paraíba. Silva & Farias (2004) reported 15 species for an Ecological Station of Caetés, Pernambuco. This low diversity of bats obtained from the scientific collection may reflect occasional sampling rather than proper inventory in Mataraca. The material of UFPB assembled 48 specimens collected on April, May and September 2002. All bats registered in Mataraca are generalist omnivorous of the family Phyllostomidae and are well adapted to edge habitats and to moderately to well disturbed environments (see Gardner, 2008), with exception of the Emballonouridae *Rhynchonycteris naso* that has insectivorous habitat.

Regarding medium and large mammals, we recorded 51.8% of the 27 species present in the Atlantic Forest on the northern bank of Rio São Francisco (Feijó & Langguth 2013). Nevertheless, it should be considered that the diversity recovered here could be underestimated, as in the case of felids. Some of the records from these taxa were obtained through footprints, precluding accurate species identification. Among the larger mammals it is also worth to mention the primates *Cebus (Sapajus) flavius* and *Alouatta belzebul*. During the three years of field work, several individuals of *C. flavius* were directly visualized in the region, suggesting a resident population of this threatened species in the area. *C. flavius* is critically endangered (de Oliveira et al. 2015) and occurs only in fragments of Atlantic Forest in the states of Alagoas, Paraíba, Pernambuco and Rio Grande do Norte (Oliveira & Langguth 2006, Feijó & Langguth 2013, Fialho et al. 2014). On the other hand, *A. belzebul* was observed only once during the study, which suggests that these animals frequent the area sporadically. Therefore, the preservation of the continuous areas of the Restinga forest of Mataraca is fundamental for the maintenance of these populations of primates.

Bradypus variegatus, *Dasypus novemcinctus*, *Tamandua tetradactyla* and *Cyclopes didactylus* were recorded only by interviews and no other considerations could be made about their conservation status. These species are widespread in the Atlantic Forest of northeastern Brazil (Feijó & Langguth, 2013) and were recorded at REBIO Guaribas (Feijó et al. 2016), only 25 km from Mataraca. Thus, it is very likely that they occur at Mataraca, and we failed to sample them, due to our unstandardized sampling effort for medium-large mammals.

The analyzes of Jaccard show that the mammalian composition of Restingas are more similar to the nearby Atlantic Forest areas than with other Restinga areas (Figures 5 and 6). However, there are still few available contributions on the mammalian diversity of Restingas in Brazil, most of them concentrated in the southeastern region. The clustering of areas of northeastern Brazil with areas of Restingas of Rio Grande do Sul could be a consequence of the incompleteness of data available about Brazilian Restinga mammalian assemblages. Bovendorp et al (2017) listed 300 locations for the entire biome, but only three of them were conducted on the Restinga. Examining their faunal elements, we can only observe the sharing of white-ear-opossum (*D. marsupialis*) and the black-footed colilargo (*O. nigripes*) (supplementary material 1). Possibly the other areas analyzed (from Rio de Janeiro and Espírito Santo) were much more similar among each other, causing the nesting of the remaining areas (Northeastern and Southern Brazil). Nevertheless, our results show that Restingas do not constitute an independent and autochthone faunal unit and support the hypothesis that Restinga areas present a mammalian assemblage similar to that found in adjacent Atlantic Forest areas (Cerqueira et al. 1990). Therefore, the preservation of the Restinga depends not only on actions focused on this coastal vegetation, but also requires a greater connectivity to nearby Atlantic Forest areas (Pardini et al. 2005).

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Supplementary material

The following online material is available for this article:
Table S1

Author's Contributions

- BC - Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.
- AF- Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation
- PGB- Contribution to data collection; Contribution to critical revision, adding intellectual content
- ARP- Contribution to data collection; Contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Short Communication

Insect galls in three species of *Copaifera* L. (Leguminosae, Caesalpinioideae) occurring sympatrically in a Cerrado area (Bahia, Brazil)

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Abstract: The aim of this study was to characterize galls of three species of the *Copaifera* genus (*C. sabulicola*, *C. luetzelburgii* and *C. depilis*), which occur sympatrically in a Cerrado area from Bahia state. A total of 23 gall morphotypes were found, 9 morphotypes on *C. sabulicola*, 7 on *C. luetzelburgii* and 7 on *C. depilis*. Galls predominated on leaves and stems. The most abundant colours found among galls were brown and green. Regarding the period of the year, from 35,511 leaflets analysed, 16,322 were collected in the dry season and 19,189 in the rainy season. In the dry season, 1,635 leaflets having galls were observed, and the highest proportion was observed on *C. luetzelburgii* (16.31%). In relation to the rainy season, there is statistical difference in the incidence of leaflets with galls between C. *sabulicola* and *C. depilis,* these two species are affected differently during the rainy season. In general, most of the gall morphotypes were more abundant in the dry season. The gallers of 18 morphotypes cannot be determined, because the gall samples were collected occupied by parasitoids or without dwellers. Cecidomyiids induced galls in five morphotypes. Associated invertebrates belonging to the orders Hymenoptera, Diptera, Coleoptera and Acari were found among all gall morphotypes. The results obtained here indicate that the three species of *Copaifera* have an expressive number of galls, but lower than reported in the literature for *C. langsdorffii*, which is considered a super host.

Keywords: *Copaifera depilis, Copaifera luetzelburgii, Copaifera sabulicola, Cecidomyiidae, insect-plant interaction*

Galhas entomógenas em três espécies de Copaifera L. (Leguminosae, Caesalpinioideae) que ocorrem em simpatria em área de Cerrado (Bahia, Brasil)

Resumo: O objetivo deste trabalho foi caracterizar as galhas de três espécies do gênero *Copaifera* (*C. sabulicola, C. luetzelburgii* e *C. depilis*) que ocorrem em simpatria no Cerrado do Estado da Bahia. Um total de 23 morfotipos de galhas foi encontrado, sendo 9 morfotipos em *C. sabulicola*, 7 em *C. luetzelburgii* e 7 em *C. depilis* As galhas predominaram nas folhas e caules e os morfotipos com coloração marrom e verde foram os mais abundantes. Em relação à época do ano, do total de 35.511 folíolos analisados, 16.322 foram coletados no período seco e 19.189 no chuvoso. Na estação seca foram observados 1.635 folíolos com galhas, sendo que a maior proporção de folíolos galhados foi observada em *C. luetzelburgii* (16.31%). Já em relação à estação chuvosa, há diferença estatística na incidência de folíolos com galhas entre *C. sabulicola* e *C. depilis,* estas duas espécies são afetadas diferentemente durante a estação chuvosa. De forma geral, a maioria dos morfotipos foi mais abundante na estação seca. Os galhadores de 18 morfotipos não foram determinados, porque as galhas coletadas apresentavam parasitóides ou estavam vazias. Os cecidomiídeos foram os responsáveis pela indução de cinco morfotipos. Os invertebrados associados às galhas pertencentes às ordens Hymenoptera, Diptera, Coleoptera e Acari. Os resultados obtidos neste estudo apontam que as três espécies de *Copaifera* possuem um número expressivo de galhas, porém menor que o relatado na literatura para *C. langsdorffii*, a qual é considerada uma super hospedeira.

Palavras-chave: *Copaifera depilis, Copaifera luetzelburgii, Copaifera sabulicola, Cecidomyiidae, interação inseto-planta*

Introduction

The genus *Copaifera* L. (Leguminosa, Caesalpinioideae) has 38 species, distributed in Central America (4 spp.), South America (29 spp.), Africa (4 spp.) and Asia (1 sp.). In Brazil, 28 species of this genus have been recorded, with 22 species restricted to Brazilian territory (Costa & Queiroz 2007). *Copaifera* species are popularly known as "copaibeiras" or "pau d'óleo" and are mainly found in the Amazon region and in Central-western Brazil (Veiga Junior & Pinto 2002). Bahia is the Brazilian state with the largest number of *Copaifera* species (13 species). Futhermore, the species *C. langsdorffii* Desf., *C. depilis* Dwyer, *C. luetzelburgii* Harms and *C. sabulicola* Costa & Queiroz occur sympatrically in western region of this state (Santos et al. 1985a).

Despite the wide distribution of *Copaifera* in Brazilian territory, studies on the relationships between galling insects and species of this genus are mainly related to *Copaifera langsdorffi* Desf. This species is considered by Costa et al. (2010), as the tropical arboreal species with the richest galling insects ever described (23 morphotypes) and can be considered a super host (Gonçalves-Alvim & Fernandes 2001). The aims of this study were to characterize galls of three species on this genus, *Copaifera depilis* Dwyer, *Copaifera luetzelburgii* Harms and *Copaifera sabulicola* Costa & Queiroz, which occur sympatrically in Western Bahia and compare their abundance.

Materials and Methods

This study was carried out from samples of three species of *Copaifera* collected in Serra da Bandeira, Barreiras, Bahia (11° 37' and 12° 25'S and 44° 34' and 46° 23W). This municipality is located in the Cerrado Biome and presents two well defined seasons, a dry and a rainy. The rainy season comprises the months from October to April, while the dry season corresponds to the months from May to September. The climatic type of the municipality, according to Köppen classification is Aw type, typical of savannah, presenting dry winter and average air temperature of the coldest month above 22ºC. The mean annual rainfall is 1500 ± 500 mm (Neto et al. 2013).

The gall characterization of *C. sabulicola*, *C. luetzelburgii* and *C. depilis* was performed by sampling 20 individuals of each species randomly selected. The collections were carried out from December 2012 to January 2013 (rainy season) and from June to July 2013 (dry season). The plants used had the following averages for height: 2.19 m (± 0.35) in *C. sabulicola*; 2.27 m (± 0.29) in *C. luetzelburgii* and 1.96 m (± 0.49) in *C. depilis*.

The richness and abundance of galls per plant were determined by collecting four branches with 50 cm length of each host plant. To avoid the effect of the position of the branches on the occurrence of the galls, each plant was divided into quadrants following the north (N), south (S), east (E) and west (W) directions, and one branch of each of these quadrants was collected. The number and size of the branches as well as the number of collections were determined in order not to damage the host plants by excessive pruning. A total of 80 branches of each species were analysed in each season and the same individuals were investigated during the dry and rainy seasons. The galls were photographed and morphologically characterized according to the shape, colour, presence of trichomes and distribution in the host plant as reported by Maia & Fernandes (2004). The occurrence of leaf galls on adaxial and/or abaxial was verified.

In order to compare the proportion of leaves with galls between seasons and species, we counted the total number of leaflets and the number of leaflets with galls, in 20 individuals of each of the three species. We applied the test of comparison between two proportions with correction of continuity, at a significance level of 5%, with decision based on p-value. Firstly, we performed the comparison test between dry and rainy seasons considering the three species together. Secondly we compare the dry and rainy seasons considering the three separate species. Finally, we compare the species to each other, both for the dry season and for the rainy season. These analyses were conducted in the software R-Gui, 3.4.0 version.

Results and Discussion

In the three *Copaifera* species, a total of 23 gall morphotypes were obtained, 9 morphotypes found on *C. sabulicola*, 7 on *C. luetzelburgii* and 7 on *C. depilis*. The characterization of the galls is presented in Table 1 and Figure 1. Galls were found in leaves and stems, being foliar the most abundant in the three *Copaifera* species. Although galls were not observed in flowers and fruits, these organs were also inspected.

In *C. sabulicola*, the most frequent morphotype found was the globoid one, which was found in 14 and 13 of the 20 plants analysed in the dry and rainy seasons, respectively (Figure 1h). Regarding the foliar surface, it was verified that four morphotypes occurred in the adaxial and abaxial surfaces. In *C. luetzelburgii* 7 morphotypes were found, however only 1 (7.42%) occurred in the stem. In this species, the parenchymal was the most frequent (Figure 1p). In *C. depilis*, 7 morphotypes were also identified, the stem types being less frequent than the foliar ones. In *C. depilis* and *C. sabulicola*, galls with trichomes were not found, whereas in *C. luetzelburgii* two morphotypes presented these structures (Table 1).

The results indicate that the pattern of occurrence of galls in the three *Copaifera* species studied is similar to that observed by Almeida et al. (2006) in *C. langsdorfii*, in which the greatest diversity and abundance of galling insects occurs in leaves, followed by branches. The occurrence of a greater number of leaf galls is corroborated by several studies in different Brazilian biomes and for several plant species (Gonçalves-Alvim & Fernandes 2001, Araújo et al. 2011; Santos et al. 2011b; Silva & Almeida-Cortez 2006), and this may be justified by the greater abundance of nutrients in this organ (Mani 1964).

According to the colour, brown and green morphotypes were the most frequent in galls on three species of *Copaifera*, however, differences were found in colour and it may be related to gall senescence. Santos et al. (2010c), in a study carried out in a Cerrado area, also verified in 20 species of plants that galls might have a wide variety of colours, especially during the maturation period. Another difference between the three species of *Copaifera* refers to the presence or absence of pubescence. In *C. sabulicola* and *C. depilis* were not observed the presence of trichomes while in *C. luetzelburgii* was observed. This result can be associated with the characteristics of the host species, due to *C. luetzelburgii* present a large number of trichomes on leaves, while the other two species are glabrous. Other possibility is that trichomes on leaves confered some protection to the galler by killing parasitoids of the larva as pointed by Fernandes et al. (1987). More detailed studies should be performed to explain this finding.

In this analysis of abundance of gall morphotypes and the relationship with leaflet numbers and period of the year, it was observed that although the collected branches had the same length (50 cm), the number of leaflets verified in the three *Copaifera* species was different depending on the species and according to the period of year. A total of 35,511 leaflets were analysed, being 16,322 collected in the dry season and 19,189 in the rainy season.

We observed that the proportion of leaves with galls during the dry season is 0.1002 (percentage approximately equal to 10%), while in the rainy season this proportion is 0.0459 (percentage approximately equal to 5%) (Table 2). The test for comparison of two proportions indicated a statistical difference between the two periods of the year (p-value approximately equal to zero), in other words, the period of year interferes with the incidence of leaves with galls in such a way that, in this study, the proportion of leaves with galls in the dry season is double the proportion in the rainy season.

The results presented in Table 3 demonstrated that *C. sabulicola* is the only species which did not present statistical difference between dry and rainy seasons, in other words the period of year did not interfere

Table 1. Gall characterization (organ, shape, colour, trichomes, foliar surface and plant frequency) in species of *Copaifera* (Serra da Bandeira, Barreiras, Bahia, Brazil). Abbreviations: Ga: galler, Pa: parasitoid, Su: successor

Gall	Organ	Shape	Colour	Trichomes	Foliar surface	Plant frequency		Associated fauna	Figure
						Dry	Rainy		
Copaifera sabulicola									
1	Stem	Discoid	Brown	Absent	-	1 (5%)	6 (30%)	Hymenoptera (Pa) Acari (Su)	1a
2	Stem	Fusiform	Brown	Absent	-	1 (5%)	0 (0%)	-	1b
3	Stem	Globoid	Brown	Absent	-	1 (5%)	0 (0%)	Formicidae (Su)	1c
4	Leaf	Parenchymatical	Green/ Brown	Absent	Adaxial	9 (45%)	10 (50%)	Cecidomyiidae (Ga)	1d
5	Leaf	Discoid	Green / Red	Absent	Both	2 (10%)	10 (50%)	Cecidomyiidae (Ga)	1e
6	Leaf	Discoid	Green / Red	Absent	Abaxial	2 (10%)	1 (5%)	-	1f
7	Leaf	Discoid	Green	Absent	Both	7 (35%)	3 (15%)	Cecidomyiidae (Ga)	1g
8	Leaf	Globoid	Green / Brown	Absent	Both	14 (70%)	13 (65%)	-	1h
9	Leaf	Cylindrical	Green /Red / Brown	Absent	Both	4 (20%)	1 (5%)	Coleoptera (Su)	1i
Copaifera luetzelburgii									
1	Leaf	Cylindrical	Green /Red/ Brown	Present	Abaxial	17 (85%)	8 (4%)	Hymenoptera (Pa)	1j
2	Leaf	Globoid	Green /Red/ Brown	Absent	Both	15 (75%)	18 (90%)	Hymenoptera (Pa)	1k
3	Leaf	Discoid	Green	Absent	Adaxial	3 (15%)	0 (0%)	Hymenoptera (Pa)	1l
4	Leaf	Discoid	Brown	Present	Abaxial	13 (65%)	5 (25%)	Hymenoptera (Pa)	1m
5	Stem	Discoid	Brown	Absent	-	12 (60%)	2 (10%)	Hymenoptera (Pa)	1n
6	Leaf	Discoid	Brown	Absent	Abaxial	4 (20%)	3 (15%)	-	1o
7	Leaf	Parenchymatical	Green / Brown	Absent	Both	18 (90%)	15 (75%)	-	1p
Copaifera depilis									
1	Stem	Discoid	Brown	Absent	-	0 (0%)	2 (10%)	Hymenoptera (Pa)	1q
2	Stem	Globoid	Brown	Absent	-	2 (10%)	1 (5%)	-	1r
3	Leaf	Globoid	Green / Brown	Absent	Both	19 (95%)	14 (70%)	-	1s
4	Leaf	Cylindrical	Green /Red/ Brown	Absent	Both	17 (85%)	13 (65%)	Cecidomyiidae (Ga)	1t
5	Leaf	Discoid	Green	Absent	Adaxial	3 (15%)	2 (10%)	-	1u
6	Leaf	Discoid	Brown	Absent	Both	15 (75%)	15 (75%)	-	1v
7	Leaf	Parenchymatical	Green / Brown	Absent	Abaxial	13 (65%)	10 (50%)	Cecidomyiidae (Ga) Hymenoptera (Pa)	1x

with the incidence of leaves with galls (p-value = 0.05417). The species *C. luetzelburgii* and *C. depilis* seemed to be sensitive to the seasons as dry and rainy seasons significantly interfered with the incidence of leaves with gall (p-value close to zero). In the *C. luetzelburgii* species, this proportion in the dry season is more than triple the rainy season, suggesting that this species is the most sensitive season of the year. In the species *C. depilis* the proportion in the dry season is double of the rainy season.

Regarding to dry season, all three species presented differences between them in relation to the incidence of leaves with gall. Specifically, during the dry season each of the three species is affected differently ($p<0.0001$) such that the most sensitive species during the dry season is *C. luetzelburgii* (16.31%), followed by *C. depilis* (8.04%). The least sensitive species among the three was *C. sabulicola* (4.36%). Referring to rainy season, there is no statistical difference in the incidence of leaves with galls between *C. sabulicola* and *C. luetzelburgii* (p-value = 0.1073, χ^2 =2.5932) and between *C. luetzelburgii* and *C. depilis* (p-value = 0.1480, χ^2 = 2.0931). However, there are differences between *C. sabulicola* and *C. depilis* (p-value = 0.002230, χ^2 = 9.3503), that is, these two species are affected differently during the rainy season.

Several hypotheses search for ecological-evolutionary evidences in order to explain the association between galling insects and their host plants. Some authors point out that species richness for galling insects is different from others insects, because they require xeric environments or dry seasons instead of mesic environments and rainy seasons (Fleck & Fonseca 2007). Although the largest number of leaflets in *Copaifera* was observed in the rainy season, the highest number of galling leaflets occurred in the dry season.

In the present study, we observed that five morphotypes were induced by Cecidomyiids and the gallers of 18 morphotypes cannot be determined, because the gall samples were collected occupied by parasitoids or without dwellers (Table 1). According to Maia (2011), Cecidomyiidae are the most important galler in all zoogeographic regions. Micro-hymenopteras identified represents possibly parasitoids, natural enemy of Cecidomyiidae, with the exception of ants (Formicidae), which were found on abandoned galls, being considered in this study as a successor for *Copaifera langsdorffii* Desf. (Fabaceae), Oliveira et al. (2013) pointed that the level of parasitoidism is very high, and sampling for cecidomyiids adults has been unsuccessful. Coleoptera and Acari were considered successors.

The results obtained in this study indicate that the three species of *Copaifera* have an expressive number of galls, but lower than reported in the literature for *C. langsdorffii*, which is considered a super host (Costa et al. 2010). Among the morphotypes found in the *Copaifera* genus, some are morphologically similar among species, such as discoid occurring in *C. depilis* (Figure 1q) and *C. luetzelburgii* (Figure 1n), which were also observed in *C. langsdorffii* by Fernandes et al. (1988). These similarities can be better understood from anatomical gall studies, identification of inducers and parasitoids, which may clarify important aspects about the co-evolution between galling species and *Copaife*ra genus.

Figura 1. Galls on C. sabulicola (a-i), C. luetzelburgii (j-p), and C. depilis (q-x) in Cerrado area (Serra da Bandeira, Barreiras, Bahia, Brazil).

Table 2. Comparison between dry and rainy seasons considering the three *Copaifera* species together (*Copaifera sabulicola*, Copaifera *luetzelburgii* and *Copaifera depilis*).

Seasons	Leaflets with galls	Total leaflets	Proportion	Confidence Interval (95%)	Test result
Dry	1,635	16,322	0.1002	[0.0956; 0.1048]	*p-value* <0.0001
Rainy	881	19,189	0.0459	[0.0430; 0.0489]	χ^2=393,6214

Table 3. Number of leaflets with galls and leaflets analyzed in *Copaifera sabulicola*, *Copaifera luetzelburgii* and *Copaifera depilis* in dry and rainy seasons in Cerrado area (Barreiras, Bahia, Brazil).

Species	Seasons	Leaflets with galls	Total leaflets (MD±SD)	Proportion	Confidence Interval (95%)	Test result
Copaifera sabulicola	Dry	193 (9.65±8.25)	4,426 (421.52±88.47)	0.0436	[0.0376; 0.0496]	*p-value*=0,05417 χ^2= 3,7073
	Rainy	321 (30.57±13.56)	6,180 (588.57±106.97)	0.0519	[0.0464; 0.0575]	
Copaifera luetzelburgii	Dry	958 (47,90±8.89)	5,874 (559.43±84.84)	0.1631	[0.1536; 0.1725]	*p-value* <0.0001 χ^2= 471,7436
	Rainy	302 (28.76±8.90)	6,615 (630.00±127.24)	0.0457	[0.0406; 0.0507]	
Copaifera depilis	Dry	484 (24.20±24.21)	6,022 (573.52±81.95)	0.0804	[0.0735; 0.0872]	*p-value* <0.0001 χ^2= 87,6910
	Rainy	258 (24.57±9.04)	6,394 (608.95±97.95)	0.0404	[0.0355; 0.0452]	

Author Contributions

Isana Martins dos Santos: contributed to the study conception and design, to data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Valdeir Pereira Lima: contributed to the data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Edyany Kellen Souza Soares: substantial contribution to data collection and data analysis.

Marcelo de Paula: contribution to data analysis and interpretation, and revision of the manuscript's intellectual content.

Daniela Cristina Calado: contributed to the study conception and design, to data analysis and interpretation, to the drafting and revision of the manuscript's intellectual content.

Conflicts of interest

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

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Density and diversity of filamentous fungi in the water and sediment of Araçá bay in São Sebastião, São Paulo, Brazil

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Abstract: Araçá Bay, located in the city of São Sebastião, São Paulo, Brazil, is a protected area of substantial complexity. It represents the last remaining mangrove swamp preserve between the cities of Bertioga and Ubatuba on the northern coast of São Paulo State. This mangrove swamp has specific physical and chemical properties, and it shelters a wide variety of life, including fungi. These microorganisms are present in a variety of species with different morphophysiological features, and they have the ability to produce enzymes of biotechnological importance. The goal of this study was to quantify, isolate, and identify filamentous fungi in water and sediment samples from the Araçá Bay mangrove swamp in São Sebastião. Two samplings were performed in the summer and two were performed in the winter. The samples were collected from intertidal zones, and dissolved oxygen (DO), temperature, salinity, and pH were measured in situ. The spread plate technique was used to inoculate the samples collected on plates with a potato dextrose agar (PDA) medium. A total of 208 colonies (68 from water samples and 140 from sediment samples) were isolated, and they were identified based on their morphological characteristics. Filamentous fungus density was higher in the sediment than in the water, and the samplings performed in the winter revealed a higher density than those performed in the summer. Though some of the environmental parameters were not ideal for fungal development, a high quantity of growth was nevertheless observed. When the isolated colonies were analyzed, the greatest diversity and species richness were found in the summer samples. The genera identified in all of the samples were *Aspergillus*, *Penicillium*, *Cladosporium*, and *Fusarium*. The pathogenic species found from these genera were *Aspergillus fumigatus*, *A. terreus*, *Penicillium citrinum*, and *P. chrysogenum*. These species are also able to produce enzymes that offer a variety of applications. The fungal community described herein represents the diversity found in this mangrove swamp during the period studied. Many of the fungus species found are pathogenic and may be useful due to their ability to produce specific enzymes applicable in the biotechnological and pharmaceutical industries.

Keywords*:* *Mangrove swamp, Environmental parameters, Mycology.*

Densidade e diversidade de fungos filamentosos na água e sedimento da Baía do Araçá, São Sebastião (SP), Brasil

Resumo: A Baía do Araçá, localizada no município de São Sebastião (SP), é uma região protegida, de grande complexidade, mantendo o último remanescente preservado de manguezal entre Bertioga e Ubatuba, litoral Norte do Estado de São Paulo. Manguezal caracteriza-se com propriedades físico-químicas específicas e abriga grande diversidade de seres vivos, entre eles os fungos. Estes microrganismos apresentam grande variedade de espécies e diferentes características morfofisiológicas com capacidade de produzir algumas enzimas de importância biotecnológica. Este trabalho teve como objetivo quantificar, isolar e identificar fungos filamentosos em amostras de água e de sedimento do manguezal da Baía do Araçá, São Sebastião, (SP). Foram realizadas 2 coletas no verão e 2 no inverno na região entremarés, medidos "in situ" os parâmetros oxigênio dissolvido (OD), temperatura, salinidade e pH. Utilizou-se a Técnica "Spread Plate" para inocular os materiais coletados, em placas com meio Potato Dextrose Agar (PDA). Foram isoladas 208 colônias (68 das amostras de água e 140 das amostras de sedimento) e identificadas pelas características morfofisiológicas. As densidades de fungos filamentosos foram maiores no sedimento em relação à água e nas coletas realizadas no inverno demonstraram maiores densidades do que no verão. Apesar de alguns parâmetros ambientais não se apresentarem ideais para o desenvolvimento dos fungos, observou-se alta quantidade de crescimento nas amostragens. Analisando as colônias isoladas, a maior diversidade e riqueza foram observadas na amostragem coletado no verão. Foram observados os gêneros *Aspergillus sp*, *Penicillium sp*, *Cladosporium sp e Fusarium sp* em todas as amostragens, dos quais as espécies *Aspergillus fumigatus*, *A. terreus*, *Penicillium citrinum e P. chrysogenum* identificados neste trabalho são considerados patogênicos mas também são espécies capazes de produzir enzimas aplicáveis em diversas atividades. A comunidade fúngica descrita apresenta a diversidade encontrada neste manguezal em relação à variedade ambiental no período estudado, sendo que muitos apresentam patogenicidade e podem ser uteis pela sua capacidade de produzir enzimas específicas aplicáveis nos estudos biotecnológicos e farmacêuticos.

Palavras-chave: *Manguezal, parâmetros ambientais, micologia.*

Introduction

Brazil possesses 1,225,444 hectares (approximately 12,250 km^2) of mangrove swamps (MMA 2010). Of these, approximately 76 km are located on the northern coast of São Paulo State, and 2.1% are located within the city of São Sebastião (Dias-Brito et al. 2014).

Araçá Bay is part of the Alcatrazes Municipal Environmental Protection Area as per Municipal Law No. 848/1992. It is also part of the Northern Coast Marine Protection Area as per State Law No. 53.525/2008 (PLDS/Araçá 2016). It represents the last remaining mangrove swamps on the São Sebastião coast, and it harbors high biological diversity (Amaral et al. 2010, PLDS/Araçá 2016). This region is considered to be relatively protected by its physical makeup and location. It includes a cove bordered by rocky walls and an extensive coastal plain with soft sand, which impedes the direct effects of tide-based hydrodynamic factors (Amaral et al. 2010, Alitto et al. 2016). The region is exposed to different types of human activity, including illegal occupation (Zanardi et al. 1999), domestic effluents (PLDS/Araçá 2016), underwater dredging (Gubitoso et al. 2008, Teodoro et al. 2011, PLDS/Araçá 2016), and activities in the nearby Port of São Sebastião (Amaral et al. 2010, PLDS/Araçá 2016).

Mangrove swamps are considered a transitory costal ecosystem between land environments and marine environments. They are typical of tropical and subtropical regions and are effected by tides (Schaeffer-Novelli 1995, Schaeffer-Novelli 2000, Andreote et al. 2012). Mangrove swamp conditions are conducive to the feeding, protection, and reproduction habits of many animal species, and they are considered an important environment for the transformation of organic matter into nutrients (Schaeffer-Novelli 1995, Schaeffer-Novelli 2000, Sridhar 2004). Mangrove swamps are also considered an important niche repository for fungi (Paul & Clark 1989, Roitman et al. 1991, Nambiar & Raveendran 2008), which play an important role in the organic matter decomposition processes present in the ecosystem (Citrón & Schaeffer-Novelli 1983, Allsopp & Seal 1986, Jones et al. 2009).

Hawksworth (1991) estimated that fungal diversity is represented by approximately 1.5 million species around the world; however, recent studies have reported that this amount may be ten times higher: between 3.5 and 5.1 million species (O'Brien et al. 2005).

The survival of mycobiota and their diversity are influenced by a number of abiotic factors, one of the most important of which is hydrogen concentrations, which influences fungal growth capacity and composition. Temperature, salinity, dissolved oxygen (DO) levels, and the availability and diversity of substrata are factors which may also influence fungal composition in a given environment (Jaitly & Rai 1982, Jaitly 1987, Jones & Alias 1997, Tucci & Mendes 2006), as is sediment texture (Roitman et al. 1991).

The Araçá Bay mangrove swamp substratum is made of sand and mud; it is relatively compact and it includes pebbles (Amaral et al. 2010). Schaeffer-Novelli (1995) described this mangrove swamp sediment as sandy and clayey with varying features, characteristics which may result from leaf and twig decomposition in the environment, as well as from animal matter. It may also contain the products of rock decay. Different types of rocks are brought into the environment by waves, winds, costal currents, and river flows (Schaeffer-Novelli 1995).

One study found that the fungi in sediment play an important role in processing plant-based organic matter (Carlile et al. 2001) and are active in the decomposition of organic compounds in both the sediment and the water (Harley 1971, Moore-Landecker 1996). Some characteristics of the waters in the environment favor the presence and propagation of fungi. Due to their importance in the ecosystem, these fungi must be evaluated using physical, chemical, and microbiological analyses (Jaitly & Rai 1982, Alexopoulos et al. 1996, Jones & Alias 1997, Duke et al. 1998). Jaitly & Rai (1982) isolated species of fungi from the Sunderbans mangrove swamp in India and found fungi that were thermophilic and thermotolerant. Their findings were based on specific parameters, including salinity, humidity, pH, and the amount of organic matter in the region.

Fungal species diversity in the world varies by location (latitude, longitude, and altitude), as well as by depth in the case of aquatic ecosystems. There is a tendency for greater species diversity in the tropics (Shearer et al. 2007), and most fungi isolated in the tropics and subtropics are anamorphic basidiomycetes and ascomycetes (Jones & Alias 1997, Sridhar et al. 2005). These fungi have been associated with numerous processes and functions, including organic matter decomposition (Tortora et al. 2005, Jones et al. 2009), geochemical cycles (Tortora et al. 2005), their usefulness in the pharmaceutical industry (Walsh et al. 2004), their value in the food industry (Molitoris 1995), and their utility in biotechnology (Atlas 1981, Carneiro & Gariglio 2011, Pereira & Freitas 2012, Houbraken et al. 2014), among other benefits (Tortora et al. 2005, Raghukumar 2008).

Some fungi are disease-causing pathogens responsible for mycoses and allergies. They are associated with diseases affecting humans, plants, and animals alike, and they are frequently detected in polluted areas (Walsh et al. 2004). However, they also produce enzymes with biotechnological applications. These microorganisms are being tested for their ability to produce enzymes, and the most frequently isolated genera are *Penicillium* and *Aspergillus*, the latter two of which are responsible for hydrocarbon assimilation (Atlas 1981). These species also degrade water and soil pollutants into less environmentally damaging forms (Vidali 2001, Novotny et al. 2004, Passos et al. 2009).

Aspergillus terreus was a fungus isolated from marine sediments and land habitats (Damare et al. 2006). It produces different secondary metabolites, but also causes infections such as aspergillosis and mycosis (Martins et al. 2005). Fungi are highly relevant for scientific research in the fields of biotechnology and bioremediation due to their potential ability to degrade organic compounds and contaminants, and they can also provide information on the environment, on biological and functional diversity, and on mangrove swamp ecosystems (Harms et al. 2011, Bonugli-Santos et al. 2015).

The objective of this study was to analyze the density and diversity of filamentous fungus species in water and sediment samples from the Araçá Bay mangrove swamp using molecular and morphophysiological methods, and to then correlate the findings with physical and chemical factors in the winter of 2015 and the summer of 2016.

Materials and Methods

In 2015 and 2016, samplings were performed at ten sites determined using a GPS device in the intertidal region at high tide (Figure 1). Two samplings were performed in the winter dry season (June and July 2015), and two were performed in the summer rainy season (January and February 2016). The water was collected in sterilized flasks from a depth of 15 to 30 cm, as per American Public Health Association standards (APHA, 2005). Surface sediment was collected from the same points and stored in sterile bags until processing in the Marine Microbiology Laboratory (MICROMAR), located on São Paulo State University's São Vicente campus (UNESP, São Vicente). The DO, temperature, pH, and salinity parameters were measured on site using a HORIBA U-50 multiparameter meter (Multiparameter Water Quality).

Twenty-gram sediment aliquots were conditioned in sterile flasks to which 180 mL of sterile purified water was added. The samples then underwent manual agitation for 10 minutes, a process which was repeated in order to loosen any microorganisms which had adhered to grains of sediment. Once the samples had been agitated, only the supernatant was used for the microbiological analyses (Oliveira & Pinhata 2008).

Next, 500 µL of sample material was inoculated using spread plate technology and potato dextrose agar (PDA) plates, to which the antibiotic chloramphenicol was added. The samples were then incubated at room temperature (25°C) for 7 to 14 days. After the incubation period, the growth of filamentous fungus colonies on the plants was determined based on the total number of mold colonies on each incubated plate (APHA 2005). These values were calculated and expressed as colony-forming units (CFUs) per 100 ml (CFU $100mL^{-1}$) in the case of the water samples and as CFUs per 100 g (CFU $100g^{-1}$) in the case of the sediment samples. A total of 208 colonies were isolated and re-isolated until purity was reached. Macromorphological features (color, diameter, and appearance of the colony) and micromorphological features (microstructures) were determined in order to identify the genera in a method similar to those provided by Raper & Fennell (1977), Nelson et al. (1983), Hanlin & Ulloa (1988), Pitt (1985), Domsch et al. (1993), Alexopoulos et al. (1996), Houbraken et al. (2014), and Talaiekhozani & Ponraj (2015).

Based on the most commonly observed microscopic characteristics, three physiological structure clusters were established. These three characteristics were the presence of vesicle on the conidiophore (7 colonies with *Aspergillus* features) the lack of a vesicle (11 colonies with *Penicillium* features), and a unique conidia format (2 colonies), which together represented the 20 fungi which were identified at the molecular level in the Microbiology Laboratory at the Center for Social Insect Studies at São Paulo State University's Rio Claro Campus (UNESP Rio Claro). The DNA was extracted and amplified using the polymerase chain reaction (PCR) technique. The genomic DNA was then sequenced using a modified version of the extraction technique method with cationic hexadecyl trimethyl ammonium bromide (CTAB), and two different nuclear DNA markers (LSU and ITS) were amplified for phylogenetic analysis (Möller et al. 1992). The PCR products were cleaned using a Wizard® SV Gel and PCR Clean-Up System (Promega). The cycle sequencing reactions were performed using a BigDye Terminator v.3.1 Cycle Sequencing Kit (Life Technologies), and the sequences were generated using an Applied Biosystems® 3500 genetic analyzer (Life Technologies). The contigs were assembled in the BioEdit sequence alignment editor, version 7.1.3 (Hall 1999) and were consulted in GenBank from the National Center for Biotechnology Information (NCBI).

The statistical analysis was performed using the Past software. To determine the normality of the data, the Kruskal-Wallis test was applied at a 95% significance level ($p<0.05$). Spearman's correlation test was used to correlate the non-parametric data on the environmental parameters and fungus density. Fungus species diversity and richness were determined using the Shannon diversity index (H) and the Chao 1 estimator, respectively.

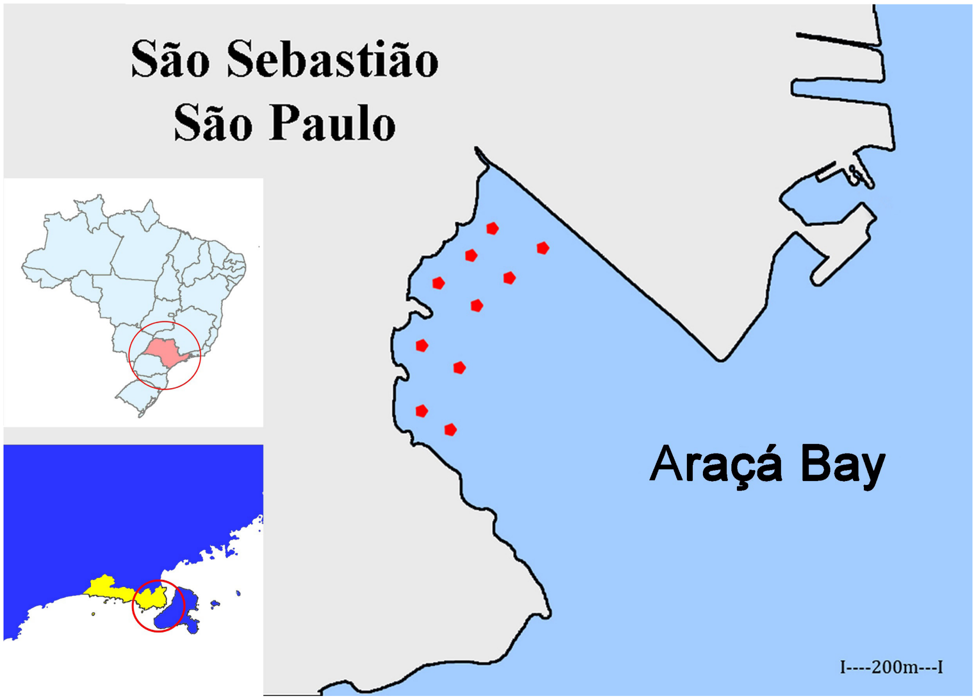

Figure 1. Map showing the research site and sampling points in the Araçá Bay mangrove swamp in the city of São Sebastião, São Paulo, Brazil.

Results and Discussion

Certain physical and chemical aspects of the environment, including temperature and pH, are important for the survival, adaptability, and growth of microorganisms (Jaitly 1987, Jones & Alias 1997, Raghukumar 2008). In this study, overall average temperature was 26°C. During the winter, this average was lower (23°C), and in the summer, it reached 27.7°C (Table 1). According to Kohlmeyer (1983) and Wong et al. (1998), the temperatures measured in this study favor fungal diversity and reproduction: these authors report that temperature affects the distribution and diversity of filamentous fungi, and that there is greater diversity in tropical areas than in temperate waters. In addition, marine fungi require temperatures between 25°C and 30°C to reproduce (Griffin 1981).

The pH levels detected herein remained slightly alkaline and ranged only from 7.7 to 8.1 during the study period (Table 1). This finding is consistent with the values reported by Sengupta & Chaudhuri (1995), who detected an alkaline pH ranging from 7.2 to 8.0 and who reported on filamentous fungi in mangrove swamp sediments from the Ganges River in India. Extracellular enzyme production and growth by marine fungi was described by Raghukumar et al. (2004), who reported a pH between 7 and 8. Jaitly (1987), Alexopoulos et al. (1996) and Raghukumar (2008) report that the best pH for the growth of different types of land fungi ranges from 4 to 7 (in the neutral to acidic range).

The salinity levels detected in this study remained below 30; the water can therefore be classified as brackish as per Brazil's National Environment Council (CONAMA) Resolution No. 357 from March 2005. The values did not vary substantially over the course of the study and ranged from 24.8 to 28.1 (Table 1). Duke et al. (1998), Bunt (1999), and Mueller & Bills (2004) report that salinity is a very important ecological parameter which is directly correlated with species distribution in mangrove swamps. Though Mueller & Bills (2004) describe salinity as a limiting factor for fungus growth, high fungal density was observed in the current study. Lorenz & Molitoris (1992) found that the ideal salinity level for the growth of certain marine fungi increases as incubation temperature increases, a finding which indicates that Araçá Bay exhibits ideal parameters for mycological development.

The DO values (mg L^{-1}) ranged from 4.0 to 6.7, with a mean value of 5.5 (Table 1). In most of the samples, dissolved oxygen values remained within the limits established by CONAMA Resolution No. 357/2005 for high-quality brackish waters. Most fungi are aerobic or microaerophilic; however, some species have been found to have a limited anaerobic metabolism, and few are capable of completely anaerobic growth (Tabak & Cooke 1968, Tortora et al. 2005). Mangrove swamps typically exhibit low levels of oxygen (Schaeffer-Novelli 1995); however, most of the samples from the Araçá Bay mangrove swamp were found to exhibit good DO quality. Because oxygen is considered an essential element for aerobic fungi, the environment analyzed herein may be considered favorable for the development of these microorganisms.

Based on the samplings, filamentous fungus colony density was found to range from 0.1x10^4 CFU 100 mL^{-1} to 4.6x10^4 CFU 100mL^{-1} in the water and from 0.4x10^4 CFU 100g^{-1} to 42.5x10^4 CFU 100g^{-1} in the sediment. Density varied considerably between the two materials. The samplings performed in the summer produced lower values both in the water (0.2x10^4 CFU 100mL^{-1}) and in the sediment (0.9x10^4 CFU 100g^{-1}). In the winter, the highest density was found in a sediment sample: 42.5x10^4 CFU 100g^{-1} (Table 1). Pupin & Nahas (2014) analyzed dry sand from the swamp on Cardoso Island and determined the number of CFUs to be between 5.2x10^5 g^{-1} and 9.7x10^5 g^{-1}, higher values than those obtained in the current study.

Filamentous fungus density was found to be much higher a total mean in the sediment samples and relative to the winter in the case of both the water samples and the sediment samples (Figure 2). Based on Spearman's correlation test, the statistical analysis did not reveal any significant correlations between the environmental parameters and fungal density in the water ($p>0.05$); however, the amount of fungi in the sediment was found to be significantly and positively correlated with salinity and temperature ($p=0.032$ and $p=0.024$, respectively). In this study, filamentous fungus colony density in the sediment was significantly higher than in the water ($p<0.05$; non-parametric Kruskal-Wallis test=9.8x10^{-8}).

Similar findings were reported by Gomes et al. (2011), who analyzed filamentous fungi isolated from sediment collected from a mangrove swamp in Barra das Jangadas, Pernambuco, Brazil. The authors reported a higher incidence of fungi during the dry season (77%) relative to the rainy season (23%) and found that the species *Aspergillus terreus*, *A. niger*, *Penicillium sp*., and *Trichoderma sp.* were common in both seasons (Gomes et al. 2011). Pinto et al. (1992) also found that rainfall significantly increases the amount of isolated fungi in both land and aquatic ecosystems.

The colonies isolated were first identified based on macromorphological and micromorphological features (Figure 3). A total of 208 filamentous fungus colonies with different morphological features were isolated, 68 of which were from water samples and 140 of which were from sediment samples. In addition to anamorphs, a total of 15 genera were found: *Aspergillus*, *Aureobasidium*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Fusarium*, *Geotrichum*, *Mucor*, *Paecilomices*, *Penicillium*, *Rhizophus*, *Sporothrix*, *Talaromyces*, *Trichophyton*, *Trichoderma.*

Of the fungi isolated, *Aspergillus* and *Penicillium* were the most commonly observed genera overall (38.9% and 35.0%, respectively). In the water, the genus *Aspergillus* was the most common (39.7%), while in the sediment, *Penicillium* was the most common (42.8%), followed by the other genera at lower frequencies (Figure 4). The colonies that were found in the asexual reproductive stage or for which microscopically differentiating features could not be detected were treated as anamorphs.

Table 1. Environmental parameters obtained from mangrove swamp samplings from Araçá Bay on the northern coast of São Paulo State, Brazil. Temp. (Temperature in °C), pH, salinity, dissolved oxygen (DO mg L^{-1}). Mean filamentous fungus density in water (expressed as CFU 100mL^{-1}) and in sediment (expressed as CFU 100g^{-1}). Min - Minimum value; Max = maximum value; x - mean; s - Standard deviation.

samples	**Environmental parameters**	**Total**		**Summer**		**Winter**	
		min - max	**x ± s**	**min - max**	**x ± s**	**min - max**	**x ± s**
water	Temp. (°C)	22.6 - 31.6	26.2 ± 2.9	24.3 - 31.6	27.7 ± 3.0	22.6 - 25.4	23.9 ± 1.0
	pH	7.7 - 8.1	7.9 ± 0.1	7.7 - 8.0	7.9 ± 0.1	7.8 - 8.1	8.0 ± 0.1
	salinity	24.8 - 28.1	27.0 ± 0.7	24.8 - 27.5	26.6 ± 0.7	27.1 - 28.0	27.4 ± 0.3
	DO (mg L^{-1})	4.0 - 6.7	5.5 ± 0.8	4.0 - 6.5	5.1 ± 0.7	5.3 - 6.7	6.2 ± 0.4
	Filamentous fungus (x10^4 UFC 100mL^{-1})	0.1 - 4.6	0.3 ± 0.8	0.1 - 1.1	0.2 ± 0.2	0.1 - 4.6	0.5 ± 1.3
sediment	Filamentous fungus (x10^4 UFC 100g^{-1})	0.4 - 42.5	5.0 ± 10.2	0.4 - 2.0	0.9 ± 0.4	0.5 - 42.5	10.4 ± 14.1

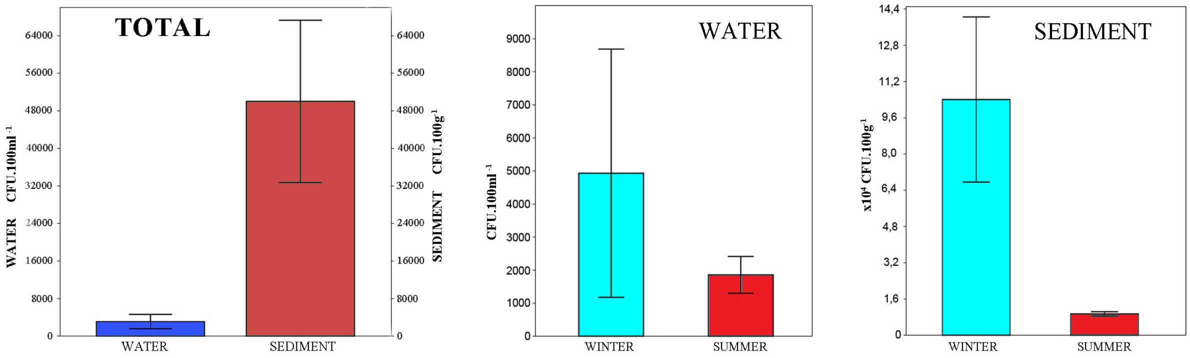

Figure 2. Mean filamentous fungus colony densities TOTAL - overall mean including the water and sediment samples; WATER (expressed as CFU 100mL^{-1}); SEDIMENT (expressed as x10^4 CFU 100g^{-1}); comparisons between the seasons.

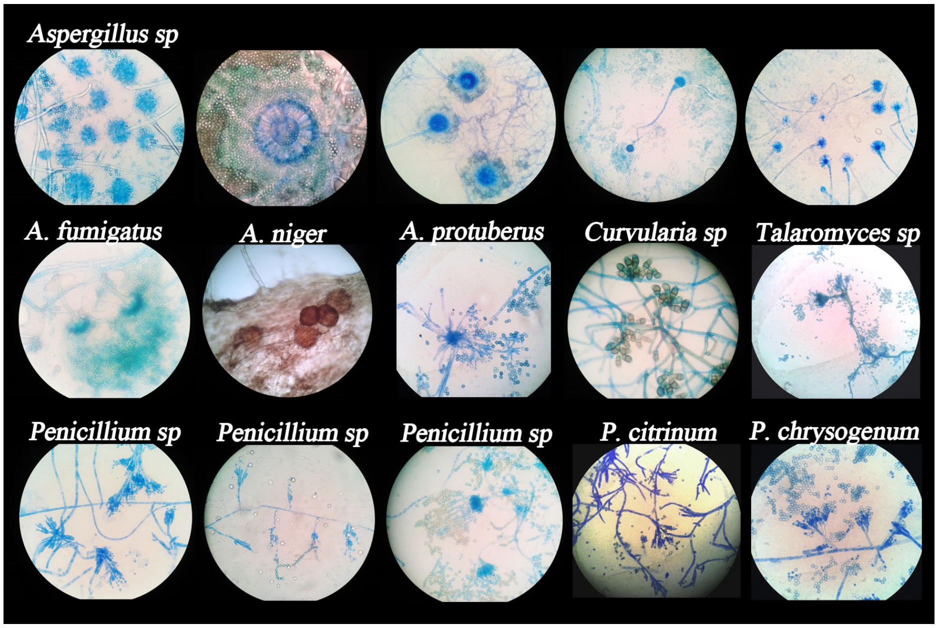

Figure 3. Isolated filamentous fungi observed microscopically.

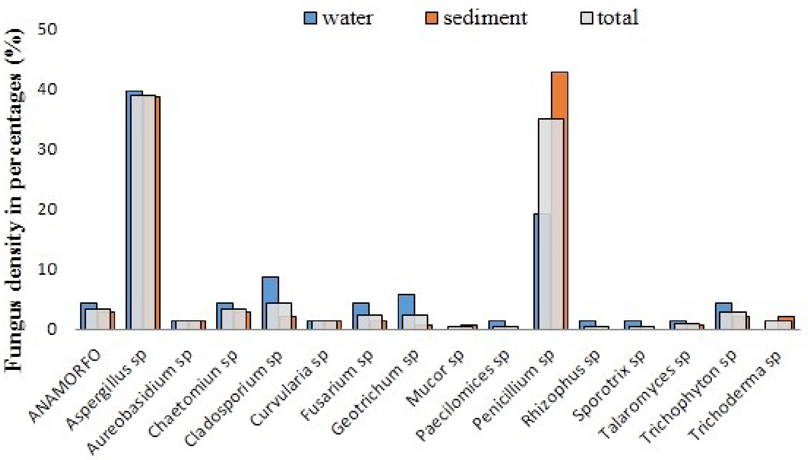

Figure 4. Percentage of filamentous fungus genera identified morphologically in samples isolated from water and sediment.

Most fungal species were observed in both materials sampled, but the genera *Paecilomices*, *Rhizophus*, and *Sporothrix* were found only in the water samples, and *Mucor* and *Trichoderma* were found only in the sediment samples.

From the colonies which had been clustered based on micromorphological characteristics, 20 fungus samples were selected for DNA extraction, amplification, and sequencing for molecular identification. Of these 20 samples, 7 had *Aspergillus* features, 11 had *Penicillium* features, and 2 had unique conidia. Based on the sequencing and GenBank results, 2 samples were identified as *Aspergillus fumigatus*, 2 samples were identified as *A. protuberus*, 1 sample was identified as *A. terreus*, 4 samples were identified as *Penicillium citrinum*, 5 samples were identified as *P. chrysogenum*, 1 sample was identified as *Talaromyces flavus*, 1 sample was identified as *Cladosporium* sp., 2 samples were identified as *Penicillium* sp., and 2 samples were identified as *Aspergillus* sp.

In the water, the genus *Aspergillus* was dominant in the winter, representing 54.5% of the specimens found, followed by *Penicillium* at 25.0%. In the summer, 20.8% of the specimens found in the water were determined to be from the genus *Cladosporium*, and 12.5% were found to be from the genus *Aspergillus*. In the sediment, the most commonly detected genera in the winter were *Penicillium* (47.2%) and *Aspergillus* (40.8%), and the most commonly detected genera in the summer sediment were *Aspergillus* (20.0%), *Cladosporium* (13.3%), and *Trichoderma* (13.3%). The genus *Aureobasidium* was detected in both the water and the sediment and only in the summer (Figure 5).

Similar results were reported by Gomes et al. (2008), who analyzed water with high salinity and alkaline pH from Casa Caiada Beach in Orlinda, Pernambuco State, Brazil. They isolated 50 species of fungi, the most common of which were from the genera *Aspergillus* and *Penicillium*. In another study by Gomes et al. (2001), *Penicillium* was the most diverse genus in the dry season sediment collected from the Barra das Jangadas swamp in Pernambuco State. Sengupta & Chaudhuri (1995) isolated fungi from the sediment and from an estuary of the Ganges River in India. The most commonly found genera were *Aspergillus* and *Penicillium*, though the salinity levels found were lower (11 to 15) than those detected in the current study.

Gomes et al. (2008) analyzed water and sediment from a beach in Olinda, Pernambuco, in both the dry season and the rainy season. They most frequently isolated and identified *Aspergillus*, *Penicillium*, and anamorphs, followed by other genera, including *Fusarium*, *Trichoderma*, *Cladosporium*, *Curvularia*, and *Paecilomyces*. Rai et al. (1981) isolated 87 saprobic fungi from mangrove swamp wood in India. The most common ascomycetes were from the genus *Chaetomium*, the most common anamorphs were from the genus *Aspergillus*, and other frequently isolated genera included *Trichoderma*, *Pestalotiopsis*, *Curvularia*, *Fusarium*, and *Penicillium*. In southern India, water samples, sediment samples, and some aquatic organisms were collected, and different *Aspergillus* species were identified. These included *Aspergillus flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. terreus*, and *A. terricola* (Babu et al. 2010).

Fungi from the genus *Cladosporium* are distributed worldwide and may be isolated from a variety of substrata, including water and sediment (Gomes et al. 2008), as well as from aquatic environments (Xiong et al. 2009, Silveira et al. 2013). Mbata (2008) isolated filamentous fungi from deep and highly saline marine waters. The species found included *Chaetomium globosum*, *Aspergillus versicolor*, *Hortaea wemeckii*, and *Aureobasidium pullulans*. *A. pullulans* is known to be distributed in marine environments with varying environmental conditions, and this species may be used in a variety of biotechnological applications (Liu et al. 2008) due to its ability to produce enzymes such as protease, lipase, and cellulase (Chi et al. 2009).

In Brazil, *Paecilomyces* fungi have been isolated from the sands of Ipanema Beach in Rio de Janeiro (Sarquis & Oliveira 1996), from the water and sediment on a beach in Olinda, Pernambuco (Gomes et al. 2008), and in the water from an estuary of the Patos Lagoon in the state of Rio Grande do Sul (Silveira et al. 2013). Richards et al. (2012) have described this genus as representing species of marine fungi, and Marante et al. (2012) isolated a new strain of *Paecilomyces variotti* from a marine environment, a strain which they determined was able to produce bioactive compounds.

Some of the fungi identified in the current study are known for being pathogenic. These include *Aspergillus fumigatus*, *A. terreus* (Martins et al. 2005, Walsh et al. 2008), *Penicillium chrysogenum* (Barcus et al. 2005), and

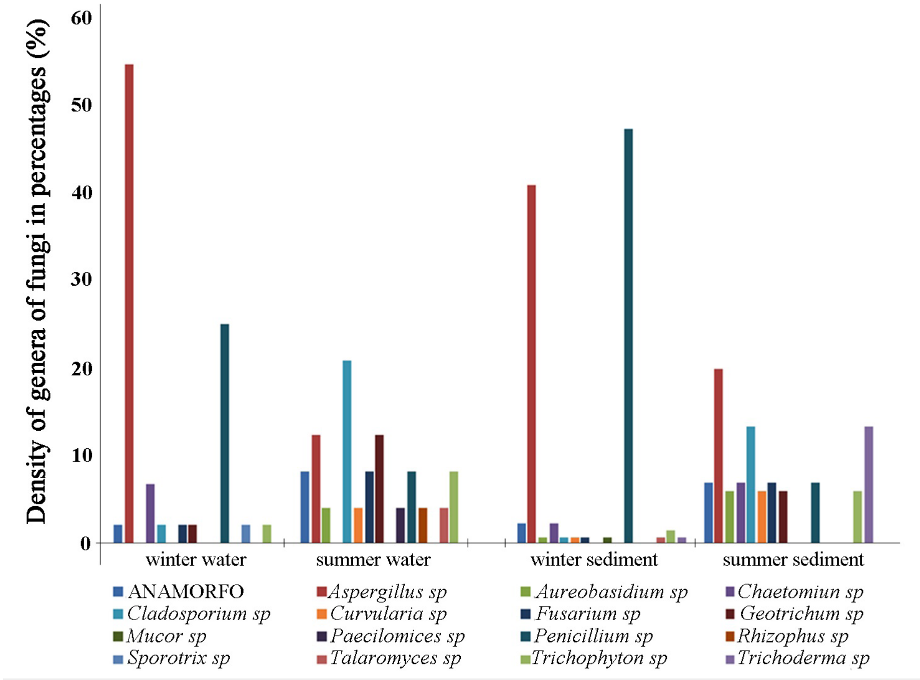

Figure 5. Filamentous fungus densities expressed as percentages (%) in water and sediment samples in the winter and summer.

Penicillium citrinum (Houbraken et al. 2010). Other studies have reported that some filamentous fungi are able to produce and assimilate enzymes for biotechnological applications. These genera include *Aspergillus* and *Penicillium*, which have proven to be important producers of enzymes such as cellulase (Zhang & Kim 2010, Castillo et al. 1994), xylanase (Zhang & Kim 2010, Shah & Madamwar 2005), lipase (Sharma et al. 2005, Zhu et al. 2009), and amylase (Prabakaran et al. 2009, Mishra & Dadhich 2010). The species *Aspergillus fumigatus* and *A. terreus* have been reported to have the potential to degrade hydrocarbon (Atlas 1981, Chaillan et al. 2004). *Penicillium chrysogenum* is able to produce amylase (Prabakaran et al. 2009, Silva et al. 2011) and alkaline protease (Zhu et al. 2009). Finally, *P. citrinum* has been found to produce both lipase (Silva et al. 2009) and cellulase (Santos et al. 2017).

In the current study, the greatest diversity was observed in the summer sediment (H'=2.4), followed by the summer water (H'=2.1), the winter sediment (H'=1.2), and finally, the winter water, in which the lowest diversity was detected (H'=0.9). According to Piedras et al. (2006), Shannon's diversity index may provide information on conservation and impacted environments. These authors also note that values between 1.0 and 3.0 reflect moderately polluted water, indicating that the region analyzed herein exhibits a good level of diversity relative to the environment. According to the Chao 1 estimator, the summer water sample exhibited the highest level of species richness among the samples (Chao 1=12). Though the summer sediment samples exhibited the highest level of diversity in this study, the water samples collected in the summer exhibited the highest amount of species richness. These findings indicate that analyses performed on summer samples may provide more information on these fungi.

Sebastianes et al. (2013) reported that the richness and diversity of endophytic fungi on leaves and branches in São Paulo swamps were higher in the summer than in the winter, results which are consistent with those of the current study. Maria & Shidhar (2003) also analyzed wood from costal mangrove swamp in southeast India and found more fungal diversity in the rainy season than in the dry season. In the study by Yadav et al. (2016), season had a significant influence over the diversity of endophytic fungi: the highest species diversity was detected during the rainy season, while the lowest was detected during the dry season. However, their data on fungal species diversity was not found to correlate with the number of isolates found in each season.

Fungi isolated from environments with extreme variations in temperature and salinity are likely to exhibit physiological adaptations that allow them to tolerate or even affect the availability of other substances in the environment (Damare et al. 2006, Stoeck et al. 2003). Studies have noted the potential of fungi from marine ecosystems to have biotechnological applications after evaluating fungal diversity and the role of fungi in the bioremediation of pollutants in contaminated environments (Gaylard et al. 2005, Passos et al. 2009, Pereira & Freitas 2012, Bonugli-Santos et al. 2015). Filamentous fungi have been found to be very effective in removing heavy metals from aqueous solutions due to their greater resistance to these elements (Kurek et al. 1982, Collins & Stotzky 1992).

Conclusion

The isolation of filamentous fungi from habitats such as mangrove swamps, which exhibit a complex variety of physical and chemical characteristics, can aid in the determination of their biological diversity and their ability to survive under adverse conditions. This knowledge may improve our understanding of the distribution of these microorganisms and their interactions with their ecosystems, as well as the human impact on these organisms, their role in conservation, and their use in a range of preservation and remediation processes. Many of the fungi found in this study may serve as potential indicators in biotechnological processes.

The preservation of these microbial communities is beneficial for biodiversity and is an important factor in sustaining ecosystems. Furthermore, studies on diversity may help us to clarify information on native microorganisms, on biological changes associated with environmental damage, and on how to protect plants specifically and ecosystems as a whole.

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Author Contributions

Sonia Assami Doi and Ana Julia Fernandes Cardoso de Oliveira: substantial contribution to the concept and design of the study; contribution to data collection, data analysis and interpretation; contribution to manuscript preparation and critical revision.

Aline Bartelochi Pinto: substantial contribution to the concept and design of the study: contribution to manuscript preparation and critical revision.

Maria Carolina Canali, Daiane Raquel Polezel and Roberta Alves Merguizo Chinellato: contribution to data collection, data analysis and interpretation.

Conflicts of interest

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

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Co-occurring morphologically distinct algae support a diverse associated fauna in the intertidal zone of Araçá Bay, Brazil

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Abstract: Species diversity is regulated by historical, neutral and niche processes, with species tolerance, dispersal and productivity guiding diversity at larger scales, while habitat heterogeneity and biotic interactions acts in smaller scales. In rocky shores, several organisms provide secondary substrates for mobile fauna, with macroalgae being the most abundant and diverse ones. The patchiness promoted by different macroalgae hosts enhances small-scale heterogeneity and may increase and maintain the diversity of the mobile organisms, since there is a close relationship between the associated fauna and its hosts. In this study we selected three morphologically different macroalgae that coexist in the same rocky shore height in the Araçá Bay, an area under the threat of the nearby harbor expansion, and evaluated the fauna associated to each algal host. Even under similar abiotic pressure (same rocky shore height), the associated fauna of each algal host varied in number and composition, revealing a close relationship. The poorly branched foliose *Ulva lactuca* sustained a lower density of organisms and was dominated by isopods, while the heavily branched turf and Bostrychietum community showed a high density of organisms, with a dominance of peracarid crustaceans and annelids on the turf and more resistant groups, such as bivalves, acaris and terrestrial insects on the Bostrychietum. Previous studies in the Araçá Bay already revealed a large spatial heterogeneity in the processes and sessile organisms distribution, and here we highlight that this heterogeneity can be observed in an even smaller scale, with different algal hosts mediating the turnover of species in a scale of centimeters and meters, resulting in diversity maintenance of the associated fauna. Since the harbor expansion may prevent the occurrence of macroalgae as a result of light limitation by suspended platforms, we may expect not only a decrease in algal cover but also in the total diversity of the associated fauna in the Araçá Bay.

Keywords: *rocky shore, phytal, peracarids, structural complexity, spatial heterogeneity*

Algas co-ocorrentes e morfologicamente distintas suportam uma diversa fauna associada na zona entremarés na Baía do Araçá, Brasil

Resumo: A diversidade de espécies é regulada por processos históricos, neutros e de nicho, com a tolerância das espécies, dispersão e produtividade do sistema guiando a diversidade em grandes escalas, enquanto a heterogeneidade do habitat e as interações bióticas atuam em escalas menores. Em costões rochosos uma série de organismos funciona como substrato secundário para a fauna móvel, sendo macroalgas o tipo mais abundante e diverso de substrato. O mosaico formado pelas diferentes macroalgas hospedeiras aumenta a heterogeneidade em pequena escala e pode aumentar e manter a diversidade dos organismos móveis, uma vez que existe uma relação próxima entre a fauna associada e seus hospedeiros. Neste estudo nós selecionamos três macroalgas diferentes morfologicamente e que coexistem na mesma altura no costão rochoso na Baía do Araçá, uma área sob a ameaça de expansão do porto que fica nas proximidades, e analisamos a fauna associada a cada alga hospedeira. Mesmo sob pressões abióticas similares (mesma altura no costão rochoso), a fauna associada a cada alga variou em número e composição, revelando de fato uma relação próxima entre fauna e alga. *Ulva lactuca*, uma alga foliosa com pouca ramificação, apresentou uma baixa densidade de organismos e foi dominada por isópodes, enquanto que o turf e o Bostrychietum, algas altamente ramificadas e mais complexas, apresentaram uma alta densidade de organismos, com uma dominância de crustáceos peracáridos e anelídeos no turf e grupos mais resistentes como bivalves, ácaros e insetos terrestres no Bostrychietum. Estudos anteriores conduzidos na Baía do Araçá já mostraram uma grande heterogeneidade espacial nos processos ecológicos e na distribuição dos organismos sésseis, e aqui nós ressaltamos que esta heterogeneidade pode ser observada numa escala ainda menor, com as diferentes algas mediando o turnover de espécies numa escala de centímetros a metros, resultando na manutenção da diversidade da fauna associada. Como a expansão do porto pode impedir a ocorrência das macroalgas devido à limitação de luz que será imposta pelas plataformas flutuantes, nós podemos esperar não apenas uma diminuição da cobertura de macroalgas mas também da diversidade total da fauna associada na Baía do Araçá.

Palavras-chave: *costão rochoso, fital, peracáridos, complexidade estrutural, heterogeneidade espacial*

Introduction

The diversity of species in an ecosystem is determined by historical, neutral and niche processes acting on both large and small spatial scales, which combined will determine the biodiversity patterns in a global scale (Ricklefs 1987, Huston 1999, Shurin & Allen 2001, Navarrete et al. 2005). While in large-scale, diversity is usually regulated by species tolerances, dispersal and productivity (Menge & Olson 1990, Whittaker et al. 2001, Hawkins et al. 2004), in small-scale, habitat heterogeneity and biotic interactions, such as predation, competition and facilitation seems to be the main drivers of diversity (Menge & Sutherland 1976, Menge & Olson 1990, Hewitt et al. 2005, Wiens 2011, Wisz et al. 2012). Although macroscale investigations on species diversity have been recognized as of major importance in a changing world (e.g. climate change: Brown et al. 2000, Harley et al. 2006; bioinvasion: Molnar et al. 2008), understanding how diversity is shaped in smaller scales may contribute to its maintenance, but is still poorly explored.

Rocky shores are dynamic coastal habitats under periodically tidal variation, and represent a transition zone between terrestrial and marine environments, which are under contrasting physical conditions (Ewel et al. 2001, Wall et al. 2001). As a result, organisms are distributed across the shore in horizontal bands according to its resistance to air exposure and competitive ability (Stephenson & Stephenson 1972, Chappuis et al. 2014). However, studies looking at the processes affecting the diversity of benthic communities in the intertidal zone showed that besides the well-known drivers of zonation, variation in biological interactions and habitat heterogeneity at a scale of few centimeters or meters may play also an important role in the patterns of diversity (McGuinness & Underwood 1986, Shurin & Allen 2001, Fraschetti et al. 2005, Le Hir & Hily 2005, Gingold et al. 2010, Valdivia et al. 2014).

A wide array of organisms acting as secondary substrate inhabit the rocky intertidal and this mosaic of hosts may help increasing diversity locally, since communities may change among the different hosts in a scale of a few meters (e.g. Parker et al. 2001, Kelly et al. 2008). Intertidal macroalgae are among the most abundant and diverse secondary substrate in rocky shores. They vary in color and shape, range from subtidal areas to the upper midlittoral, and can harbor diverse assemblages, providing both shelter and food for the associated fauna (Christie et al. 2009). The tridimensional complexity may create micro-habitats that protect associated organisms against predators (Duffy & Hay 1991), wave action (Sotka 2007) and desiccation (Davenport et al. 1999), and may also trap sediments (Airold & Virgilio 1998), which may favor deposit feeder species (Prathep et al. 2003). Besides, the alga itself and the epiphytic assemblage supported by it may be used as food by associated animals (Viejo 1999), and differences in diet preferences and algal palatability may favor different faunas (Duffy & Hay 1991, Zamzow et al. 2010, Tavares et al. 2013, Machado et al. 2017). Thus, variation in both algal host attributes, and associated fauna needs in terms of habitat and food, results on species-specific interactions and, consequently, on a taxonomic diversity of associated organisms among coexisting macroalgae in the shore (Engelen et al. 2013, Best et al. 2014). In this way, the spatial heterogeneity of hosts and the algal identity may play a crucial role in maintaining the biodiversity in intertidal areas, reinforcing the idea that conservation efforts should take into account different spatial scales (Thompson et al. 2002).

Coastal areas are among the ecosystems deeply affected by human activity, since most of the world's population inhabits coastlines (Dafforn et al. 2015). The introduction of man-made structures, such as harbors, piers, marinas and breakwaters (Bulleri & Chapman 2010, Mineur et al. 2012), and also the increased discharge of pollutants (Addison et al. 2008, Piola et al. 2009) are leading to a severe modification of coastal ecosystem functioning, mainly resulting in a loss of biodiversity (Airoldi et al. 2008, Magurran et al. 2015, Bianchelli et al. 2016). The Araçá Bay in the São Sebastião Chanel, São Paulo – Brazil, is an area under intense anthropogenic influence as a result of São Sebastião Harbor proximity, which leads to frequent sewage discharges and oil spills for decades. However, the area still holds a high biodiversity and has socio-economic importance for the local fisherman community that conduct artisanal fishery and shellfish harvesting in the area (Amaral et al. 2010). Recently, the Araçá Bay system has being threatened by the plan of expansion of São Sebastião Harbor (for details check Pardal-Souza et al. 2017), which would lead to irreversible damages to this ecosystem. Although the plan consists in covering the bay with suspended platforms, a recent study conducted in the area has shown that the limitation of light imposed by the platforms may lead to significant changes in the structure of sessile and mobile community, reducing the occurrence of macroalgae (Pardal-Souza et al. 2017).

Since heterogeneous micro-habitats can sustain a variable community, our goal was to verify if coexisting macroalgal hosts support different communities, contributing to the maintenance of biodiversity in the Araçá Bay. We selected three distinct macroalgae occurring at the same zone in the shore and compared the associated community to understand the importance of the algal identity on setting the patterns of diversity of associated fauna. We expected to find distinct patterns of abundance of organisms, richness, evenness and diversity of groups, and community structures among hosts, reinforcing the idea that heterogeneity at small scales may help maintaining biodiversity in intertidal systems.

Material and Methods

1. Study Area

Sampling was conducted in the rocky shores of Pernambuco Island (23º48'54.9"S, 45º24'24.6"W), inside the Araçá Bay in the São Sebastião Chanel, São Paulo – Brazil (for details check Amaral et al. 2010, Dias et al. in press). The Araçá Bay is a shallow bay that includes sandy and rocky shores, mud flats and mangroves (Dias et al. in press), sustaining a high biodiversity (Amaral et al. 2010). The rocky shore is composed by both platforms and variable sized boulders, which creates a heterogeneous and complex habitat (Amaral et al. 2016).

2. Sampling Procedure

To evaluate how the identity of co-occurring macroalgae can affect the associated fauna living on it, we selected three distinct algae: *Ulva lactuca* (Linnaeus 1753) – an ephemeral green alga with poorly branched foliose fronds; Bostrychietum community – a group of red algae with heavily branched fronds, dominated by *Bostrychia* sp. (Montagne 1842) and that normally occurs on the upper portions of the intertidal zone (García et al. 2016); algal turf – dominated by the red filamentous algae *Gracilaria* sp. (Greville 1830) that mostly occurs in the intertidal fringe.

The samples consisted of 30 cores (10 for each alga) with an area of 36 cm^2 scraped along 20 m of a uniform rocky shore during a spring tide in July 2013. Bostrychietum beds extend from the supralittoral to the midlittoral while *Ulva* and turf beds extends from the midlittoral to the sublittoral fringe, so any difference in the associated fauna could be the result of habitat-specific traits or caused by the height of the algae on the shore. Because we were interested in the differences among associated fauna caused by the identity and the complexity created by secondary substrata, and not by differences in shore height, all samples were randomly collected in the midlittoral, where species distributions overlapped. Scraped samples were individually kept in alcohol 70% and then, in the laboratory, all organisms were separated and classified in major taxonomic groups. After separating the organisms, we quantified the volume of each sample in order to standardize the variables measured; however, since the volume did not differ among the three different algae ($F_{2,27} = 1.60$; $p = 0.221$), we decided to use the raw data, with no volume standardization.

3. Data Analyses

We compared the richness of taxonomic groups, evenness, Shannon diversity and total abundance of organisms with one-way ANOVA considering alga identity as a fixed factor. In order to achieve normality and homoscedasticity, abundance data was log transformed. For significant effects, we performed Tukey's test *a posteriori* for pairwise comparisons. Additionally, we performed separate analysis comparing the abundance of the most abundant groups among the algal species using the same ANOVA model.

To evaluate how the algal identity affects the structure of the associated fauna community, we used the abundances of all taxonomic groups in a multivariate approach, building a resemblance matrix using Bray-Curtis distance. The resemblance matrix was used in a PERMANOVA test with 999 unrestricted permutations of the raw data (Anderson 2001) using the same model explained above, and to build an n-MDS plot to better visualize groups relationship (Clark 1993). For significant factors, we performed a pairwise comparison (Anderson 2001) and then a SIMPER test to obtain the groups that most contributed to such significant effects (Clark 1993).

Results

We recorded 13,689 individuals of the following taxonomic groups: Amphipoda, Tanaidacea, Isopoda, Ostracoda, Cirripedia, Copepoda, Bivalvia, Gastropoda, Hydrozoa, Polychaeta, Oligochaeta, Nematoda, Rhabditophora, Ascidiacea, Bryozoa, Acari, Pycnogonida, Collembola, Diptera, Coleoptera and Lepidoptera. Amphipoda was the most abundant group, with 135±43 individuals per sample, followed by Bivalvia, with 85±18 individuals per sample, and Acari, with 60±22 individuals per sample.

Algal identity did not affect richness of groups, evenness and Shannon diversity, but influenced total abundance, with more individuals being observed on turf and Bostrychietum when compared to *Ulva* (Figure 1, Table 1). Each algal species also showed a specific community structure for the associated fauna, clustering separately in the n-MDS plot (Figure 2, Table 1). The groups that most contributed to the differences among algal species were Amphipoda, Tanaidacea, Oligochaeta and Polychaeta, more abundant on turf; Isopoda, more abundant on turf and *Ulva*; Nematoda, with similar abundance on the three algae; Bivalvia, more abundant on turf and Bostrychietum; and Acari and Diptera, more abundant on Bostrychietum (Figure 3, Table 2 and 3).

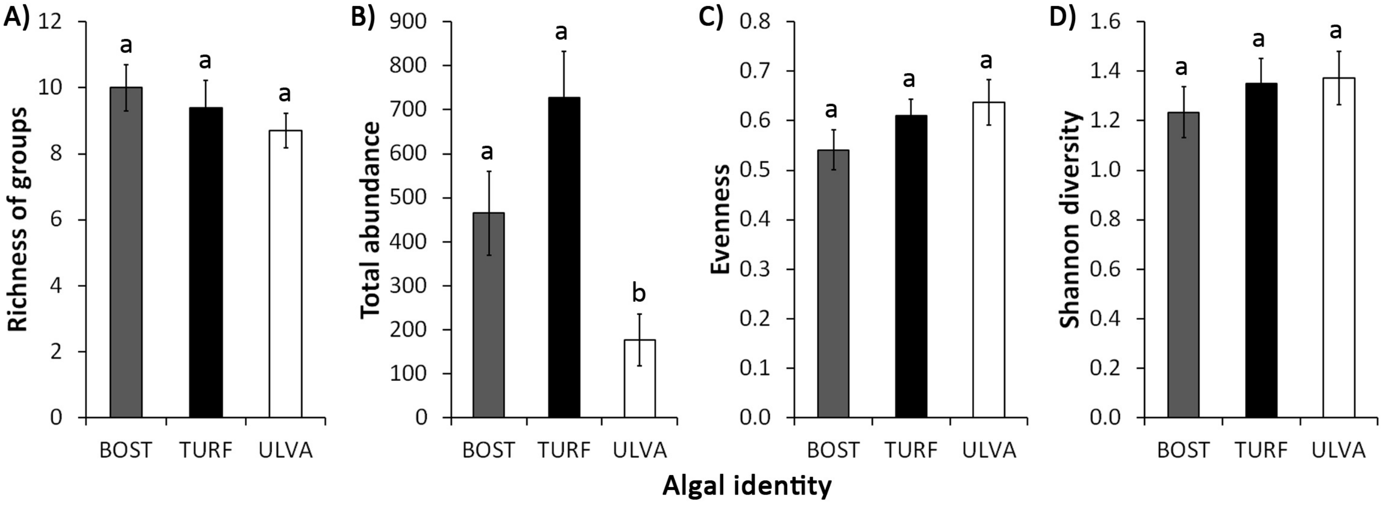

Figure 1. Mean (±SE) A) richness of groups, B) total abundance, C) evenness and D) Shannon diversity of faunal community associated to Bostrychietum (BOST), algal turf (TURF) and *Ulva lactuca* (ULVA) in the intertidal fringe of Araçá Bay. For each variable, differences between algae sharing a single letter are not significant ($p > 0.05$).

Table 1. Summary results of ANOVAs for richness of groups, total abundance, evenness and Shannon diversity, and PERMANOVA for community structure, comparing these attributes of associated fauna among Bostrychietum, algal turf and *Ulva lactuca*. 'ns' – non-significant; '***' – $p < 0.001$.

Source	*Richness of Groups*				*Total Abundance*			*Evenness*			*Shannon Diversity*			*Community Structure*		
	DF	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*Pseudo-F*	*P*
Algal Identity	2	4.23	0.89	ns	6.25	13.8	***	0.024	1.5	ns	0.055	0.5	ns	8733	12.6	***
Error	27	4.76			0.45			0.016			0.107			695		

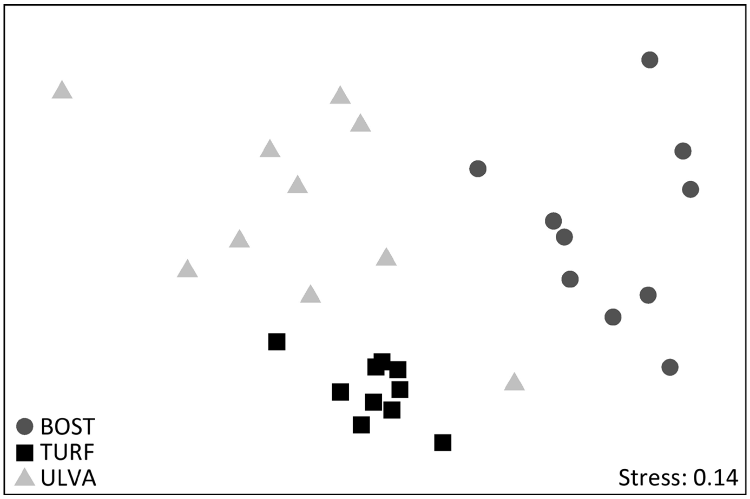

Figure 2. nMDS plot of abundance of all taxonomic groups of faunal communities associated to Bostrychietum (BOST – dark gray circles), algal turf (TURF – black squares) and *Ulva lactuca* (ULVA – light gray triangles) in the intertidal fringe of Araçá Bay.

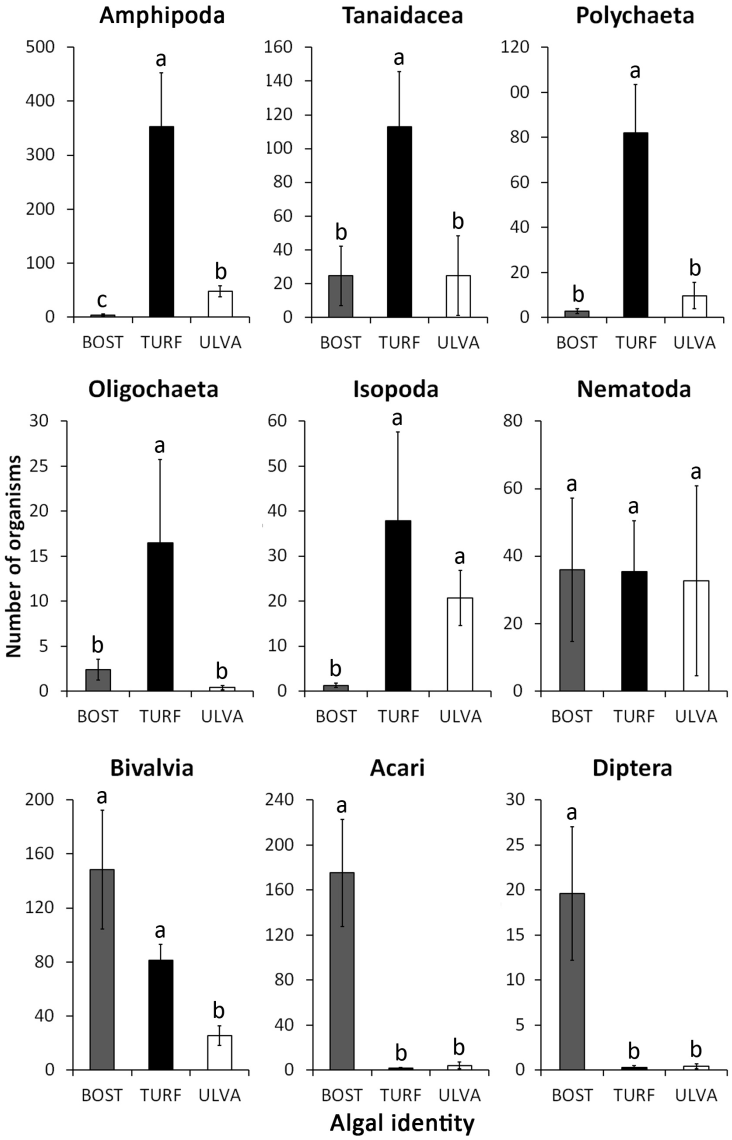

Figure 3. Mean abundance (±SE) of the most abundant groups of faunal communities associated to Bostrychietum (BOST – dark gray), algal turf (TURF – black) and *Ulva lactuca* (ULVA – light gray) in the intertidal fringe of Araçá Bay. For each taxonomic group, differences between algae sharing a single letter are not significant ($p > 0.05$).

Table 2. SIMPER results showing the relative contribution (%) of the five taxonomic groups that most contributed to significant differences among associated fauna of Bostrychietum (B), algal turf (T) and *Ulva lactuca* (U). Abbreviations in front of each taxa stand for the alga in which the group was more abundant.

B x T		***B x U***		***T x U***	
Group	***(%)***	***Group***	***(%)***	***Group***	***(%)***
Amphipoda (T)	15.16	Acari (B)	16.61	Tanaidacea (T)	17.48
Acari (B)	14.95	Amphipoda (U)	11.70	Polychaeta (T)	12.55
Polychaeta (T)	11.43	Diptera (B)	8.62	Nematoda (T)	9.66
Tanaidacea (T)	10.34	Bivalvia (B)	8.41	Amphipoda (T)	8.75
Diptera (B)	7.62	Isopoda (U)	8.26	Oligochaeta (T)	8.42

Table 3. Summary results of ANOVAs comparing the abundance of the most abundant groups of associated fauna among Bostrychietum, algal turf and *Ulva lactuca*. 'ns' – non-significant; '**' – $p < 0.01$; '***' – $p < 0.001$.

Source	***Amphipoda***				***Tanaidacea***			***Isopoda***		
	DF	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Algal Identity	2	8.87	35.8	***	5.77	12.98	***	2.19	7.13	**
Error	27	0.25			0.45			0.31		
Source	***Oligochaeta***				***Polychaeta***			***Nematoda***		
	DF	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Algal Identity	2	1.49	7.50	**	5.48	26.90	***	0.74	1.57	ns
Error	27	0.20			0.20			0.47		
Source	***Bivalvia***				***Acari***			***Diptera***		
	DF	*MS*	*F*	*P*	*MS*	*F*	*P*	*MS*	*F*	*P*
Algal Identity	2	1.83	10.06	***	10.30	54.80	***	2.91	21.55	***
Error	27	0.18			0.19			0.14		

Discussion

We show that even under similar abiotic pressure (same rocky shore height), different coexisting algae influenced the fauna associated to them. Although we did not observe the effect of algal identity on all community metrics, the abundance of organisms and the community structure were particular for each algal host, showing a strong taxon-specificity between host and associated fauna, in a way that the coexisting algae increase the turnover and total diversity of animals in the Araçá Bay. Besides, more complex algae (Bostrychietum and turf) sustained higher abundances, reinforcing the idea that complexity creates an array of micro-habitats in different scales that supports more individuals.

Habitat complexity has been recognized as of major importance on characterizing the spatial distribution of species from intertidal areas (Beck 1998, 2000). Complex substrates are expected to provide several resources, allowing the maintenance of more abundant and diverse assemblages with different survival requirements (e.g. Hicks 1977). Within the macroalgae, higher branching can support higher faunal abundances by supplying additional surface for attachment (Hacker & Steneck 1990) or by offering higher interstitial volume (Bueno et al. 2017). In our study, the highly branched turf and Bostrychietum hosted higher abundances of faunal groups, while the smooth sheet-like blades of *Ulva* supported less individuals, both accounting for the total as well as for the most abundant groups (Amphipoda, Bivalvia and Acari). The lower number of individuals on *Ulva* may be an effect of the reduced surface rugosity, demanding much effort for animals to keep anchored, as observed for amphipods (Hacker & Steneck 1990). Besides, it does not provide as many refuges as intricate canopies (Zamzow et al. 2010), resulting in a very specific fauna inhabiting *Ulva* blades (Corte et al. 2012). Habitat selection on macroalgae can be size-dependent (Hacker and Steneck 1990), and the spaces between the overlapping blades of *Ulva* could be sufficient as shelter from predation for small, but not for large species (Holmlund et al. 1990).

The turf, mainly composed by *Gracilaria* in our study, hosted the highest abundances of most faunal groups, especially the peracarid crustaceans (Amphipoda, Tanaidacea and Isopoda) and annelids (Polychaeta and Oligochaeta). In shores from the Northwestern Atlantic Ocean, patches of *Gracilaria vermiculophylla* (Papenfuss 1967) are effective shelters for the amphipod *Gammarus mucronatus* (Say 1818) regarding both desiccation and predation risks (Wright et al. 2014). Turfs intricate canopy can retain water and ameliorate conditions during periods of air exposure (Bertness et al. 1999), enhancing the survivorship of *G. mucronatus* during low tides (Wright et al. 2014). During high tides, *G. mucronatus* on algal turf, similar to the studied here, were less prone to be detected by predators (Wright et al. 2014). We believe that such algal attributes would work for most of the groups found on the algal turf in Araçá Bay, representing then a suitable refuge from biotic and abiotic pressures.

Algae belonging to the Bostrychietum community are frequently found on upper intertidal areas on both rocky shores and mangroves. Since this region is under high abiotic stress (Connell 1972), it is expected that Bostrychietum species living in such region show adaptations to deal with emersion periods, as observed to *Bostrcyhia radicans* (Montagne 1842), which sustained maximum photosynthetic rates, constant respiratory rates and a fast recovery after relatively long periods of desiccation (Mann & Steinke, 1988). Since the spatial distribution of macroalgae associated fauna can be related to host identity and vertical position on the shore (Bueno et al. 2017), faunal assemblages could develop a close relation with their host and occupy different levels on the shore. Mites (Acari), for example, use Bostrychietum as habitat and source of food and their spatial distribution on rocky shores is closely related to the host position (Pfingstl 2013). As previously observed for Bostrychietum covering mangrove pneumatophores in the same area (García et al. 2016), the rocky shore Bostrychietum in our study showed the presence of more resistant organisms like bivalves, acaris, and typically terrestrial animals, such as insects. The occurrence of these organisms in the lower limit of

Bostrychietum bed in the midlittoral zone may reflect a close algal-fauna association and, in turn, a side-effect of insects using the algae as a proxy of the height in the shore.

While the turf and Bostrychietum are more perennial algae, *Ulva* is an ephemeral species that commonly increases the occurrence or abundance after nutrient input (Chávez-Sánchez et al. 2017, Dias et al. in press). The variation in abundance and occurrence of ephemeral algal species may contribute to promote temporal heterogeneity that was not explored here. The temporal variability of host coupled with the spatial heterogeneity imposed by perennial hosts may enhance diversity maintenance (Chesson 2000). Although we do not know if the differences of associated fauna composition observed among the three algal hosts sampled here will be maintained through time, the alternation between higher discrepancy and higher similarity among the associated fauna of different algal hosts will also impose temporal variation and does contribute to heterogeneity in such scale, maintaining diversity (Menge & Sutherland 1976).

Benthic communities in the entrance of the Araçá Bay are more dynamic and controlled by oceanic processes operating at the São Sebastião Channel, while those at the inner portion of the bay are under the influence of Mãe Isabel River (Corte et al. 2017, Gorman et al. 2017, Dias et al. in press). As a consequence of this variable influence, the bay becomes shallower and richer in organic matter (Corte et al. 2017), and also receives a higher nitrogen input (Gorman et al. 2017) towards the inner portion. This environmental gradient spatially affects population dynamic of barnacles (Dias et al. in press) and also the community structure of sessile species in spatial and temporal scales (Kitazawa et al. *in prep.*). Here we show that the heterogeneity in the Araçá Bay is also observed in an even smaller scale, being mediated by the patchiness promoted by different algal hosts to the associated fauna. While the maintenance of such small-scale heterogeneity is important to guarantee refugees, acting as reservoirs to rare species (Hewitt et al. 2005, 2010), the maintenance of variation in a larger scale is also crucial, since local diversity does not depend solely on ecological processes, but is linked to the regional pool of species in marine communities, which highlights the importance of protecting larger areas (Witman et al. 2004) such as the Araçá Bay.

Our results corroborate the idea that community organization, in terms of abundance and community structure here, can vary in a considerable small-scale in the intertidal system (algal patches within the same rocky shore height) and, in our study, this is related to the heterogeneity promoted by different algal host holding specific associated faunas. Although we did not observe differences of richness, evenness and Shannon diversity, we believe that increasing the taxonomic resolution would result in differences in such metrics too. This is crucial to diversity maintenance not only in the local scale (among patches), but also in larger scales (regional diversity), with broad implications to conservation, especially today with the anthropogenic threat that most coastal systems have been facing, such as the Araçá Bay. The expansion of São Sebastião Harbor may lead not only to a homogenization of conditions, which may result on the survivor of only few resistant species, but will also preclude algae survivorship through light limitation caused by the suspended platforms (Pardal-Souza et al. 2017). As a consequence, following the decrease of diversity of macroalgae species, we may also expect a great loss of diversity of associated organisms, since they are closely related to their hosts.

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Authors' Contributions

HRF and GMD substantially contributed in the concept and design of the study and to data collection.

EAV, HRF and GMD contributed to data analysis and interpretation.

EAV, HRF, MB, FPPL and GMD contributed 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.

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Fauna of mosquitoes (Diptera: Culicidae) in Goytacazes National Forest and surrounding area, State of Espírito Santo, Southeastern Brazil

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Abstract: Mosquitoes comprehend a group with a major impact on public health, because some species transmit parasites vertebrate hosts. Comparative studies between preserved environment and disturbed areas provide important epidemiological information, due to the major knowledge on species populational dynamics and the possibility of a contact between vector species and human population. This work aims to characterize and compare the fauna of mosquitoes in the Goytacazes National Forest (GNF) and surrounding area, in the State of Espírito Santo, in Brazil. Collections occurred simultaneously in a preserved environment and in a peridomicile, from 15:00 to 18:00, between July 2008 and May 2009. In each environment, it was used a modified trap of Shannon type. As a result, 1,490 specimens from 14 genera and 19 identified species were collected. The species diversity (H = 1.95), species richness (S = 17), equitability index (J = 0.68) and the number of collected specimens (n = 1,100) were higher in the forest environment. The plentiful species were respectively *Aedes* (*Ochlerotatus*) *scapularis* (Rondani, 1848), *Culex* (*Culex*) *nigripalpus* Theobald, 1901 and *Aedes* (*Ochlerotatus*) *fulvus* (Wiedemann, 1828). Our results demonstrate that the occurrence of vector species in this region increases the potential risk of diverse arboviruses occurrence, especially wild-type yellow fever.

Keywords: *Atlantic Forest, Disease Vector, Diversity, Richness, Yellow Fever.*

Fauna de mosquitos (Diptera: Culicidae) na Floresta Nacional de Goytacazes e área adjacente, estado do Espírito Santo, sudeste do Brasil

Resumo: Os mosquitos compreendem um grupo importante para a saúde pública, porque algumas espécies estão envolvidas na transmissão de várias doenças para os seres humanos. Estudos comparativos entre ambiente florestal e áreas perturbadas fornecem importantes informações epidemiológicas, dado o maior conhecimento da dinâmica populacional das espécies e a possibilidade de contato entre as espécies de vetores e população humana. O objetivo deste estudo foi caracterizar e comparar a fauna de mosquitos na Floresta Nacional de Goytacazes e área adjacente, no estado do Espírito Santo, Brasil. As coletas ocorreram simultaneamente no ambiente florestal e peridomicílio, de 15:00 às 18:00 horas, entre junho de 2008 e maio de 2009. Em cada ambiente foi utilizada uma armadilha do tipo Shannon modificada. Como resultado, foram coletados 1.490 espécimes pertencentes a 14 gêneros e 19 espécies identificadas. A diversidade de espécies (H = 1,95), riqueza de espécies (S = 17), índice de equitabilidade (J = 0,68) e o número de espécimes coletados (n = 1.100) foram maiores no ambiente florestal. As espécies mais abundantes foram respectivamente *Aedes* (*Ochlerotatus*) *scapularis* (Rondani, 1848), *Culex* (*Culex*) *nigripalpus* Theobald, 1901 and *Aedes* (*Ochlerotatus*) *fulvus* (Wiedemann, 1828). Os resultados demonstram que a ocorrência de espécies vetores na região, aumenta o risco potencial de ocorrência de diversas arboviroses, com destaque para a febre amarela silvestre.

Palavras-chave: *Mata Atlântica, Vetores de doenças, Diversidade, Riqueza, Febre Amarela.*

Introduction

The Atlantic Forest is one of the world's biodiversity hotspots (Myers et al. 2000, Orme et al. 2005). The environmental degradation suffered by this biome has caused changes in the natural landscape, with consequent alteration of the fauna and population dynamics of different living organisms, including insects such as mosquitoes and sandflies (Virgens et al. 2008, 2015).

Mosquitoes (Diptera: Culicidae) consist of a group with high impact to public health, because some species are involved in the transmission of pathogens responsible for human diseases, such as dengue, zika, chikungunya, yellow fever, filariasis and malaria (Consoli & Lourenço-de-Oliveira 1994, Forattini 2002, Wilkerson et al. 2015). The association between mosquitoes and these diseases has stimulated researches on the fauna, geographic distribution and ecology of these insects, especially in areas with potential risk of pathogen transmission.

In the State of Espírito Santo, the few existing studies on the fauna characterization and diversity of mosquitoes are directed to Anophelinae subfamily and areas of potential risk of malaria (Andrade & Brandão 1957, Deane et al. 1968, Natal et al. 2007, Meneguzzi et al. 2009, Rezende et al. 2009a, b, 2013, Silva et al. 2013). In the mountainous region of Espírito Santo, where sporadic cases of malaria are notified, studies have incriminated *Anopheles* (*Kerteszia*) *cruzii* Dyar & Knab, 1908 as the main vector of *Plasmodium vivax* (Grassi & Feletti, 1890) (Rezende et al. 2009b, 2013). In the flat region, it is possible incriminating *Anopheles* (*Nyssorhynchus*) *darling* Root, 1926 as a vector in imported malaria cases (Meneguzzi et al. 2009, Silva et al. 2013). In addition, there are several areas with potential occurrence of *An.* (*Nys.*) *darlingi* in the State of Espírito Santo, which deserve additional studies to understand the *Anopheles* Meigen, 1818 fauna (Meneguzzi et al. 2009).

Regarding Culicinae subfamily, studies mainly have focus in *Aedes* (*Stegomyia*) *aegypti* (Linnaeus, 1762) (Varejão et al. 2005, Mendonça et al. 2011, Santos et al. 2011) and the knowledge on the occurrence of species belonging to this subfamily in wild environment is scarce (Deane et al. 1968, Alencar et al. 2004, Rezende et al. 2011). Therefore, studies on the Culicidae fauna in Espírito Santo wild areas, mainly in flat areas, should be encouraged.

Considering the epidemiological importance of mosquitoes (Diptera: Culicidae) and the lack of invertigations in the region, this study aims to characterize and compare the diurnal fauna of mosquitoes in the Goytacazes National Forest (GNF) and surrounding area, in the State of Espírito Santo, Brazil.

Material and Methods

1. Study Area

Samples in two ecologically distinct environments were collected: 1) forest environment represented by the GNF and 2) GNF adjacent anthropic environment, represented by peridomicile occupied in general by cocoa crops grown in *cabruca* agroforest system, where cacao trees are planted under thinned-out native forests, and presenting human habitations with the presence of attached livestock (pigsty, chicken coop and barn).

The GNF (19º 28' 01" S; 40° 04' 18" W; elevation of 10 meters [a.s.l]) is a unit of federal conservation, linked to the Chico Mendes Institute for Biodiversity Conservation, which granted permission to carry out this research. The GNF is located in Linhares, Espírito Santo, Southeast Brazil (Figure 1). It has an estimated area of 1,423 hectares located between the Doce River and the Federal Highway BR 101, lying about 1 kilometer from Linhares downtown.

The GNF is located about 30 km from the Vale Natural Reserve and 50 km from the Sooretama Biological Reserve, representing the last remaining Atlantic Forest of the Doce river alluvial floodplain (Rolim et al. 2006). The region has a tropical monsoon climate, according to Köppen-Geiger's climatic classification (Peel et al. 2007). Average temperatures range from 11 to 18°C in the coldest months (June to August) and 30-34°C in the warmer months (December to March), with about 1,200 mm of annual rainfall (Hijmans et al. 2005).

2. Sampling

The collections simultaneously occurred in the forest and in the peridomicile, between June 2008 and May 2009. In each environment, it was used a modified Shannon trap (Shannon, 1939), of dimensions

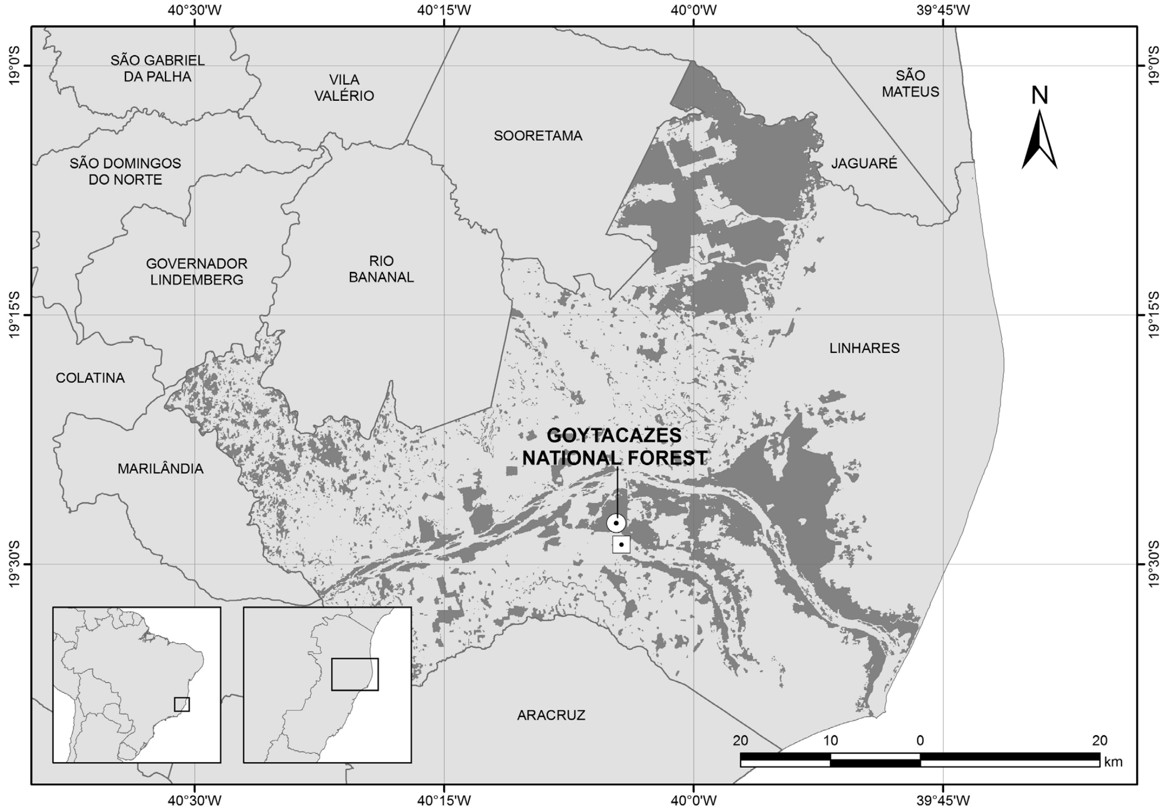

Figure 1. Collection sites (square – peridomiciliary environment; circle – forest environment represented of the Goytacazes National Forest) in the Atlantic Forest, State of Espírito Santo, Southeastern Brazil.

1 m X 1 m X 1.9 m (topped with an overhanging square roof of side 1.6 m) (Ferreira et al. 2001). The culicids were collected using a Castro manual suction catcher and tubes impregnated with ethyl acetate. These samples were collected once (one day) a month, from 15:00 to 18:00, with a total exposure of 36 hours in each environment.

The collected specimens were sent to the Parasitology Laboratory at the Federal University of Espírito Santo (UFES), and the morphological identification followed the taxonomic criteria proposed by Consoli & Lourenço-de-Oliveira (1994), Forattini (2002), and Marcondes (2011). The abbreviations of genera and subgenera followed Reinert (2001). A sample of the collected material was sent to the Entomology Laboratory at the Public Health School of the University of São Paulo, to confirm the species identification. Lastly, we have deposited the collected material in the entomological collection of Tropical Medicine Unit at UFES.

3. Climatic data

Climate precipitation data (mm^3), average temperature (°C) and relative humidity (%) were obtained from the National Institute of Meteorology Station, located in the Capixaba Institute of Research, Technical Assistance and Rural Extension (INCAPER), in Linhares, about 8 kilometers of distance from the studied area.

4. Statistical analysis

The specific richness (S), evenness indexes (J), and Shannon diversity indexes (H) were evaluated for each environment using PAST 2.09 software (Hammer et al. 2001). In addition, it was used a Biostat 5.0 software to evaluate the difference between the number of specimens collected in both environments by Mann-Whitney statistical test and the association between the climate data and the most abundant species in each environment by Spearman nonparametric test. Differences were considered significant when the probability (p) of error was less than 5% ($p < 0.05$). It was only performed the analysis with specimens identified into specific level.

Results

A total of 1,490 culicids were collected (S = 20, J = 0.58, H = 1.74), in which 1,483 specimens (99.5%) belonging to the subfamily Culicinae and seven specimens (0.5%) belonging to the subfamily Anophelinae. The Aedini and Sabethini tribes represented 76.0% of the total, with 604 and 529 specimens, respectively (Table 1). The most abundant genera were *Aedes* Meigen, 1818 (37.1%), *Trichoprosopon* Theobald, 1901 (28.0%) and *Culex* Linnaeus, 1758 (17.2%). These collected specimens belong to 14 genera and 19 identified species (Table 1).

The most abundant species were *Aedes* (*Ochlerotatus*) *scapularis* (Rondani, 1848), with 408 specimens (27.3%), *Culex* (*Culex*) *nigripalpus* Theobald, 1901 with 230 specimens (15.4%), and *Aedes* (*Ochlerotatus*) *fulvus* (Wiedemann, 1828), with 123 specimens (8.2%). *Aedes* (*Ochlerotatus*) *scapularis* and *Cx.* (*Cux.*) *nigripalpus* were the most abundant species in both studied environments and there was no significant difference in the numbers of specimens between these two environments. The species diversity (H = 1.95), species richness (S = 17), equitability index (J = 0.68) and the number of collected specimens (n = 1,100) were higher in the forest environment.

The species, *Aedes* (*Ochlerotatus*) *taeniorhynchus* (Wiedemann, 1821), *Anopheles* (*Nyssorhynchus*) *evansae* (Brèthes, 1926), *Haemagogus capricornii/janthinomys, Haemagogus* (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924), *Psorophora* (*Janthinosoma*) *ferox* (Humboldt, 1819) and *Trichoprosopon digitatum* (Rondani, 1848) were collected exclusively in the forest environment, while *Anopheles* (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga, 1878, *Anopheles* (*Nyssorhynchus*) *argyritarsis* Robineau-Desvoidy, 1827, *Coquillettidia* (*Rhynchotaenia*) *chrysonotum* (Peryassú, 1922), *Coquillettidia* (*Rhynchotaenia*) *venezuelensis* (Theobald, 1912) and *Mansonia* (*Mansonia*) *humeralis* Dyar & Knab, 1916 were collected only in the peridomicile.

In the forest environment, *Ae.* (*Och.*) *scapularis* showed a positive and significant correlation with the monthly precipitation (R = 0.84; P = 0.00) and monthly average temperature (R = 0.62; P = 0.02). In the peridomicile,

Table 1. Number of mosquitoes (Diptera, Culicidae) collected in Shannon traps between June 2008 to May 2009 and ecological indices from two environments (F = Forest; P = Peridomicile) at Goytacazes National Forest, municipality of Linhares, State of Espírito Santo, southeastern Brazil. (T = Total)

	Number		
	F	**P**	**T**
Subfamily Anophelinae	3	4	7
Genus *Anopheles*			
Anopheles (*Nyssorhynchus*) *albitarsis* Lynch-Arribalzaga, 1878	-	2	2
Anopheles (*Nyssorhynchus*) *argyritarsis* Robineau-Desvoidy, 1827	-	1	1
Anopheles (*Nyssorhynchus*) *evansae* (Brèthes, 1926)	1	-	1
Anopheles (*Nyssorhynchus*) *oswaldoi* (Peryassú, 1922)	2	1	3
Subfamily Culicinae	1097	386	1483
Tribe Aedini	375	229	604
Genus *Aedes*			
Aedes (*Ochlerotatus*) *fulvus* (Wiedemann, 1828)	111	12	123
Aedes (*Ochlerotatus*) *scapularis* (Rondani, 1848)	192	216	408
Aedes (*Ochlerotatus*) *taeniorhynchus* (Wiedemann, 1821)	11	-	11
Aedes spp. *	11	-	11
Genus *Haemagogus*			
Haemagogus (*Conopostegus*) *leucocelaenus* (Dyar & Shannon, 1924)	3	-	3
Haemagogus (*Haemagogus*) *capricornii/janthinomys*	3	-	3
Haemagogus (*Haemagogus*) *spegazzinii* Brèthes, 1912	1	1	2
Genus *Psorophora*			
Psorophora (*Janthinosoma*) *ferox* (Humboldt, 1819)	28	-	28
Psorophora spp. *	15	-	15

* Refers to damaged specimens, not being possible identified to species level.

Table 1. Continued...

	Number		
	F	**P**	**T**
Tribe Culicini	141	111	252
Genus *Culex*			
Culex (*Culex*) *nigripalpus* Theobald, 1901	125	105	230
Culex spp. *	16	6	22
Tribe Mansoniini	61	36	97
Genus *Coquillettidia*			
Coquillettidia (*Rhynchotaenia*) *chrysonotum* (Peryassú, 1922)	-	5	5
Coquillettidia (*Rhynchotaenia*) *shannoni* (Lane & Antunes, 1937)	30	6	36
Coquillettidia (*Rhynchotaenia*) *venezuelensis* (Theobald, 1912)	-	3	3
Genus *Mansonia*			
Mansonia (*Mansonia*) *humeralis* Dyar & Knab, 1916	-	7	7
Mansonia (*Mansonia*) *titillans* (Walker, 1848)	27	15	42
Mansonia spp. *	4	-	4
Tribe Sabethini	519	10	529
Genus *Johnbelkinia*			
Johnbelkinia sp. *	1	-	1
Genus *Limatus*			
Limatus durhami Theobald, 1901	53	7	60
Limatus sp. *	-	1	1
Genus *Runchomyia*			
Runchomyia spp. *	22	-	22
Genus *Sabethes*			
Sabethes sp. *	1	-	1
Genus *Trichoprosopon*			
Trichoprosopon digitatum (Rondani, 1848)	21	-	21
Trichoprosopon spp. *	394	2	396
Genus *Wyeomyia*			
Wyeomyia (*Phoniomyia*) spp.	23	-	23
Wyeomyia (*Wyeomyia*) spp. *	4	-	4
Tribe Uranotaeniini	1	-	1
Genus *Uranotaenia*			
Uranotaenia sp. *	1	-	1
Total	**1100**	**390**	**1490**
Species richness (S)	17	14	20
Equitability index (J)	0.68	0.49	0.58
Shannon's diversity index (H)	1.95	1.31	1.74

* Refers to damaged specimens, not being possible identified to species level.

Ae. (*Och.*) *scapularis* showed a positive and significant correlation with monthly rainfall (R = 0.68; P = 0.01), and *Cx.* (*Cux.*) *nigripalpus* showed a positive and significant correlation with relative humidity (R = 0.88; P = 0.00). Results were not significant in other analysis of *Ae.* (*Och.*) *scapularis*, *Cx.* (*Cux.*) *nigripalpus*, and climate data.

Discussion

The culicides fauna observed in the forest environment presented a composition of bioindicators of anthropogenic areas, represented by *Ae.* (*Och.*) *scapularis* and the Tribe Mansoniini species (Dorvillé 1996, Guimarães et al. 2000, Cardoso et al. 2011), indicating that the area presents a significant degree of environmental degradation, despite being a unit of Atlantic Forest Federal Conservation.

The number of specimens of Anophelinae subfamily collected in this study was relatively low. However, the occurrence of *An.* (*Nys.*) *albitarsis s.l.*, *An.* (*Nys.*) *evansae* and *Anopheles* (*Nyssorhynchus*) *oswaldoi* (Peryassú, 1922), even in low frequency, presents epidemiological importance, due to the potential transmission of malaria by these species (Consoli & Lourenço-de-Oliveira 1994, Forattini 2002). Silva et al. (2013), in a study conducted in Sooretama Biological Reserve, in the State of Espírito Santo, incriminated *An.* (*Nys.*) *darlingi* as a primary vector and *An.* (*Nys.*) *albitarsis s.l.* as a secondary vector of malaria in the region. Thus, the region has characteristics compatible with the re-emergence of malaria, especially considering that the area has a potential risk of disease transmission due to the occurrence of *An.* (*Nys.*) *darlingi* (Meneguzzi et al. 2009).

In relation to Culicinae subfamily, genera *Aedes*, *Trichoprosopon* and *Culex* were the most abundant, respectively. Several species of *Aedes* and *Culex* have epidemiological importance, once we can incriminate them in the transmission of pathogens to humans and other animals (Consoli & Lourenço-de-Oliveira 1994, Forattini 2002).

The three most abundant species observed in this study were *Ae.* (*Och.*) *scapularis*, *Cx.* (*Cux.*) *nigripalpus* and *Ae.* (*Och.*) *fulvus*. Cardoso et al. (2011) also observed the abundance of *Ae.* (*Och.*) *scapularis* and *Cx.* (*Cux.*) *nigripalpus* in the Atlantic Forest from the State of Rio Grande do Sul.

Aedes (*Ochlerotatus*) *scapularis* was the most abundant species in the study area, and its occurrence in both environments seems to indicate that this species has a higher degree of eclecticism in relation to their eating habits, since it presents compatibility with both artificial and natural breeding (Forattini 2002). In this context, it performs blood meal in peridomicile and in the altered areas, and then it returns to the adjacent Atlantic Forest, a place that serves as a shelter (Consoli & Lourenço-de-Oliveira 1994, Forattini 2002). The species has vector competence for the transmission of several arboviruses, such as Ilhéus virus, Rocio virus and the yellow fever virus (Forattini 2002, Wilkerson et al. 2015). It also presented a positive and significant correlation with the monthly rainfall in both studied environments, which demonstrates that rain influences directly and positively in its density.

Culex (*Culex*) *nigripalpus* was the second most abundant species, presenting no significant differences in the number of collected specimens among the studied environment. This result can be explained by the presence of chickens in peridomicile and high diversity of birds in the GNF, once *Cx*. (*Cux*.) *nigripalpus* has an ornitophilic behavior (Guimarães et al. 1987, Consoli & Lourenço-de-Oliveira 1994, Forattini 2002). In this context, the mosquito has a potential for transmission of some arboviruses, even if we consider its low anthropophily (Laporta et al. 2008, Cardoso et al. 2011).

Aedes (*Ochlerotatus*) *fulvus* represented the third most abundant species, being mainly collected in the forest environment. This is an essentially wild species and possibly presents an association with the transmission of arboviruses, including yellow fever (Consoli & Lourenço-de-Oliveira 1994, Forattini 2002, Fé et al. 2003).

Regarding *Haemagogus* spp., it was registered a low number of specimens. However, the occurrence of *Hg*. (*Con*.) *leucocelaenus* and *Haemagogus* (*Haemagogus*) *spegazzinii* Brèthes, 1912 has a higher epidemiological importance, due to its involvement with the transmission of wild-type yellow fever (Forattini 2002, Vasconcelos et al. 2003). Rezende et al. (2011) reported the occurrence of *Hg*. (*Con*.) *leucocelaenus* colonizing artificial container (sectioned tire) in the same study area of our research.

Aedes (*Ochlerotatus*) *taeniorhynchus* is a common species in tide-affected areas, where its immatures develop in brackish water (Forattini 2002). Marcondes et al. (2012) collected winged specimens to evaluate the differences on eggs kinds in Vitória, Espírito Santo. The species has high flight capacity. However, the species occurrence in the study area, distant about 40 km from the Atlantic Ocean, appears to be associated with breeding present in the area, which have fluid collections with some degree of salinity.

The data obtained in this study show that the occurrence of vector species in the region increases the potential risk of several arboviruses, mainly due to the occurrence of *Ae*. (*Och*.) *scapularis*, *Ae*. (*Och*.) *fulvus*, *Hg*. (*Con*.) *leucocelaenus* and *Hg*. (*Hag*.) *spegazzinii*, species incriminated as vectors of wild-type yellow fever etiological agents. These results demonstrate a need for further entomological studies on bio-ecological aspects of local fauna, including the possible spread to the anthropic environment.

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Author Contributions

T. M. Virgens, I. S. Pinto and H. R. Rezende were responsible for conception, field work, data analysis, identification of specimens and the writing manuscript. A. Falqueto coordinated the study and final editing of the manuscript.

Conflicts of interest

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

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