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Inventory

New records of dragonflies and damselflies (Insecta: Odonata) from Amapá state, Brazil

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Abstract: The Odonata Order comprises one of the largest groups of aquatic insects, 7,000 species are described worldwide, with approximately 860 species registered in Brazil, around 14% of the global fauna known to date. However, there are still great gaps in the knowledge of Odonata fauna in many areas of Brazil. This study aims to present the results of the survey of Odonata species sampled in three counties in the state of Amapá. The state is located in the north of the country, inserted in the Amazon Biome. Odonata were caught between January and December 2018, with 472 specimens being sampled, belonging to seven families, 36 genera and 53 species. In total, 27 of the species found during the study correspond to new records for the state of Amapá. Due to the lack of information on the diversity of the Odonata order in Amapá, the list of species presented should serve as input for new studies contributing to the knowledge of the order in the state.
Keywords: *Inventory; Amazônia; Diversity; Fauna.*

Libélulas e donzelinhas (Insecta: Odonata) novos registros para o estado do Amapá, Brasil

Resumo: A ordem Odonata compreende um dos maiores grupos de insetos aquáticos, mundialmente são descritas 7.000 espécies, sendo que, para o Brasil estão registradas aproximadamente 860 espécies, algo em torno de 14% da fauna global conhecida até o momento. No entanto, ainda existem grandes lacunas no conhecimento da odonatofauna em muitas áreas do Brasil. Este estudo tem como objetivo apresentar o resultado do levantamento de espécies de Odonata amostradas em três municípios do estado Amapá. O estado se encontra na região norte do país, inserido no Bioma amazônico. As capturas de Odonata foram realizadas entre os meses de janeiro e dezembro de 2018, sendo amostrados 472 espécimes pertencentes a sete famílias, 36 gêneros e 53 espécies. No total, 27 das espécies encontradas durante o estudo correspondem a novos registros para o estado do Amapá. Devido à falta de informação sobre a diversidade da ordem Odonata no Amapá, a lista de espécies apresentada deve servir de aporte para novos estudos contribuindo com o conhecimento da ordem no estado.
Palavras-chave: *Inventário; Amazônia; Diversidade; Fauna.*

Introduction

The Odonata order comprises the second largest group of aquatic insects (Djikstra et al. 2014). In Brazil, the group has a great diversity of names, the most common being dragonfly, hyacinth and lava-bunda (Brasil & Vilela 2019). The immatures are aquatic, and the adult individuals are winged terrestrial, organisms with predatory habits, both in their larval and adult stages (Corbet 1980).

Dragonflies occur on all continents, with the exception of Antarctica (Trueman 2007). Worldwide, the order comprises about 7,000 species (Kalkman et al. 2008), the number of species of Odonata in the neotropical region is close to 1,800 (Von Ellenrieder 2009), in Brazil 860 species are known, representing about 14% of the global fauna (Pinto 2018), or approximately 50% of the neotropical species.

Because of their close relationship with the aquatic environment, insects of the order Odonata are strongly affected by the physical and chemical changes in these environments, and some species of the order have environmental and ecological specificities for their occurrence (Nessimian et al. 2008). In particular, the species of the suborder Zygoptera are those that demonstrate the greatest environmental restrictions (Monteiro-Junior et al. 2014, 2015, Oliveira-Junior et al. 2015). These characteristics allow the use of dragonflies as bioindicators of the health of water bodies (Ferreira-Peruquetti & De Marco 2002, Monteiro-Junior et al. 2015). Different factors have been affecting Odonata populations; according to Clausnitzer et al. (2009) one out of ten species of Odonata is threatened with extinction, among aquatic insects, dragonflies are one of the few organisms that have the conservation status evaluated in Brazil by ICMBIO and globally by IUCN.

Dragonflies are charismatic insects (Corbet 1999), easily observed and handled in the field (De Marco & Vianna 2005). Even so, only 29% of the Brazilian territory presents data on the Odonata order (De Marco & Vianna 2005). In recent years, several studies have been published, increasing even more the knowledge of odonatafauna in Brazil, especially in regions with lesser knowledge: Takiya et al. (2016) in the state of Ceará, Rodrigues & Roque (2017) in Mato Grosso do Sul, Dalzochio et al. (2018) in Rio Grande do Sul, Koroiva et al. (2020) in Amazonas, Santos et al. (2020) in the state of Sergipe.

With the exception of the states of Amazonas and Pará, which have established large groups of studies with aquatic insects, studies with Odonata are still scarce in the Brazilian Amazon. Among the states of the northern region, Acre, Amapá, Rondônia and Roraima have a high lack of knowledge about the order. Amapá has a large area of land destined for preservation, approximately 70% of its territory is protected environments (Oliveira 2010). Even with this large area preserved, the state has a high lack of knowledge for numerous groups; among them, the insects of the Odonata order stand out for the lack of information. Therefore, this study aims to present a list of Odonata species collected in three counties in the state, reporting a series of new occurrences.

Material and Methods

1. Study area

The collections were carried out in three counties (Table 1) in the state of Amapá, located in the Amazon region in northern Brazil (Figure 1). The climatic classification of the state according to Köppen is of the type Am (Tropical humid), the average annual temperature is 27ºC and the average rainfall is 3330 mm.

In Macapá, Odonata were collected in an environment of dry forest island (Ilha de Mata Seca), which according to Santos et al. (2013) is an area of less structural complexity, where the vegetation is small due to several factors. The part of forest sampled has approximately 6 Km^2 and is located in an urban area within the Campus Marco Zero of the Federal University of Amapá-UNIFAP. The Ilha de Mata has no connection with any water course; the collections of water when present are temporary, occurring more frequently in the rainiest period. Still in the county, collections were made in the Rio Curiaú Environmental Protection Area (Rio Curiaú APA). The APA has predominantly Cerrado areas, floodplain forest in addition to large extensions of flooded fields, and during the rainy season these environments flood completely and remain like this during much of the year (Chagas 1997). In Oiapoque, collections were carried out in the village of Clevelândia do Norte, which borders French Guiana. The village is located on the right bank of the Oiapoque River, about 5 km south of the urban area of the county. The environment is bordered by an area of primary forest, part of the Amazon Forest, and the dense forest of solid ground is predominant in the place. The sampling of Odonata in Porto Grande was carried out in the Amapá Forestry and Cellulose SA (AMCEL) area; the study region

Table 1. Location of points and type of environment sampled.

Sample Point	County of Sample	Type of sampling environment	Coordinates
P1	Macapá	Brazilian Cerrado	0°00’19.5”S 51°05’06.5”W
P2	Macapá	Brazilian Cerrado	0°00’18.5”S 51°05’14.0”W
P3	Macapá	Brazilian Cerrado	0°00’24.8”S 51°05’13.9”W
P4	Macapá	Lotic Environment	0°16’17.5”N 50°54’58.0”W
P5	Macapá	Lotic Environment	0°14’48.0”N 50°57’26.4”W
P6	Macapá	Floodplain	0°11’21.7”N 51°00’03.5”W
P7	Oiapoque	Lotic Environment	3°48’38.5”N 51°51’55.7”W
P8	Oiapoque	Lotic Environment	3°48’36.0”N 51°51’48.8”W
P9	Oiapoque	Lotic Environment	3°47’46.1”N 51°51’47.9”W
P10	Porto Grande	Lotic Environment	0°43’51.30”N 51°21’36.6”W
P11	Porto Grande	Lotic Environment	0°37’26.79”N 51°21’3.11”W
P12	Porto Grande	Floodplain	0°39’20.68”N 51°22’49.8”W

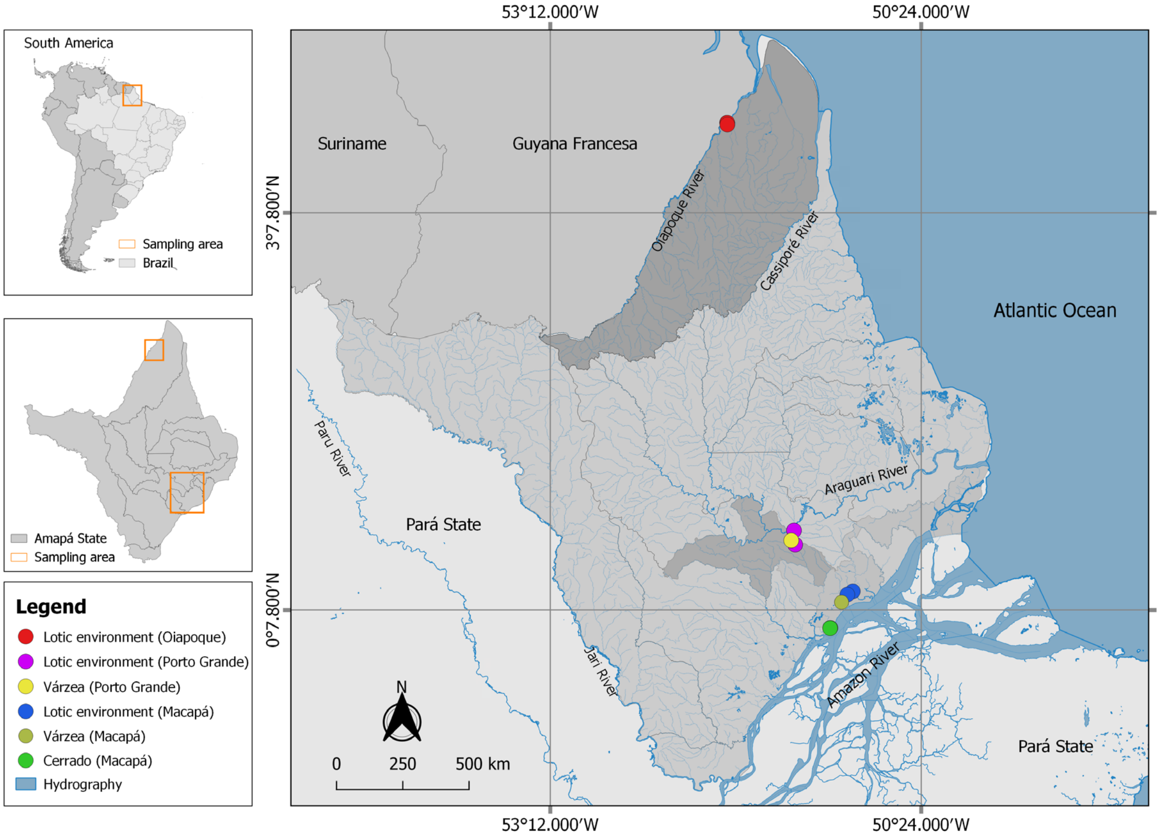

Figure 1. Map with the location of the counties of Macapá, Oiapoque and Porto Grande (AP) Brazil where the Odonata collections were carried out.

is formed by a mosaic comprising natural vegetation and extensive areas of monocultures, predominantly eucalyptus. According to Mustin et al. (2017) the natural vegetation occurring in the area comprises Amazonian savanna environments cut by stretches of gallery forests and flooded fields.

2. *Data collection*

The collections of Odonata were made between the months of January and December 2018. For the sampling of adults, entomological nets were used and the collections occurred between 10.00 and 16.00 P.M. of sunny days. The collected specimens were treated with acetone PA following the methodology proposed by Lencioni (2005), later identified according to the taxonomic keys of Garrison et al. (2006, 2010) and Lencioni (2005, 2006, 2017) for genera and species, and additional Anisoptera literature pertinent to each genera to identify at the species level. The collected specimens were deposited in the ArthroLab entomological collection at the Federal University of Amapá-UNIFAP.

3. *Statistical analysis*

To measure the sampling effort and number of species collected, the non-parametric Jackknife1 estimator was used, and the rarefaction curve was generated from the estimator. Jackknife 1 and the rarefaction curve were calculated using software R (R Core Team 2019) using the vegan package (Oksanen et al. 2019).

Results

In total, 472 specimens belonging to seven families, 36 genera and 53 species were sampled (Table 2), with 27 of the species found during the study corresponding to new records for the state of Amapá. Among the sampled families, Libellulidae showed the greatest diversity of species (n = 27), followed by Coenagrionidae (n = 17), Calopterygidae (n = 3), Aeshnidae, Gomphidae and Heteragrionidae (n = 2) and Dicteriadidae with one species.

The individuals of the suborder Anisoptera represented the greatest abundance of collected specimens (n = 280), followed by Zygoptera (n = 192). Among the Anisoptera, the family Libellulidae was the one that comprised the largest number of captured specimens (n = 269). The most abundant families among the Zygoptera were Coenagrionidae (n = 150), Calopterygidae (n = 26) and Heteragrionidae (n = 15). The most representative genera were *Erythrodiplax* (n = 67), *Ischnura* (n = 46) and *Orthemis* (n = 36). Among the sampled species *Miathyria marcella* (Selys in Sagra, 1854) (n = 29), *Erythrodiplax umbrata* (Linnaeus, 1758) (n = 28) and *Ischnura fluviatilis* Selys, 1876 (n = 24) were the ones with the highest number of collected individuals.

The rarefaction curve, based on the analysis of the data sampled during the study, showed good results for the sampling efforts, the collection efficiency was close to 84%. The Jackknife 1 estimator (Figure 2) demonstrated that the richness of species found corresponded to 73% (± 1,4).

Table 2. List of species registered for the state of Amapá. Location of the collection points present in table 1. ICMBIO red book status 2019, LC (Least Concern) and DD (Insufficient Data). * New records for the State.

Suborder / Family	Species	Collection points	ICMBIO Status
Zygopetra			
Calopterygidae	*Hetaerina amazonica* Sjöstedt, 1918	P10, P11	LC
	Hetaerina mortua Hagen in Selys, 1853	P8	LC
	Hetaerina moribunda Hagen in Selys, 1853	P10	LC
Coenagrionidae	*Acanthagrion amazonicum* Sjöstedt, 1918*	P4, P6	LC
	Acanthagrion egleri (Santos, 1961)*	P6	DD
	Acanthagrion kennedii Williamson, 1916*	P5, P6	LC
	Aceratobasis macilentum (Rambur, 1842)*	P6	LC
	Argia fumigata Hagen in Selys, 1865	P11	LC
	Epipleoneura kaxuriana Machado, 1985*	P5, P10, P11	LC
	Inpabasis rosea (Selys, 1877)*	P10	LC
	Ischnura capreolus (Hagen, 1861)	P1, P6, P4, P9, P10, P9	LC
	Ischnura fluviatilis Selys, 1876	P5, P6, P7, P12	LC
	Homeoura nepos (Selys, 1876)	P5, P6	LC
	Mecistogaster lucretia (Drury, 1773)*	P9	LC
	Neoneura bilinearis Selys, 1860*	P7, P8	LC
	Phasmoneura exigua (Selys, 1886)*	P10	LC
	Phoenicagrion flammeum (Selys, 1876)	P4	LC
	Psaironeura tenuissima Selys, 1886*	P7, P9	LC
	Telebasis carminita Calvert, 1909	P4, P6	LC
	Telebasis griffinii (Martins, 1896)*	P6	LC
Dicteriadidae	*Heliocharis amazona* Selys, 1853*	P8	LC
Heteragrionidae	*Heteragrion ictericum* Williamson, 1919*	P7, P8, P11	LC
	Oxystigma petiolatum (Selys, 1862)*	P10	LC
Anisoptera			
Aeshnidae	*Coryphaeschna adnexa* (Hagen, 1861)*	P6	LC
	Gynacantha mexicana Selys, 1868*	P3, P5, P10	LC
Gomphidae	*Aphylla janirae* Belle, 1994*	P5	DD
	Phyllocycla sp.	P8	LC
Libellulidae	*Anatya guttata* (Erichson in Schomburgk, 1848)*	P1, P3, P4	LC
	Brachymesia herbida (Gundlach, 1889)	P1, P2, P9, P11, P12	LC
	Diastatops obscura (Fabricius, 1775)	P5, P7, P8, PP11	LC
	Diastatops dimidiata (Linnaeus, 1758)*	P8	LC
	Erythemis peruviana (Rambur 1842)	P2, P3, P8, P11	LC
	Erythemis vesiculosa (Fabricius, 1775)	P1, P2, P6, P7, P11	LC
	Erythrodiplax basalis (Kirby, 1897)*	P5, P7, P8	LC
	Erythrodiplax famula (Erichson in Schomburgk, 1848)	P3, P12	LC
	Erythrodiplax fusca (Rambur, 1842)	P2, P5, P8, P11, P12	LC
	Erythrodiplax umbrata (Linnaeus, 1758)	P2, P3, P9, P11, P12	LC
	Erythrodiplax unimaculata (de Geer, 1773)	P6, P10, 12	LC
	Miathyria marcella (Selys in Sagra, 1857)	P1, P2, P9 ,P12	LC
	Nephepeltia flavifrons (Karsch, 1889)	P4, P6	LC

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Nephepeltia phryne (Perty, 1834)*	P5	LC
Orthemis attenuata Erichson in Schomburgk, 1848*	P9	LC
Orthemis concolor Ris, 1919*	P2, P3, P7, P10	LC
Orthemis discolor (Burmeister, 1839)	P1, P6, P9, P10, P11, P12	LC
Orthemis sulphurata Hagen, 1868	P3	LC
Pantala flavescens (Fabricius, 1798)	P3, P6, P7, P11	LC
Perithemis lais (Perty, 1834) *	P4	LC
Tholymis citrina Hagen, 1867*	P3, P8, P10	LC
Tramea calverti Muttkowski, 1910	P1, P8, P10, P11	LC
Tramea rustica DeMarmels & Rácinis, 1982	P6	LC
Uracis fastigiata (Burmeister, 1839)*	P5, P8, P10, P11	LC
Uracis ovipositrix Calvert, 1909	P7, P8, P10	LC
Zenithoptera fasciata (Linnaeus, 1758)*	P3, P6, P7, P11, P12	LC
Zenithoptera viola Ris, 1910	P6, P10	LC

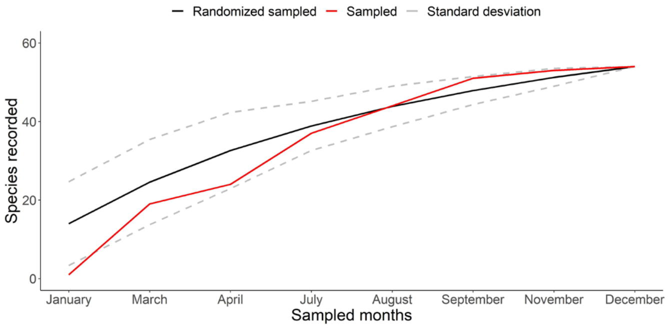

Figure 2. Rarefaction curve and its confidence interval (95% CI) of Odonata species collected in the counties of Macapá, Oiapoque and Porto Grande (AP), Brazil.

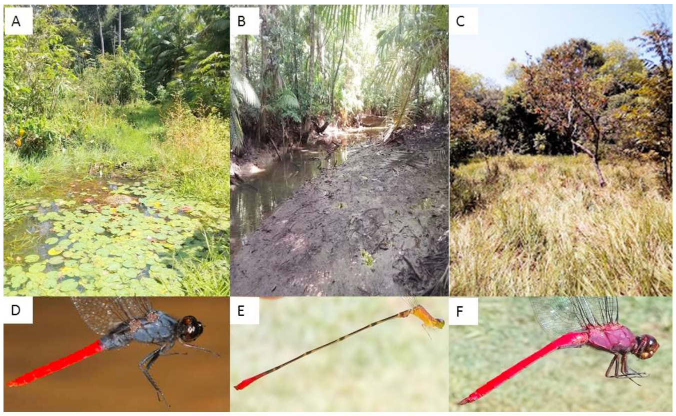

Figure 3. Sample locations: A, Macapá, Rio Curiaú Environmental Protection Area - APA do Curiaú; B, Porto Grande, Amapá Forestry and Cellulose SA (AMCEL); C, Macapá, Ilha de Mata seca in the Amazon savanna. Odonata: D, Libellulidae, Erythemis peruviana (Rambur, 1842); E, Coenagrionidae, *Psaironeura tenuissima* Selys, 1886 and Libellulidae, F, *Orthemis discolor* (Burmeister, 1839).

Discussion

The study was carried out in a small sample area in three counties in the state of Amapa; in total 53 species were found, a result that suggests that the region's Odonata fauna is relatively richer. Representatives of the suborder Anisoptera comprised approximately 60% of the samples sampled, with 280 specimens collected, the Libellulidae family (n = 269) was the most abundant during the study. This high number can be explained by the fact that many species of this family are opportunistic organisms (Costa & Oldrini 2005, Boti et al. 2007), frequently prevalent in open vegetation environments (Dutra & De Marco 2015, Rodrigues & Roque 2017) or even in anthropized environments (Damaceno et al. 2014). This fact is due to its ability to control body temperature (Corbet & May 2008), in addition to having great dispersion capacity (Monteiro-Júnior et al. 2013, Oliveira Júnior et al. 2017).

The families Aeshnidae and Gomphidae had only two species collected each, totaling 11 individuals sampled, eight specimens belonging to Aeshnidae and three to Gomphidae.This low number can be associated with the flight habits of these organisms, which are usually extremely fast. In addition, species of Aeshnidae usually reach great heights and some species are crepuscular (Bedê et al. 2000), a fact that makes it difficult to capture them. According to Almeida et al. (2013) these low numbers may be linked to the type of collection performed, a diversification of sampling methods may allow obtaining species rarely captured using the standard methodology.

Coenagrionidae, the family with the largest number of species for the suborder Zygoptera in Brazil (Lencioni 2006), presented the greatest diversity of species (= 17) and abundance (n = 150) among zygopterans, with almost 78% of the collected specimens. The genera *Ischnura*, *Acanthagrion* and *Telebasis* with 46, 22 and 22 individuals collected respectively were the most abundant for the family, comprising approximately 60% of the Coenagrionidae sampled. The specimens of these genera are highly representative organisms in many studies and in different environments such as: (Cerrado, Ferreira-Peruquetti & Fonseca-Gessner 2003, Borges et al. 2019; Veredas, Vilela et al. 2016; Pampa, Garcia Junior et al. 2019; Atlantic Forest, Ferreira-Peruquetti & De Marco 2002).

With the exception of *Oxystigma petiolatum* (Selys, 1862) (Heteragrionidae) and *Mecistogaster lucretia* (Drury, 1773) (Coenagrionidae) found exclusively within the forest area during this study, the other specimens of zygopterans were collected close to water bodies, generally in shaded areas. This fact can be attributed to ecophysiological restrictions of the group (Corbet 1999). These organisms generally have a small size, which represents a high conductance, so they tend to look for places with less thermic variation, such as places with less sunlight (Brasil et al. 2019).

Most of the species found during the study, according to De Marco et al. (2018) in the red book of Brazilian fauna threatened with extinction fall into the level of least concern. However, *Aphylla janirae* Belle, 1994 and *Acanthagrion egleri* (Santos, 1961) are species that have insufficient data to classify the level of vulnerability; these new distribution records may contribute in the future to the threat level of these species.

In conclusion the diversity of Odonata found and the report of the new occurrence records for the state indicate that the fauna of the region deserves more attention, and the establishment of new collection points can further expand the list of species occurring in Amapá.

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Author Contributions

Manoel Daltro Nunes Garcia Junior: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Monique Telcia dos Santos Damasceno: Contribution to data collection. Contribution to manuscript preparation.

Maria Jeovana Lima Martins: Contribution to data collection. Contribution to manuscript preparation.

Tiago Silva da Costa: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Ricardo Marcelo dos Anjos Ferreira: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Raimundo Nonato Picanço Souto: Substantial contribution in the concept and design of the study. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

Conflicts of Interest

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

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Article

Anuran fauna of the Parque Estadual Carlos Botelho - Núcleo Sete Barras, southeastern Brazil: species composition, use of breeding sites, and seasonal patterns of breeding activity

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Abstract: The goal of this work was to study the species composition, the use of breeding sites, and the seasonal patterns of breeding activity of the anuran fauna from Parque Estadual Carlos Botelho - Núcleo Sete Barras, state of São Paulo, southeastern Brazil. Fieldwork was carried out from September 2005 to October 2007 through two main methods: active visual search inside a 10 ha- permanent plot and aural and visual search in seven previously selected aquatic breeding habitats. Species richness was related to the sampling effort by means of species accumulation curve and through non-parametric estimators. Thirty-three species distributed in 12 families were recorded, from which 69% are endemic to the Atlantic Forest. *Trachycephalus mesophaeus* represents a new record for the park. *Cycloramphus lutzorum* is included as Data Deficient in the IUCN list. The species accumulation curve did not stabilize, showing some tendency to rise. The use of breeding sites by 20 species was compared using cluster analysis, which revealed two major groups: the first with five species (two habitat generalists and three stream specialists) and the second composed by the other species (with different reproductive modes associated with flooded environments). The breeding period of most species was associated to the rainy season (October to February), and only *Scinax hayii* showed continuous breeding activity during the entire period of study.

Keywords: *anuran amphibians; Atlantic Rainforest; diversity; breeding sites; spatial distribution; seasonal breeding patterns.*

Anurofauna do Parque Estadual Carlos Botelho – Núcleo Sete Barras, sudeste do Brasil: composição de espécies, uso de sítios reprodutivos e padrões sazonais de atividade reprodutiva

Resumo: O objetivo deste trabalho foi estudar a composição de espécies, o uso de habitats reprodutivos e os padrões sazonais de atividade reprodutiva da anurofauna do Parque Estadual Carlos Botelho - Núcleo Sete Barras, estado de São Paulo, sudeste do Brasil. O trabalho de campo foi desenvolvido de setembro de 2005 a outubro de 2007 por meio de dois métodos principais: procura visual ativa no interior de uma parcela permanente de 10 ha e busca visual e acústica em sete habitats aquáticos de reprodução previamente selecionados. A riqueza de espécies foi relacionada com o esforço amostral por meio de curvas de acumulação de espécies e estimadores não-paramétricos. Foram registradas 33 espécies distribuídas em 12 famílias, 69% das quais são endêmicas da Mata Atlântica. *Trachycephalus mesophaeus* representa um novo registro para o parque. *Cycloramphus lutzorum* está incluída como Deficiente em Dados na lista da IUCN. A curva de acumulação de espécie não se estabilizou, mostrando alguma tendência de crescimento. A similaridade no uso dos ambientes aquáticos por 20 espécies foi avaliada por meio de uma análise de agrupamento, que revelou dois grupos: o primeiro com cinco espécies (duas generalistas de habitat e três especialistas de riachos) e o segundo formado pelas demais espécies (com diferentes modos reprodutivos associados a ambientes aquáticos lênticos). O período reprodutivo da maioria das espécies esteve associado à estação chuvosa (outubro a março), e apenas *Scinax hayii* mostrou atividade reprodutiva contínua durante todo o período de estudo.

Palavras-chave: *anfíbios anuros; Floresta Atlântica; diversidade; sítios reprodutivos; distribuição espacial; padrões reprodutivos sazonais.*

Introduction

The Atlantic Rainforest is home to 528 species of anurans, of which about 80% are endemic (Vancine et al. 2018). This astonishing diversity is commonly attributed to the highly rugged terrain and the existence of geographical barriers that isolate populations and create high levels of speciation and endemism; the stratified physiognomy of the vegetation, with a great diversity of epiphytes, together with high levels of rainfall and humidity, result in intense partitioning of habitats and microhabitats by anurans and the evolution of specialized reproductive modes (see Haddad & Prado 2005 and references herein).

In the last decades many studies were developed on anuran amphibian communities from different physiognomies of the Atlantic Rainforest biome (e.g, Heyer et al. 1990, Canelas & Bertoluci 2007, Araújo et al. 2010, Brassaloti et al. 2010, Forlani et al. 2010, Vilela et al. 2011, Figueiredo et al. 2019). Population declines and even local extinctions were detected in some localities, such as Santa Tereza, in Espírito Santo state (Weygoldt 1989), and Serra do Japi (Haddad & Sazima 1992), Estação Biológica de Boracéia (Heyer et al. 1988, 1990, Bertoluci & Heyer 1995, Verdade et al. 2011) and Estação Biológica do Alto da Serra de Paranapiacaba (Verdade et al. 2009), in São Paulo state, which show the importance of ecological studies, even in well-studied regions, such as southeastern Brazil (Haddad & Prado 2005, Brito 2008, Verdade et al. 2012).

The anuran fauna of the state of São Paulo is well known (about 230 species) and represents 27% of the Brazilian diversity and 4% of world diversity (Rossa-Feres et al. 2011). Most species are found in Atlantic Rainforest areas, including the Estação Biológica de Boracéia (66 espécies; Heyer et al. 1990; Verdade et al. 2011), the Parque Estadual Intervales (Bertoluci & Rodrigues 2002b; 48 species), the Estação Biológica do Alto da Serra de Paranapiacaba (Verdade et al. 2009; 69 species), the Parque Estadual Turístico do Alto Ribeira - PETAR (Araújo et al. 2010; 60 species), and the Parque Natural Municipal Nascentes de Paranapiacaba (Trevine et al. 2014; 80 species).

The greatest diversity in São Paulo state was recorded in the Parque Estadual Carlos Botelho, with about 85 anuran species, which is perhaps due to the abundance of water bodies and the excellent state of conservation of this conservation area (e.g., Guix et al. 1994, 2000, Bertoluci et al. 2007, Forlani et al. 2010).

Spatial distribution and reproductive success of amphibians are strongly dependent on the physical characteristics of reproductive environments, varying in space and time. Environments with similar abiotic factors harbor different species compositions, which indicates that there is more than one factor influencing the choice of reproductive sites, such as habitat heterogeneity and degree of preservation of terrestrial habitats around aquatic breeding sites (Wells 1977, Gascon 1991, Rudolf & Rödel 2005), which affect assemblage diversity, local abundance of some species and interspecific interactions (Underwood 2000).

Rainfall distribution and air temperature variation along the year strongly influence anuran breeding activity (Wells 2007). A higher number of species is able to reproduce throughout the year in aseasonal (Hero 1990, Gascon 1991) than in seasonal environments, where the calling and breeding activity of most species are associated with the rainy season (Aichinger 1987, Bertoluci 1998, Bertoluci & Rodrigues 2002b, Canelas & Bertoluci 2007, Narvaes et al. 2009).

The present study aimed to investigate the composition of the anuran fauna of the Núcleo Sete Barras of the Parque Estadual Carlos Botelho, São Paulo state, southeastern Brazil, the use of breeding sites by the species and their seasonal patterns of breeding activity.

Materials and Methods

1. *Study site*

The Parque Estadual Carlos Botelho (PECB) (24º00' - 24º15' S, 47º45' - 48º10' W) is part of an ecological complex of the Atlantic Rainforest in the southern part of the State of São Paulo, encompassing an area of approximately 38.000 ha, with altitudes that very between 30 to 1600 m above sea level, covered by Dense Ombrophylous Forest (Ferraz & Varjabedian 1999) (Figure 1). According to Veloso et al. (1991), the vegetation at PECB may perform three different physiognomic and floristic compositions: montane rainforest, sub-montane and low lands. The Núcleo Sete Barras (NSB) comprises two forest formations (sub-montane and low lands) and is located in the municipality of Sete Barras, Vale do Ribeira de Iguape, in a contact zone between two geomorphological formations, Guapiara Plateau (low lands) and Serra de Paranapiacaba (sub-montane) (24º11'37" S, 47º55'11" W) (Ferraz & Varjabedian 1999).

The climate is Cfa of Köppen, mesothermal, subtropical, hot and humid, with no dry season (Setzer 1946). In the study period, the mean annual temperature was 21.8° and the annual accumulated precipitation was 1,582 mm (Bertoluci et al. 2007).

2. *Data sampling and collection*

The anuran fauna sampling was carried out using two main methods: active visual (Crump & Scott 1994) in a 2500 m transect inside a permanent plot of 10 ha (more detail in Bertoluci et al. 2007a); and male vocal activity monitoring in seven different previously selected breeding habitats (Table 1). In the plot area, we carried out two visits a month between September 2005 and October 2006, always two researchers (20h/person), totalizing a sampling effort of 240h/person. The breeding habitats were visited for two consecutive nights per month between September 2005 and October 2007, totaling 52 visits in the period. In addition, we recorded all individuals found by chance in the sampling areas. All selected reproductive habitats were located along the main track called "Trilha das Figueiras", which is located along the main road that connects the municipalities of São Miguel Arcanjo and Sete Barras (Figure 1).

Seasonal patterns of breeding activity were established by estimating the number of males (per species) calling synchronously at each aquatic site (Table 1) in the following classes of abundance: (1) 1-2, (2) 3-5, (3) 6-10, (4) 11-20, (5) 21-50, (6) more than 50 males (Bertoluci 1998, Bertoluci & Rodrigues 2002b, Canelas & Bertoluci 2007, Narvaes et al. 2009). The activity of species whose males did not vocalize in the selected sites was recorded in a qualitative way, and we considered the presence in the sites of gravid females, amplectant pairs, and egg clutches as evidences of actual reproduction, as already done

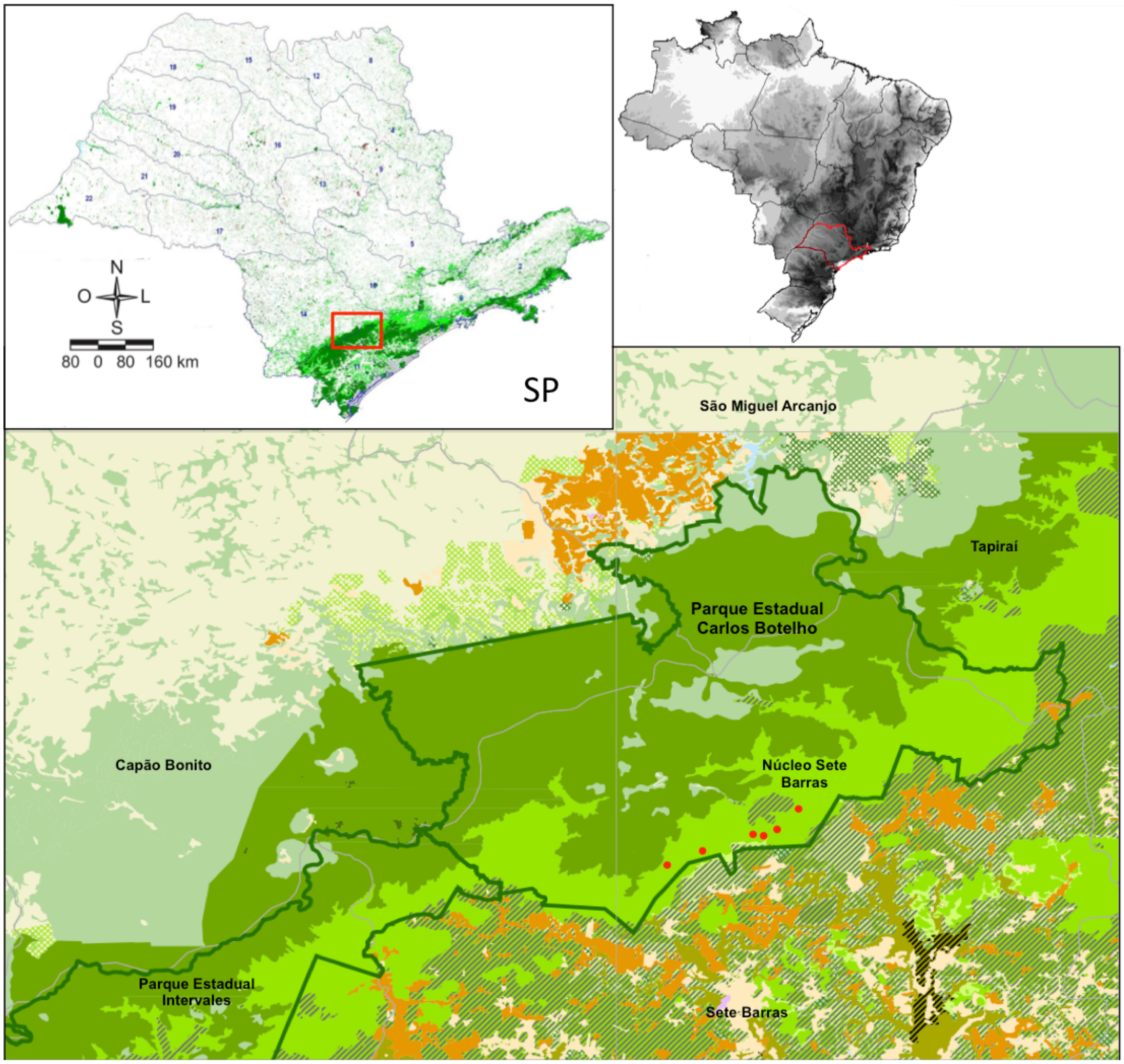

Figure 1. Geographical location of the study area showing the location of the sampled breeding sites (●).

Table 1. Physical characteristics of the seven water bodies studied in the Núcleo Sete Barras, Parque Estadual Carlos Botelho, southeastern Brazil. Legend: VAR – arboreal vegetation; VAT – understory bush vegetation; VHR – herbaceous vegetation (Poaceae); VHA – aquatic herbaceous vegetation. *Transect 100 m.

Sites	Geographic coordinates	Altitude (m)	Hydroperiod	Type	Substrate	Vegetation type
L1	24º11’36” S, 47º55’43” W	241	Semipermanent	Pond	Argiloso	VAR, VAT
L2	24º12’13” S, 47º55’56” W	52	Semipermanent	Pond	Muddy	VAR, VAT, VHA
L3	24º12’08” S, 47º56’30” W	44	Permanent	Pond	Muddy	VAR, VAT, VHA
L4	24º12’21” S, 47º57’16” W	48	Permanent	Pond	Muddy	VAR, VAT, VHA
L5	24º11’37” S, 47º55’11” W	65	Permanent	Reservoir	Argiloso	VAT, VHR
R1	24º11’35” S, 47º55’11” W	53	Permanent	Stream*	Rocky	VAR, VAT
R2	24º11’39” S, 47º55’55” W	48	Temporary	Stream*	Muddy	VAR, VAT, VHA

by Bertoluci (1998), Bertoluci and Rodrigues (2002b), and Canelas & Bertoluci (2007).

Some individuals were collected and submitted to a saturate atmosphere of CO_2 (resolution Nº 714 of 20 June 2002 of CFMV), fixed in formal 10% and preserved in alcohol 70% (under license IBAMA n° 430/05). Voucher specimens were deposited in the herpetological collection of Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo (Appendix I).

3. *Data analysis*

The species richness registered in relation to the sampling effort was assessed by the species accumulation curve (*sensu* Colwell et al. 2004), calculated through the *exact* method (Kindt 2004) using the function “specaccum”. To estimate the richness we used the function “specpool” and “poolaccum” and the non-parametric estimators Bootstrap and Jacknife *I* (Santos 2003) with 10,000 randomizations with sample reposition, “vegan” package (Oksanen et al. 2011), developed for R environment (R Development Core Team 2012). A matrix with monthly occurrence was used for all species registered in the reproductive habitats and chance encounters.

To evaluate the similarity in the use of the breeding sites, we considered only species that show reproductive activity in the selected aquatic sites between September 2006 and October 2007. Based on the breeding sites use, species were classified as habitat generalists and specialists. Dissimilarity between species pairs was also calculated by the complement of the Jaccard index (DJ = 1– J), and this triangular matrix was submitted to a cluster analysis by the UPGMA method (e.g., Sneath & Sokal 1973), which calculates the coefficient of cofenetic correlation (r), indicating the degree of representativeness of the similarity matrix in the dendrogram, so that values of $r \geq 0.8$ allow to consider that the dendrogram adequately represents the dissimilarity matrix (Rohlf 2000). For this analysis we use the “hclust” function, applying the “average” method, also from the “vegan” package (Oksanen et al. 2011), developed for the R-environment (R Development Core Team 2012).

Results

1. *Species composition*

During the study period we recorded to the NSB 33 species distributed in 12 families: Brachycephalidae (1 species), Bufonidae (4), Centrolenidae (1), Craugastoridae (1), Cycloramphidae (1), Hemiphractidae (1), Hylidae (15), Hylodidae (1), Leptodactylidae (5), Microhylidae (1), Odontophrynidae (1), and Phyllomedusidae (1) (Table 2, Figure 2). *Trachycephalus mesophaeus* represents a new record to the area. The species accumulation curve did not show stabilization (Figure 3), with certain trend towards ascension, which is evident by the high confidence interval. The Jackknife estimator I, based in the rare species occurrence, showed higher value in the last month of our sampling ($S_J = 35.88 \pm 1.665$ standard error), and the Bootstrap estimator, considering the full data set (repeated sampling with reposition), for 10000 randomization, was $S_B = 34.34 \pm 1.004$ (Figure 4).

2. *Use of breeding sites*

Twenty species were recorded in the seven breeding sites we monitored (Table 3). The number of species in the sites varied from four to 12. Site L1 showed the highest number of species (12), followed by Site L2 (11). The lowest number of species was found in Site R2 (4), the only temporary site studied. *Fritziana fissilis*, *Dendrophryniscus brevipollicatus* and *Physalaemus spiniger* were recorded in the surroundings of water bodies. Cluster analysis identified two major groups: (I) formed by *Rhinella ornata* and *R. icterica* (Ia) and *Cycloramphus lutzorum, Vitreorana uranoscopa* and *Bokermannohyla hylax* (Ib); (II) formed by the four species of *Dendropsophus* (IIa) and other species (IIb) (Figure 5).

3. *Seasonal breeding patterns*

Table 4 summarizes the annual breeding patterns of the 20 anuran species whose males vocalized in the seven aquatic sites showed in Table 3. Classes shown here are the highest classes of the number of calling males recorded each month considering all monitoring sessions and all sites. Evidences of actual reproduction (amplectant pairs, oviposition behavior or egg clusters) are indicated. Anurans called in almost every month of the two-years period, with higher number of active species (more than 10) being recorded in the rainy seasons (October to February) of both years. Only *Scinax hayii* displayed a continuous breeding pattern, with males calling in 22 of the 24 samples.

Discussion

1. *Species composition*

The species composition at the NSB is typical of Neotropical sites, with high representativeness of Hylidae (Duellman 1999). In addition, 69% of the species are endemic to the Atlantic Rainforest; the high degree of endemism is also typical of this biome (Vancine et al. 2018), however the geomorphological characteristics of NSB, with altitudes close to the sea level, rising in a gradient that reaches 1,000 m in altitude, influenced the composition of the anuran fauna, which is composed by species typical of the low Atlantic Rainforest (*Rhinella hoogmoedi*, *Ololygon argyreornata* and *O. littoralis*) (Pombal & Gordo 1991, Caramaschi & Pombal 2006, Bertoluci et al. 2007) alongside of species restricted to the hillside forest (e.g., *Ischnocnema* aff. *guentheri*) (Bertoluci et al. 2007).

The estimated richness values theoretically indicate the possibility of increasing at least three species in the sampling survey, which can be observed when considering the high ß diversity of anuran fauna found in PECB in its different phytophysiognomies or even in an altitudinal gradient (e.g. Guix et al. 1994, Guix et al. 2000, Bertoluci et al. 2007, Moraes et al. 2007, Forlani et al. 2010).

The anuran fauna of different portions of PECB was described in four other studies (Guix et al., 1994; Guix et al., 2000; Bertoluci et al., 2007; Forlani et al., 2010). The compilation of these lists by Forlani et al. (2010) resulted in a richness of 85 species for the entire park. We add here one species to that list, *Trachycephalus mesophaeus*, maybe due to our higher sampling effort compared to those of the studies cited

Table 2. Anuran species recorded in the Núcleo Sete Barras, Parque Estadual Carlos Botelho, southeastern Brazil, between September 2005 and October 2007.

Brachycephalidae	*Ischnocnema* aff. *guentheri* (Steindachner, 1864)
Bufonidae	*Dendrophryniscus brevipollicatus* Jiménez de la Espada, 1870 1871 “1870”
	Rhinella hoogmoedi Caramaschi & Pombal, 2006
	Rhinella icterica (Spix, 1824)
	Rhinella ornata (Spix, 1824)
Centrolenidae	*Vitreorana uranoscopa* (Müller, 1924)
Craugastoridae	*Haddadus binotatus* (Spix, 1824)
Cycloramphidae	*Cycloramphus lutzorum* Heyer, 1983
Hemiphractidae	*Fritziana fissilis* (Miranda Ribeiro, 1920)
Hylidae	*Boana albomarginata* (Spix, 1824)
	Boana bischoffi (Boulenger, 1887)
	Boana faber (Wied-Neuwied, 1821)
	Boana semilineata (Spix, 1824)
	Bokermannohyla hylax (Heyer, 1985)
	Dendropsophus berthalutzae (Bokermann, 1962)
	Dendropsophus elegans (Wied-Neuwied, 1824)
	Dendropsophus minutus (Peters, 1872)
	Dendropsophus seniculus (Cope, 1868)
	Ololygon argyreornata (Miranda-Ribeiro, 1926)
	Ololygon littoralis (Pombal & Gordo, 1991)
	Ololygon rizibilis (Bokermann, 1964)
	Scinax aff. *alter* (B. Lutz, 1973)
	Scinax hayii (Barbour, 1909)
	Trachycephalus mesophaeus (Hensel, 1867)
Hylodidae	*Hylodes* cf. *phyllodes*
Leptodactylidae	*Adenomera marmorata* Steindachner, 1867
	Leptodactylus latrans (Steffen, 1815)
	Leptodactylus notoaktites Heyer, 1978
	Physalaemus olfersii (Lichtenstein & Martens, 1856)
	Physalaemus spiniger (Miranda-Ribeiro, 1926)
Microhylidae	*Myersiella microps* (Duméril & Bibron, 1841)
Odontophrynidae	*Proceratophrys boiei* (Wied-Neuwied, 1825)
Phyllomedusidae	*Phyllomedusa distincta* A. Lutz in B. Lutz, 1950

above, raising the overall richness of PECB to 86 species. This richness corresponds to 37% of the anuran known for the state of São Paulo (Rossa-Feres et al. 2011). As expected the composition of the anuran fauna of NSB is similar to those of other localities of southeastern Brazil covered by the Dense Ombrophilous Forest of São Paulo state (Bertoluci et al. 2007), like the Estação Biológica de Boracéia (Heyer et al., 1990; Bertoluci et al., 2005), the Parque Estadual Intervales (Bertoluci, 1998), and the Reserva Biológica do Alto da Serra de Paranapiacaba (Verdade et al. 2009). The different physiognomies of the Atlantic Forest, the large altitudinal gradient (Guix et al. 1994, 2000) and the good state of conservation of PECB areas, and habitat heterogeneity (e.g., Silva et al. 2011 and references therein) are also relevant factors in the interpretation of this high richness.

Although none of the species recorded in the present study were included in the Brazilian red list of endangered fauna, *Cycloramphus lutzorum* is considered Data Deficient with decreasing populations by IUCN (2019); major threats to this frog are residential and commercial development, agriculture and aquaculture, and biological resource use.

2. *Breeding site utilization*

Species that used the monitored reproductive habitats did not distribute homogeneously among sites. *Rhinella ornata*, *R. icterica* and *Ololygon littoralis* were classified as habitat generalists (they used all habitat types); the pattern of habitat use of these species has already been observed in other taxocenoses (e.g., Izecksohn & Carvalho-e-Silva 2001, Bertoluci & Rodrigues 2002c, Narvaes et al. 2009).

Vitreorana uranoscopa, Cycloramphus lutzorum and *Bokermannohyla hylax* were considered stream specialists. *Vitreorana uranoscopa* lays eggs on leaves of the marginal vegetation suspended over streams, where eggs fall and tadpoles complete development to

Figure 2. Anuran species recorded in the Núcleo Sete Barras (PECB): (a) *Ischnocnema* aff. *guentheri,* (b) *Dendrophryniscus brevipollicatus,* (c) *Rhinella icterica,* (d) *Rhinella ornata,* (e) *Haddadus binotatus,* (f) *Cycloramphus lutzorum,* (g) *Proceratophrys boiei*, amplectant pair, (h) *Hylodes* cf. *phyllodes,* (i) *Physalaemus spiniger,* (j) *Adenomera marmorata,* (k) spawn of *A. marmorata*, (l) *Myersiella microps,* (m) *Bokermannohyla hylax,* (n) *Dendropsophus elegans,* (o) *Dendropsophus seniculus,* (p) *Boana albomarginata,* (q) *Boana bischoffi*, (r) fighting males of *Boana faber,* (s) *Boana semilineata,* (t) *Phyllomedusa distincta,* (u) *Scinax* aff. *alter*, (v) *Scinax hayii,* amplectant pair, (w) *Ololygon littoralis,* (x) *Trachycephalus mesophaeus*.

metamorphosis (Heyer 1985a, 1990). *Cycloramphus* species lay their eggs on rocks and in wet rock crevices, with semi-terrestrial tadpoles living at the water-land interface, microhabitats commonly found on the banks of rocky streams (Haddad & Prado 2005), as sampled in PECB. *Bokermannohyla hylax* is a typical species of dense ombrophilous forest streams (Heyer 1985b; Bertoluci 2002; Bertoluci et al. 2003). Males vocalize from the vegetation on the edge of forest streams, tadpoles seek shelter under rocks, dead leaves or burrow into the mud (Bertoluci et al. 2003). The presence of this species in Site L1 (classified as a pond) is probably due to the interruption of the stream course by the road slope, altering the original structure of the habitat. Changes caused by anthropogenic disturbances modify the local structure of the forest, form new microhabitats and make different resources available, which influences community structure (Connell & Slatyer 1977, Pickett & White 1985, Tocher et al. 2001).

Species of the genus *Rhinella* were considered habitat generalists, showing the primitive reproductive mode for the group, which favors the exploration of different habitat types (Haddad & Prado 2005). Subgroup Ib of the dendrogram includes only species with specialized reproductive modes associated with streams (Heyer et al. 1990, Bertoluci et al. 2004,

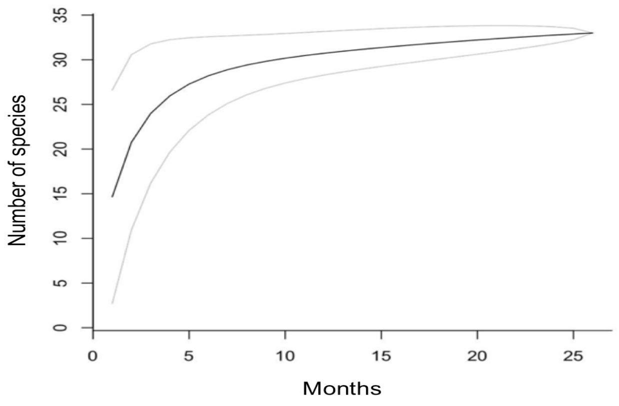

Figure 3. Species accumulation curve between September 2005 and October 2007.

Haddad & Prado 2005). Group II includes only species that breed in lentic water bodies, all of the family Hylidae, except *Leptodactylus latrans* (Leptodactylidae). In subgroup IIa are *Dendropsophus* species, typical of lagoons (e.g., Bertoluci & Rodrigues 2002c, Narvaes et al. 2009) and with generalized reproductive mode (e.g., Haddad & Prado 2005). In subgroup IIb there are two smaller branches; the former includes all species of Scinaxinae (*Scinax* and *Ololygon*), as well as *Leptodactylus latrans* and *Phyllomedusa distincta*, pond-associated species. Among the species of *Scinax* all have generalized reproductive mode except *Ololygon rizibilis*, which produces an aquatic foam nest (Haddad & Prado 2005). *Leptodactylus latrans* builds floating foam nests in permanent or semipermanent environments (Vaz-Ferreira & Gerhau 1975) and *P. distincta* lays eggs on leaves hanging over water in permanent aquatic environments (Woehl & Woehl 2000). The most intimate grouping between *O. rizibilis* and *L. latrans* may be due to the use of semi-permanent environments, suitable for their specialized reproductive mode. The second branch is formed by all *Boana* species, among which only *Boana faber* has a specialized reproductive mode (eggs in nests dug in mud in the banks of lentic water bodies; Martins & Haddad 1988), despite being habitat generalist.

The high degree of overlap in the use of reproductive habitats corroborates the well-established idea that suitable water bodies for reproduction behave as limiting factors for anurans (e.g., Bertoluci & Rodrigues 2002c). However habitat heterogeneity permits anurans use specific microhabitats as calling, oviposition and developmental sites with low or no overlap, influencing anuran richness and the composition of the assemblages (e.g., Bertoluci & Rodrigues 2002c, Vasconcelos & Rossa-Feres 2008, Vasconcelos et al. 2009, Figueiredo et al. 2019).

3. *Seasonal patterns of breeding activity*

The breeding activity of anurans at PECB-NSB was closely related to the rainy season. This general pattern is commonly found in seasonal tropical areas of both Amazonia (Toft & Duellman 1979, Aichinger, 1987, Hero, 1990, Gascon 1991, Duellman, 1995) and southeastern Brazil (Bertoluci 1998, Eterovick & Sazima 2000, Bertoluci & Rodrigues 2002b, Canelas & Bertoluci 2007, Narvaes et al. 2009). Only

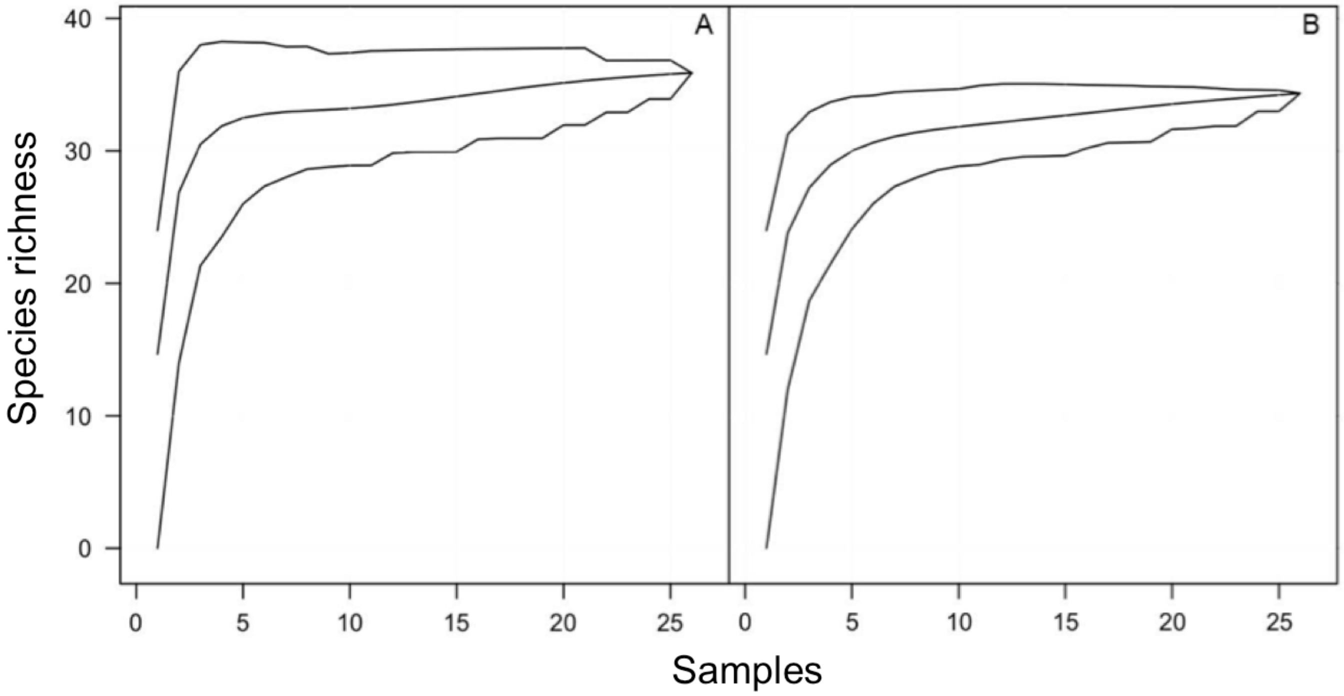

Figure 4. Richness estimators (A) Bootstrap (SB = 32.53) and (B) Jacknife I (SJ = 35.88 ± 1.665 standard error) built from 10,000 randomizations, during 26 months (September 2005 to October 2007).

Table 3. Distribution of 20 anuran species in seven aquatic breeding sites of the Núcleo Sete Barras, Parque Estadual Carlos Botelho, southeastern Brazil.

Species	**L1**	**L2**	**L3**	**L4**	**L5**	**R1**	**R2**
Rhinella ornata	●	●	●	●	●	●	●
Boana albomarginata	●	●	●	●	●		
Boana semilineata	●	●	●	●	●		
Ololygon littoralis		●	●	●	●	●	●
Dendropsophus berthalutzae	●	●	●	●			
Phyllomedusa distincta	●	●		●			
Scinax hayii	●	●		●			
Boana bischoffi	●	●	●				
Bokermannohyla hylax	●					●	●
Rhinella icterica	●				●		●
Dendropsophus minutus	●				●		
Dendropsophus seniculus		●			●		
Leptodactylus latrans		●	●				
Dendropsophus elegans			●		●		
Vitreorana uranoscopa				●		●	
Boana faber	●	●					
Ololygon rizibilis	●						
Ololygon argyreornata				●			
Scinax aff. *alter*					●		
Cycloramphus lutzorum						●	
Total	12	11	8	9	9	5	4

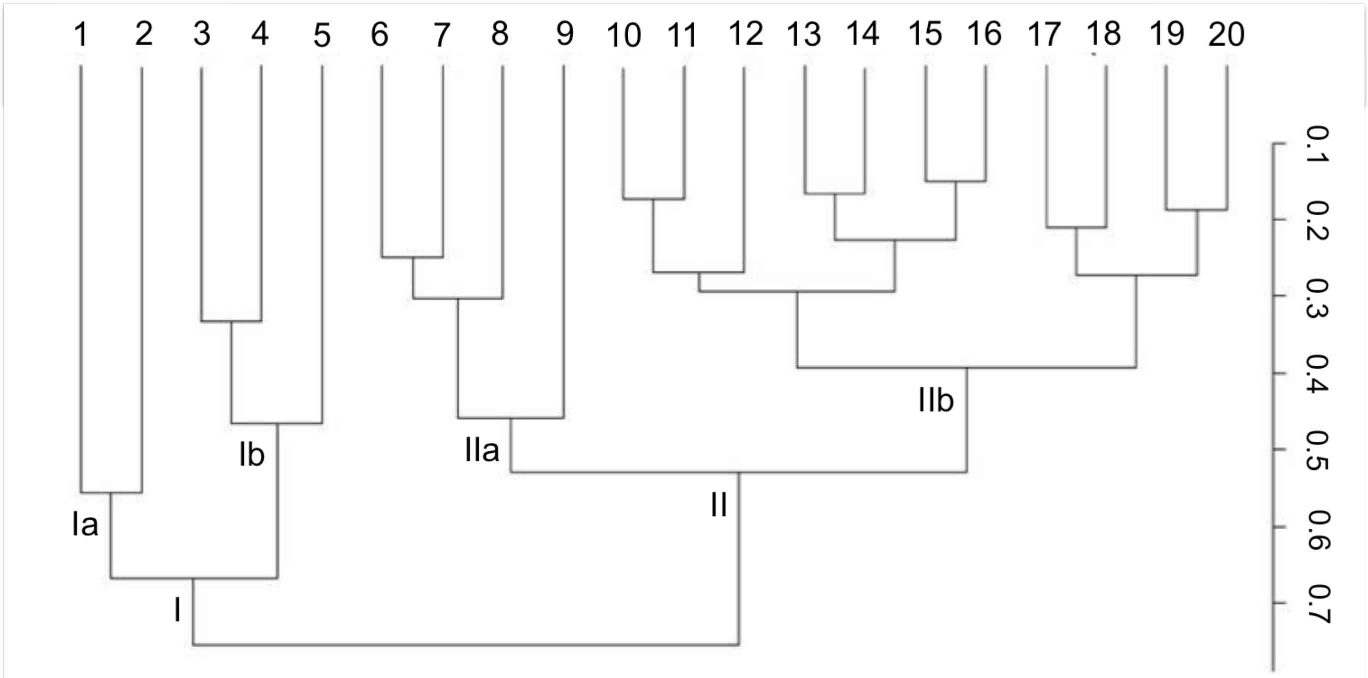

Figure 5. Cluster analyses of 20 anuran species based on their occurrence in seven breeding sites of the Núcleo Sete Barras-Parque Estadual Carlos Botelho (SP). (1) *Rhinella ornata,* (2) *R. icterica,* (3) *Cycloramphus lutzorum,* (4) *Vitreorana uranoscopa,* (5) *Bokermannohyla hylax,* (6) *Dendropsophus seniculus,* (7) *D. minutus,* (8) *D. elegans,* (9) *D. berthalutzae,* (10) *Leptodactylus latrans,* (11) *Ololygon rizibilis,* (12) *O. littoralis,* (13) *O. argyreornata,* (14) *Scinax alter,* (15) *S. hayii,* (16) *Phyllomedusa distincta,* (17) *Boana semilineata,* (18) *B. albomarginata,* (19) *B. faber,* (20) *B. bischoffi.*

Table 4. Seasonal calling and breeding patterns of 20 anuran species in seven aquatic breeding sites of Parque Estadual Carlos Botelho – Núcleo Sete Barras, southeastern Brazil, between September 2005 and September 2007. Classes for the number of synchronously calling males: (1) 1-2, (2) 3-5, (3) 6-10, (4) 11-20, (5) 21-50, and (6) more than 50; V, abundance of calling males not estimated; * evidence of actual reproduction (amplexes or egg clusters); + presence of individuals without breeding activity.

	2005									2006										2007					
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Scinax hayii	v	v	6	6	3		2	2		5	2	5	6	5	6	1	2	5		4	3	1	5*	6	5
Rhinella icterica	+	v	2	1	+		+			*		1	1	2	3	1	+	3	2*	+	1	2*		1	3
Boana faber		6*	6*	5*	6*	4*	2					2*	1			4	2*								1
Dendropsophus elegans		v*	6	5	5	4								6	5	3	2								2
Ololygon littoralis		2	2	1	v	2		+		2	2	1		1	4*	2	4	+	+	2	2	1	4	6*	6
Rhinella ornata		v	v	4	2	+				1		5	5		v	+		1	1			*	4	3*	3
Bokermannohyla hylax		v	2	1	2	2				+	2	4	2	4	2	1	4	+					3		2
Boana albomarginata		1		1	1	3								1	4	+	+								2
Scinax aff. *alter*		v*	6*	4	*	2*						1	3	4	*	1	*					+			4
Boana bischoffi		+	1		4	2								5	5	4	5*	3	2	3	2	2	2	1	3
Boana semilineata			1			+	+				+	2	2	+	2	4	1	2	2	3	3	1	4	5	4*
Dendropsophus minutus			2	v	3	1				1		1	1	3	4		1	1						2	2
Phyllomedusa distincta					3	2								2	2	1	1				+				1
Ololygon rizibilis					2										1										
Cycloramphus lutzorum										+						+					+		+		
Dendropsophus seniculus													3												
Dendropsophus bertalutzae															6*	3	2	5					6	6	5
Ololygon argyreornata																		2							
Leptodactylus latrans																			+	1					
Vitreorana uranoscopa																									*
Total number of species	**2**	**10**	**11**	**10**	**13**	**11**	**4**	**2**	**0**	**7**	**4**	**9**	**9**	**11**	**14**	**14**	**13**	**10**	**6**	**6**	**7**	**7**	**8**	**8**	**15**

Scinax hayii showed calling activity almost throughout the entire period of study (22/24 months), a continuous breeding pattern already observed in the populations of the Estação Biológica de Boracéia (Ombrophylous forest; Bertoluci & Rodrigues 2002b) and Estação Ecológica Juréia-Itatins (Restinga forest; Narvaes et al. 2009), both sites located is São Paulo state, southeastern Brazil. A breeding pattern associate to the rainy season was showed by *S. hayii* in the Parque Estadual Intervales (Ombrophylous forest, Bertoluci 1998), also in São Paulo state. These differences seems to be related to the different climate regimes of these localities. A high proportion of continuous breeders is characteristic of aseasonal environments (e.g. Crump 1974).

We conclude claiming attention to the importance of preserving all types of habitats and microhabitats in the entire altitudinal gradient of PECB as a strategy for the conservation of frogs of southeastern Brazil, one of the richest regions of the world.

Supplementary Material

The following online material is available for this article:
Appendix I - Voucher material.

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Author Contributions

Jaime Bertoluci: Substantial contribution in the conception and design of the work; Contribution in the acquisition of data; Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in the critical review adding intellectual content.

Henrique Oliveira Sawakuchi: Substantial contribution in the conception and design of the work; Contribution in the acquisition of data; Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in the critical revision adding intellectual content.

Carolina Ortiz: Contribution in the analysis and interpretation of data; Contribution in the writing of the work.

Ricardo Augusto Brassaloti: Substantial contribution in the conception and design of the work; Contribution in the acquisition of data; Contribution in the analysis and interpretation of data; Contribution in the writing of the work; Contribution in the critical review adding intellectual content.

José Wagner Ribeiro-Júnior: Contribution to data acquisition; Contribution to the writing of the work.

Shirley Famelli: Contribution on data acquisition; Contribution on data analysis and interpretation; Contribution to 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.

Ethics

Permits necessary to this study in the time it was developed were collection license from IBAMA and license to study in conservation units (COTEC). We had these two licenses.

Data availability

Results from this study should be stored in the *Sistema de Informação Ambiental do Programa Biota/Fapesp/SinBiota*, since it was part of Programa Biota SP.

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Article

Trophic overlap of lionfish (*Pterois volitans*) and two native predators (*Lutjanus apodus* and *Cephalopholis cruentata*) in the western Caribbean

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Abstract: Impact of invasive species on native biota may be due to predation, competition for space or food, or indirect effects. Lionfish (*Pterois volitans*), invasive in the western Atlantic, is a voracious generalist predator, so it is expected to have a significant trophic overlap with native fishes of comparable size and habits. The goal of this study was to determine the diets of potential competitors of the lionfish, in particular a grouper, *Cephalopholis cruentata* (Graysby), and a snapper, *Lutjanus apodus* (Schoolmaster), and to compare them to the diet of lionfish in Xcalak, southern Mexican Caribbean. Stomach contents were analyzed and electivity and diet overlap were estimated. The trophic overlap between the lionfish and the two putative competitors, especially the grouper, was high, including prey that was consumed by the predators in higher proportion than the relative abundance of the prey in the environment, and probably at the same time of day. Lionfish and grouper shared as important diet items *Stegastes* sp., *Halichoeres* sp., Brachyura, and Palaemonidae, and most full stomachs were found during the early morning. The hypothesis of competition between them for particular prey is supported, so we advise to continue the culling programs of lionfish and also to monitor the abundance of the possible native competitors.

Keywords: *Feeding habits; native predators; invasive species; competition; Mexico.*

Traslape trófico del pez león (*Pterois volitans*) y dos depredadores nativos (*Lutjanus apodus* y *Cephalopholis cruentata*) en el Caribe occidental

Resumo: El impacto de las especies invasoras sobre la biota nativa puede deberse a depredación, competencia por espacio o alimento, o efectos indirectos. El pez león (*Pterois volitans*), invasor en el Atlántico occidental, es un voraz depredador generalista, de modo que se espera que tenga un notable sobrelape trófico con peces nativos de tamaño y hábitos comparables. El objetivo de este estudio fue determinar las dietas de competidores potenciales del pez león, en particular un mero, *Cephalopholis cruentata* (payaso), y un pargo, *Lutjanus apodus* (canchix), y compararlas con la dieta del pez león en Xcalak, sur del Caribe mexicano. Se analizaron los contenidos estomacales y se estimó la selectividad y el traslape de dietas. El traslape trófico entre el pez león y los dos posibles competidores, sobre todo el mero, fue amplio, incluidas presas consumidas por los depredadores en proporción mayor a la abundancia relativa de éstas en el ambiente, y probablemente a la misma hora del día. El león y el mero compartieron como presas importantes *Stegastes* sp., *Halichoeres* sp., Brachyura, y Palaemonidae, y la mayoría de los estómagos llenos se encontraron en horas tempranas de la mañana. Se apoya entonces la hipótesis de competencia entre ellos por presas específicas, de modo que recomendamos continuar el programa de extracción de pez león y también el monitoreo de la abundancia de los posibles competidores nativos.

Palavras-chave: *Hábitos alimenticios; depredadores nativos; especies invasoras; competencia; México.*

Introduction

Invasive species have the potential to alter the structure and functioning of communities and ecosystems, displacing native species by predation or competition for habitat or food (McCleery 2011; Ruttenberg et al. 2012). The competitive advantages of invaders over natives may be due to the absence of predators and other natural limits in the invaded areas (Weis 2011; Layman & Allgeier 2012; Paolucci et al. 2013; Raymond et al. 2015). Introduced exotic species may induce trophic cascades (Muñoz et al. 2011; Lönnstedt & McCormick 2013).

The reduction of available prey induced by the invasive predator can also have an impact on a native predator, and the damage may be further compounded by fishing pressure on the native predator (Albins, 2013; Leung et al., 2015). In addition, the fact that lionfish can both prey on and compete with some native species makes the impact more complex, even stronger, and harder to model and predict (Mills et al. 2004): White et al. (2006) found no clear patterns in the literature that they reviewed about indirect effects of invasive species.

Lionfishes (*Pterois volitans* and *P. miles*) are scorpaenids native to Indo-Pacific coral reefs, escaped from the aquarium trade and presently invading the western Atlantic (Semmens et al. 2004). In the western Caribbean, *P. miles* is known from very few records (Guzmán-Méndez et al. 2017), but lionfish has become abundant and widespread from subtropical USA to Brazil (Ferreira et al. 2015). They are generalist predators that consume a great diversity and abundance of mostly demersal fishes (Cobián-Rojas, Schmitter-Soto, Aguilar Betancourt, et al. 2018), in a variety of habitats (Jud et al. 2014).

Following Albins' (2013) experience with lionfish and Coney, *Cephalopholis fulva*, our hypothesis is that lionfish should overlap broadly in diet with native predators of comparable size, e.g. groupers and snappers. Most groupers in the western Caribbean are larger than adult lionfish, except the two species of *Cephalopholis*, *C. fulva* and *C. cruentata*, which often are also the most abundant ichthyobenthophagous fishes in the reef (Loreto-Viruel et al. 2003). The former species used to be more common in past decades, dominance shifting recently towards the latter (Schmitter-Soto et al. 2017). As for snappers, many species fall within the size range of the lionfish, the most abundant ones being *L. apodus* in the reef and *L. griseus* in mangroves; most individuals found in the reef lagoon tend to be large juveniles or young adults, with larger specimens dwelling deeper in the front reef and younger fish taking refuge among mangrove roots (Nagelkerken 2007).

Stomach contents analysis is the method of choice for identifying specific prey items in diets; Cortés (1997) has advised that data based on volume, based on number of individuals and number of stomachs can be combined in an index of relative importance (e.g. Pinkas et al. 1971). The list of prey species can be greatly expanded if genetic barcoding methods are applied (Valdez-Moreno et al. 2012). On the other hand, a different approach, stable isotope analysis, although losing in taxonomic information, can detect origin signatures of the carbon in fish tissue, thereby determining habitat changes e.g. from mangrove to reef, as well as estimating the trophic level via the isotopes of nitrogen, and combining these to depict trophic niches (Cocheret de la Morinière et al. 2003).The aim of this work is therefore to compare the diets of two potential competitors of lionfish for food resources, grouper *C. cruentata* (Serranidae) and snapper *Lutjanus apodus* (Lutjanidae), in Xcalak, Mexican Caribbean.

Materials and methods

1. *Study area*

Field work was carried out in the reef lagoon and the shallower front reef of Xcalak, Mexican Caribbean (Figure 1). The locality, a marine protected area, is part of the Mesoamerican Barrier Reef System, which in its northern part has a well-developed fringing reef. At Xcalak, the reef barrier is punctuated by a few channels, locally termed "quebrados". The reef lagoon is about 1000 m wide and on average 3 m deep, with seagrasses and coral patches, as well as sand flats and isolated gorgonians (Ruiz-Zárate et al. 2003).

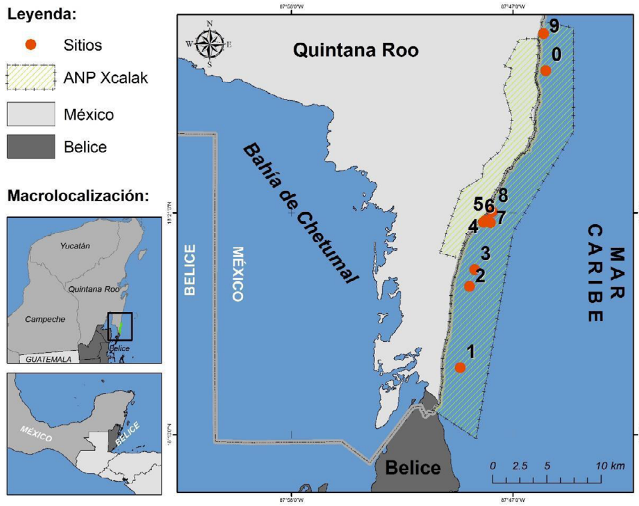

Figure 1. Collection localities (numbered) for *Cephalopholis cruentata*, *Lutjanus apodus*, and *Pterois volitans*, in Xcalak, Mexican Caribbean.

The region has a dry season, usually from February to April, followed by rains in May to September, and a cold-fronts season in October-January (Espinoza-Ávalos et al. 2009). Surface water temperature is high, always above 26 °C, on average 29 °C. Tides are semidiurnal and moderate (a variation of just 20-50 cm), but they induce outward and inward currents through the above mentioned "quebrados" (Hernández-Arana et al. 2009). The coastal current usually flows north, although there can be local eddies and countercurrents. Hurricanes occur from June to October, and they can strongly affect the structure of the reef (Jordán-Dahlgren & Rodríguez-Martínez 1998).

2. *Field and laboratory work*

We sampled in September and October 2014, and March and April 2015, thus representing both the rainy and dry seasons of the region. We actively searched for lionfish, the grouper, and the snapper in the reef lagoon and the shallow front reef of Xcalak, in coral patches and over algae, sand, and seagrass, at depths from 2 to 10 m; we omitted mangrove, where juveniles of the three species are predominant, and deeper reaches of the reef, where larger adults of the three can be found. We used a variety of fishing gear, including cast nets and hook-and-line, but harpoon where snorkeling was most effective. We also acquired specimens of grouper and snapper from local fishermen, and most individuals of lionfish were donated to us by the lionfish control program of the marine park. Given our choice of sampling habitat, most snapper were juveniles (fork length [FL] at first maturity, 25 cm: Froese

& Pauly 2020), whereas most grouper and lionfish were adults (FL at first maturity, 16 cm and 11 cm, respectively: Froese & Pauly 2020). Each sampling expedition lasted 4-5 days, and every sampling event took about 2 hours, during daylight (8:00-18:00 h).

The fish were weighed wet in the field with a scale (to the nearest 0.1 g) and their total length (TL) measured with an ichthyometer (to the nearest 1 mm). Then the stomach was extracted, from the esophagus to the beginning of the intestine (McCleery 2011), and placed in jars with 96% ethanol, which were kept in ice.

In the laboratory, the stomachs were flushed with water and the contents emptied in a petri dish, where they were classified by recognizable items under a binocular microscope (ca. 10X). The prey were identified using appropriate keys and guides, and the number of individuals counted when possible. Also, the volume of every item, from every stomach, was measured by displacement of water in a graduated cylinder (to the nearest 1 ml).

3. *Data analysis*

The percentage of empty stomachs for each species was graphically examined to establish whether foraging activity fluctuated by time of day. To do this, the number of collected specimens was controlled by the number of hours and fishers at every time of day (i.e. two-hour intervals, from 8-10 h to 16-18 h).

Prey accumulation curves were fitted as a function of the number of stomachs, using the Clench model with 100 aleatorizations, a sigmoid curve that incorporates a "learning window" at the start and predicts the "true" richness asymptotically (Jiménez-Valverde & Hortal 2003). For every diet item, three variables were calculated: frequency of occurrence as the percentage of stomachs with the item (F), percent volume (V), and percent abundance, i.e., number of individuals (N). The three variables were examined separately and also combined as the Index of Relative Importance (Pinkas et al. 1971), $IRI = F\ (N + V)$, expressed as a percentage by dividing the IRI of every item by the sum of the IRI values for all items (Dahl & Patterson III 2014).

We also evaluated electivity, by means of Ivlev's index (as modified by Jacobs 1974), $E = (r - p) / (r + p)$, where r is the fraction (relative abundance) of an item in the stomachs and p is the fraction of the same item in the environment. Environmental abundance of prey (only fish prey) was based on the data of a simultaneous study in Xcalak, with visual censuses of fish (Schmitter-Soto et al. 2017; Cobián-Rojas et al. 2018). Values of E toward -1 mean avoidance or inaccessibility, whereas values approaching 1 indicate active selection, and values around zero imply random feeding.

The food items shared by the studied predators were compared by means of Kruskal-Wallis tests (Zar 1998), to detect differences ($p < 0.05$) in abundance, volume, frequency, IRI, or E, using the software Statistica (Statsoft 2007), A complementary, more integral approach to examine trophic overlap of the three species (pairwise) was the use of Schoener's index (Schoener 1968): $D_{jk} = 1 - ½\ Σ|p_{ij} - p_{ik}|$, where p_{ij} and p_{ik} are the fractions of each food item i in the stomachs of species j and k, respectively. Zero implies no overlap at all, and $D = 1$ represents complete overlap between the species being compared; values of $D > 0.6$ are considered to signal substantial dietary overlap (Layman & Allgeier 2012).

Results

We caught 60 specimens of grouper (129-270 mm LT, mean 199 mm; 30-282 g, mean weight 136 g; 97% juveniles), 59 of snapper (110-312 mm LT, mean 213 mm; 21-520 g, mean weight 180 g; 90% adults), and 131 of lionfish (70-380 mm LT, mean 184 mm; 10-712 g, mean weight 106 g; 96% adults). According to the prey accumulation curves, our data represent about 80% of the diet composition for each species.

Empty stomachs reached 44% in lionfish, 53% in the grouper, and 68% in the snapper. For the snapper there was no clear relationship to time of day, whereas for the grouper most of the full stomachs occurred during the early morning (Figure 2).

We identified 16 taxa in stomachs of lionfish, 13 in the grouper, nine in the snapper (Table 1). All three species are predators of fish and crustaceans, although the snapper tended to prefer the latter, whereas the lionfish and the serranid were mostly piscivores (Figure 3). Because there were no environmental data available for crustaceans, electivity could be evaluated only for two species (Table 2): the grouper actively predated on *Hypoplectrus* sp. (E=1), unlike *Halichoeres* sp. and *Stegastes* sp., whereas lionfish selected *Coryphopterus glaucofraenum, C. personatus*, *Holacanthus ciliaris*, *Halichoeres maculipinna*, and *Cantherhines*

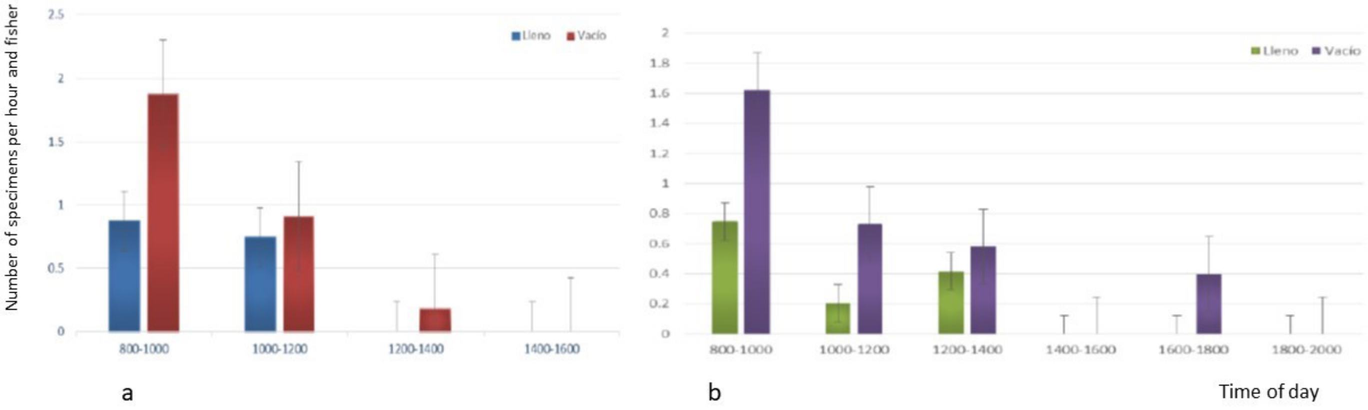

Figure 2. Specimens collected by time of day, controlled by number of hours and fishers, for (a) *Cephalopholis cruentata* and (b) *Lutjanus apodus* in Xcalak, Mexican Caribbean. Bars are standard errors.

pullus, all with E=1. Some lionfish prey occurred in lower abundance in the stomachs than in the environment: *Stegastes partitus*, *Acanthurus coeruleus*, *Halichoeres garnoti*, and *Thalassoma bifasciatum*.

Table 1. Food items and their percent Index of Relative Importance in the diets of grouper *Cephalopholis cruentata*, snapper *Lutjanus apodus*, and lionfish *Pterois volitans* in Xcalak, Mexican Caribbean.

Prey	*C. cruentata*	*L. apodus*	*P. volitans*
Brachyura	8.09	51.95	0.07
Fish remains	14.89	8.96	7.46
Portunidae	0.14	13.16	0
Coryphopterus personatus	0	0	12.95
Palaemonidae	2.50	1.46	10.88
Unidentifiable organic matter	2.04	0.97	10.72
Hypoplectrus sp.	1.46	0	0
Lyosquilla sp.	1.40	0	0
Stegastes sp.	1.09	0	0
Sipunculus cf. *nudus*	0	0.88	0
Gobiidae	0	0	0.60
Thalassoma bifasciatum	0	0	0.59
Cronius sp.	0	0.47	0
Portunus vocans	0.34	0	0
Callinectes sp.	0.15	0.29	0
Coryphopterus glaucofraenum	0	0	0.28
Hydrozoa	0	0.24	0
Stenopus sp.	0.19	0	0
Panulirus sp.	0.14	0	0
Halichoeres sp.	0.12	0	0
Acanthurus coeruleus	0	0	0.05
Balistes vetula	0	0	0.05
Cantherhines pullus	0	0	0.05
Halichoeres maculipinna	0	0	0.05
Halichoeres garnoti	0	0	0.03
Holocanthus ciliaris	0	0	0.03
Stegastes partitus	0	0	0.01
Pomacanthidae	0	0	0.01

The lionfish and the grouper shared as important diet items *Stegastes* sp., *Halichoeres* sp., Brachyura, and Palaemonidae. With the snapper, the lionfish shared as important prey fish remains, Brachyura, and Palaemonidae. In both cases, D > 0.6 (0.86 and 0.99, respectively), which implies a very high overlap in the consumption of these prey.

Most fish prey were consumed in different proportions by lionfish and snapper (usually lower consumption of fishes by the snapper), both by volume and by abundance, except for *Stegastes* sp. and *Halichoeres* sp. On the contrary, palaemonids and brachyurids were eaten in higher volume and abundance by the snapper.

Discussion

We find a substantial overlap, both in the overall diet and in particular prey items, mostly between the lionfish and the grouper, less so between lionfish and snapper. This includes also active electivity of some prey by lionfish and grouper, and foraging probably occurred at the same time of day. Trophic overlap, especially when coupled with active electivity of prey, supports the hypothesis of competition between predators (Barley et al. 2017). Competition of invasive lionfish with native predators has not been definitely proven (Côté et al. 2013); in fact, "proving" competition is controversial, because of "repeated attempts to infer causality from community patterns, without first having understood the mechanisms of the interaction" (Murray & Illius 2000). We do not claim to "prove" competition; however, our study provides evidence of diet overlap, especially in the case of the grouper. In spite of differences in diet composition, lionfish and the grouper significantly share such prey as *Halichoeres* sp. and *Stegastes* sp., as well as brachyurans and palaemonids, which moreover are not captured opportunistically, but actively selected. *Lutjanus apodus* also shares with the lionfish the mentioned crustaceans, although the reliance of the snapper on them is higher.

Time of feeding directly determines percentage of empty stomachs. Both the snapper and the grouper are reported to have crepuscular habits (Randall 1967; Sierra et al. 1994), although we found more empty stomachs for the snapper during the morning, as well as an important proportion of stomachs with already digested contents, which suggests that foraging is mostly nocturnal for the snapper in Xcalak. Lionfish is also thought to hunt mostly at dawn (Green et al. 2011), although Morris & Akins (2009) found them to be more active during the day, and García-Rivas et al. (2018) discovered that younger lionfish search for prey during the night, whereas larger individuals do so during the day. This temporal factor in habitat use contributes to minimize competition of lionfish with the snapper, but probably not with grouper.

We sampled in two seasons of the year, dry and rainy, which differ not just by the influence of rainwater on salinity close to the coast, but also because of the organic enrichment by runoff; we omitted the colder season, which in terms of precipitation is intermediate. These seasonal changes can induce shifts in dominance, especially of benthic invertebrates; Pimentel & Joyeux (2010) did find differences in trophic niche between juvenile snappers due to this seasonal change.

One additional factor that minimizes competition between lionfish and snapper, but not between lionfish and grouper, is that the latter share fishes as preferential prey, whereas the snapper preferred crustaceans, as reported also by Muñoz et al. (2011) and Arredondo-Chávez et al. (2016). Sierra et al. (1994) observed that fish prey have a greater energy content than crustaceans. There are also differences in hunting strategy: snappers tend to hunt in group rather than individually (pers. obs., although up to one-third of lionfish hunts occur in groups, according to García-Rivas et al. 2018). These findings coincide with research in Cuba (Sierra et al. 1994) and the Bahamas (Layman & Allgeier, 2012).

On the other hand, the diets of snappers and also of lionfish shift in varying degrees from crustaceans at young stages towards piscivory at larger sizes (McCleery 2011). This was not evident in the present study

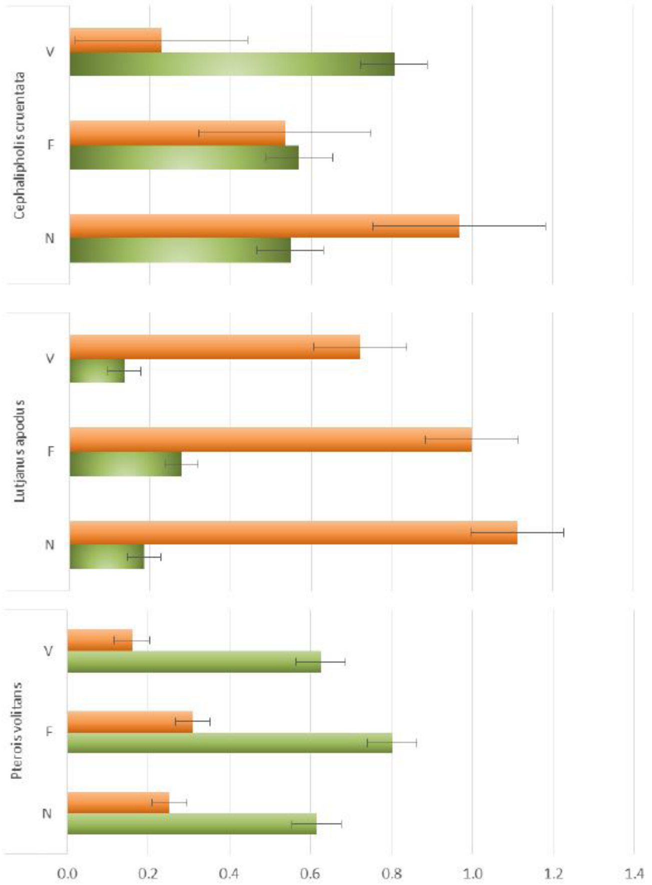

Figure 3. Importance by relative volume (V), frequency of occurrence (F) and abundance (N) of fish (green) and crustaceans (orange) in the diet of *Pterois volitans, Cephalopholis cruentata*, and *Lutjanus apodus* in Xcalak, Mexican Caribbean. Bars are standard errors.

because of the exclusion of the smaller juveniles and the larger adults, but ontogenetic diet changes are common in fishes and are often coupled with habitat shifts, e.g. as juveniles move from mangrove to the reef as they grow (Cocheret de la Morinière et al. 2003; Nagelkerken & van der Velde 2004). Since our study concentrated in just one habitat (the habitat where the three studied species tend to have the same sizes), the results are less confounded by ontogenetic changes, although this means also that the conclusions cannot be extrapolated to other habitats. Prey size, related to gape, can be another way to minimize competition (Barley et al. 2017), but it was uniform as well.

Predation and competition are the main ecological interactions that define community structure (Lönnstedt and McCormick, 2013). The presence of an invasive predator alters prey abundance directly, and competitor abundance indirectly, but both can be significant (Arias-González et al. 2011; Green et al. 2012; Leung et al. 2015). The impact of the invader depends on its life history, including its trophic dynamics, and also on the ecology of the invaded community (Jones & Gomulkiewicz 2012): if the integrity of the invaded community is high (especially in terms of its species richness and the balanced presence of trophic guilds), it can be expected to display biotic resistance to invaders (Albins & Hixon 2011). In particular, it has been suggested that large groupers and sharks could exert some biological control on lionfish (Mumby et al. 2011). However, in the western Caribbean, this has been shown not to be the case in the Bahamas (Anton et al. 2014), Mexico and Cuba (Cobián-Rojas, Schmitter-Soto, Aguilar Betancourt et al. 2018), and also Belize (Hackerott et al. 2013).

The conclusion of Valdivia et al. (2014) is that managers should not rely on native predators to outcompete lionfish, and, same as Cobián-Rojas, Schmitter-Soto, Aguilar Betancourt, et al.(2018), that the biotic resistance hypothesis is most often not supported. Hackerott et al. (2013) found that the density of lionfish had no relationship to the density of native predators. This implies a gloomy outlook for local faunas; generalist predators tend to be more successful as invaders (Muñoz et al. 2011), especially when their strategies are unknown to native prey (Layman & Allgeier, 2012). Because of the similarity in hunting habits, rather than competing with snappers and groupers, the lionfish could be anticipated to compete with the native Caribbean scorpaenid of comparable size, *Scorpaena plumieri*. Arredondo-Chávez et al. (2016)

Table 2. Relative abundance of prey of lionfish *Pterois volitans* and grouper *Cephalopholis cruentata* in Xcalak, Mexican Caribbean, in two natural habitats and in the stomachs. Ivlev's electivity index (E) also shown. Full names of species, in Table 1.

Prey	Reef lagoon	Frontal reef	*P. volitans*	*C. cruentata*	*E*
A. coeruleus	2.5	2.0	1.0	-	-0.3
B. vetula	0	1.0	1.0	-	0
C. pullus	0	0	1.0	-	1.0
C. glaucofraenum	0	0	1.0	-	1.0
C. personatus	0	0	2.9	-	1.0
H. garnoti	2.3	1.5	1.0	-	-0.2
H. maculipinna	0	0	1.0	-	1.0
Halichoeres sp.	2.2	0	-	1.0	-0.4
H. ciliaris	1.0	0	1.0	-	1.0
Hypoplectrus sp.	0	0	-	1.0	1.0
S. partitus	1.8	3.2	1.0	-	-0.5
Stegastes sp.	2.3	0	-	1.0	-0.4
T. bifasciatum	5.7	7.8	1.3	-	-0.7

found that *S. plumieri* preferred octopi, which are rare or absent from lionfish diet; however, based on analyses of stable isotopes of nitrogen and carbon, the same author proved niche overlap not just between both scorpaenids, but also among both the grouper and the snapper.

Raymond et al. (2015) found that prey tend to be captured preferably by the largest predator. Although the size intervals overlapped completely in our samplings, lionfish was largest (380 mm TL), followed by the snapper (310 mm) and the grouper (199 mm). *Pterois volitans* can reach 490 mm in invaded habitats (Darling et al. 2011). This is an additional advantage for the invader in Xcalak and many other Caribbean localities, where larger snappers and groupers, as well as sharks, have become very scarce, due mainly to historical overfishing in addition to coastal development and other impacts on the coral reef (Schmitter-Soto et al. 2017).

Our prey accumulation model fell short of the results of Arredondo-Chávez et al. (2016), who found 79 prey items in lionfish stomachs, quite above the prediction of our model, and also much more than what was reported by Muñoz et al. (2011), who listed only 18 prey items, or even Valdez-Moreno et al. (2012), with 34 species, most of these identified using molecular markers. We think that the difference is due not to sample size (number of stomachs), but rather to geographic diversity: Arredondo-Chávez et al. (2016) worked in six localities spread over the Caribbean coast of Mexico, two of them insular. It is known that lionfish diet varies opportunistically by region and habitat (Pimiento et al. 2013; García-Rivas et al. 2017).

According to Lönnstedt and McCormick (2013), invasive predators with generalist feeding habits are expected to have more severe effects on the invaded community, because of the multiple and often more complex roles that they can play. The wide trophic niche of the lionfish has been confirmed by many authors (McCleery 2011; Muñoz et al. 2011; Ruttenberg et al. 2012; Valdez-Moreno et al. 2012). Notwithstanding, even generalist or opportunistic predators can display some active prey selection. The preference for a given food item can be due to its being more profitable in energetic terms (Begon et al. 1987), or else a methodological artifact, especially in the case of cryptic prey, such as gobies, underestimated in visual censuses (Green et al. 2012). Other important food items for lionfish and its putative competitors at Xcalak, prey such as *S. partitus, A. coeruleus, H. garnoti*, and *T. bifasciatum*, are among the most abundant fish species in the environment (Schmitter-Soto et al. 2017), and yet they had a negative electivity index, which does not support the idea of opportunistic predation.

Prey naïveté has been invoked as an explanation for the competitive advantage of invasive predators: the grouper or the snapper may be recognized as potential predators, whereas lionfish is not, because it has not coevolved with the local ichthyofauna (Côté et al. 2013; Leung et al. 2015). Nevertheless, it is remarkable that local fish species have quite quickly "learned" to "use" the presence of lionfish to their benefit, in spite of facing a new predator: such potential prey as the fairy basslet (*Gramma loreto*) have been found to share refugia with lionfish at night, presumably seeking protection from nocturnal marauders (García-Rivas et al. 2017).

Dahl & Patterson (2014) predicted that the negative impacts of lionfish will surely increase as their populations grow. In addition, the historic or continuing overfishing of native piscivores, such as *Epinephelus guttatus, E. striatus, C. cruentata, C. fulva, L. apodus, L. griseus*, and *L. jocu,* has affected their abundance in Xcalak and generally in the western Caribbean in the last decades (Schmitter-Soto et al. 2017). Moreover, lionfish preys on fish from different trophic guilds (herbivores, detritivores, small predators), as well as diverse macroinvertebrates. Therefore, its impact extends across trophic levels (Lönnstedt & McCormick 2013).

In conclusion, the trophic overlap between the lionfish and the two putative competitors, especially the grouper, was high, including prey that were selected by the predators, and probably at the same time of day, so the hypothesis of competition between them for particular prey is supported. Although the expansion of invasive lionfish in the Caribbean is likely reaching a limit in distribution, abundance, and body size (Cobián-Rojas et al. 2016; Sabido-Itzá et al. 2016), and albeit the diet overlap here shown does not necessarily imply a "domino effect" across the trophic web, as modelled by Arias-González et al. (2011), we think it is most prudent to continue the culling programs of lionfish (Côté et al. 2014), and also to monitor the abundance of possible competitors, such as snapper and grouper, in such long-term endeavors as the Atlantic and Gulf Rapid Reef Assessment Program, AGRRA (Ginsburg & Lang 2003).

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Author Contributions

Brenda Iveth Murillo-Pérez: Substantial contribution in the concept and design of the study and contribution to data collection, as well as contribution to data analysis and interpretation and to manuscript preparation.

Juan Jacobo Schmitter-Soto: Substantial contribution in the concept and design of the study and contribution to data collection, as well as contribution to data analysis and interpretation and to manuscript preparation.

Dorka Cobián-Rojas: Contribution to data collection and adding intellectual content.

Roberto Luis Herrera-Pavón: Contribution to data collection and adding intellectual content.

Conflicts of Interest

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

Ethics

The paper does not involve human beings or clinical trials.

Data availability

The authors are willing to share their data in a public repository, although the data are already available to the public as part of the dissertation by the first author at the Universidad Nacional Autónoma de México.

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Article

Hair as a tool for identification of predators and prey: a study based on scats of jaguars (*Panthera onca*) and pumas (*Puma concolor*)

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Abstract: Microscopic hair identification is a non-invasive, simple, and economical method applied in scientific studies to identify mammal species. In ecology, this method is used mainly in mastofaunistic inventories and dietary studies. In the last decade, the number of dietary studies using the microscopic identification of hairs has grown substantially, but the application of this technique as a tool for the identification of both predators and prey species is still scant. Thus, the aim of this study was to identify predator and prey hairs in scat samples from the two largest species of carnivores in the Neotropical region, the jaguar (*Panthera onca* Linnaeus, 1758) and the puma (*Puma concolor* Linnaeus, 1771). We examined a total of 100 scat samples being 50 from the Pantanal of Mato Grosso do Sul and 50 from the Atlantic Forest of Paraná. We used different identification categories that included the hair microscopic and macroscopic identification, as well as the use of hooves and nails present in the scats associated with tracks and kills found in the field. We identified 57 prey items in the Pantanal samples and 61 in the Atlantic Forest samples. Predator´s hairs were identified in 34% of Pantanal samples and in 46% of Atlantic Forest samples. The combination of hair microscopic and macroscopic characteristics was efficient in the identification of different taxonomic levels, with most identifications reaching the level of the species. However, the methodological protocol for microscopic hair identification was not fully effective in obtaining all the microstructural patterns of the studied mammals. Adjustments in the technique are necessary to differentiate microstructural characteristics of species belonging to the same family. We recommend macroscopic identification of scat content items (hairs, hooves or nails) of both prey and predators to be used to complete the microscopic hair identification technique in dietary ecological studies.

Keywords: *Macroscopic identification; Microstructure hair; Tricology; Guard-hair; Predator hairs; Brazil.*

Pelos como ferramenta de identificação de predadores e presas: um estudo baseado em fezes de onças-pintadas (*Panthera onca*) e pumas (*Puma concolor*)

Resumo: A identificação microscópica do pelo é um método não invasivo, simples e econômico, aplicado em estudos de identificação de várias espécies. Em ecologia, esse método é usado principalmente em inventários mastofaunísticos e estudos de dieta. Na última década, a identificação microscópica de pelos em estudos de dieta tem crescido substancialmente, porém ainda conhecemos muito pouco sobre o uso das características microscópicas e macroscópicas dos pelos para identificação tanto de presas quanto de predadores. Assim, o objetivo deste estudo foi identificar pelos de predadores e presas em amostras fecais das duas maiores espécies de carnívoros da região Neotropical, onças (*Panthera onca* Linnaeus, 1758) e pumas (*Puma concolor* Linnaeus, 1771). Foram examinadas um total de 100 amostras de fezes, provenientes do Pantanal de Mato Grosso do Sul e da Mata Atlântica do estado do Paraná, sendo 50 amostras de cada local. Utilizamos diferentes categorias de identificação que incluíam a identificação microscópica e macroscópica do pelo, bem como o uso de outros vestígios como cascos e unhas presentes nas fezes e pegadas e carcaças encontradas em campo. Nós identificamos 57 itens de presas nas amostras do Pantanal e 61 itens de presas nas amostras da Mata Atlântica. Pelos dos predadores foram identificados em 34% das amostras do Pantanal e 46% das amostras da Mata Atlântica. A combinação de características microscópicas e macroscópicas dos pelos foi eficiente na identificação de diferentes níveis taxonômicos, com a maioria das identificações atingindo o nível da espécie. No entanto, o protocolo metodológico de identificação microscópica dos pelos não foi totalmente eficaz para obter todos os padrões microestruturais dos mamíferos estudados. São necessários ajustes na técnica para diferenciar características microestruturais de espécies pertencentes à mesma família. Recomendamos que a identificação macroscópica de itens alimentares (pelos, cascos ou unhas) tanto de presas quanto de predadores seja usada para completar a técnica de identificação microscópica dos pelos em estudos de ecologia alimentar..

Palavras-chave: *Identificação macroscópica; Microestrutura do pelo; Tricologia; Pelos-guarda; Pelos de predadores; Brazil.*

Introduction

Populations of carnivore species are vital in the dynamic of their communities, exerting a direct influence on prey density or indirect in the plant community (Terborgh 1992, Borer et al. 2005). This top-down effect is based on the trophic cascade model in which the presence of predators affects the regulation of population density at other trophic levels (Terborgh et al. 2001). In this sense, felines are considered key species within an ecosystem because their presence controls the population size of their available prey and their absence causes population imbalance at other trophic levels (Schonewald-Cox et al. 1991). In addition, the conservation of feline species is crucial for biodiversity protection, as they require large areas with diverse habitat types and prey and the protection of these areas increases the chance of survival of other species (Carroll et al. 2001). Indeed, felines are considered bioindicators of the environment and studies related to ecological aspects of this group are important for the conservation of biodiversity (Santos et al. 2019).

Knowledge about dietary habits contributes for the understanding of ecological processes necessary for the protection of biodiversity, such as reproductive and social behavior, distribution, population density and habitat use (Juarez & Marinho-Filho 2002, Bueno & Motta-Junior 2004, Azevedo 2008). For this purpose, fecal screening methods and hair microstructure analysis have been conducted in studies on dietary ecology of felines (Chakraborty & Chakraborty 1996, Martins et al. 2008, Tortato 2009, Perilli et al. 2016, Tirelli et al. 2018). The analysis of internal hair morphology using optical microscopy is a simple and economical noninvasive method applied in several species' identification studies (Dagnall et al. 1995, Wolfe & Long 1997, Alberts et al. 2017, Felix et al. 2019, Mandoreba et al. 2019). In predator dietary studies, bone and tooth fragments found in fecal specimens may have little diagnostic value because they are often damaged due to intense fragmentation due to chewing (Chehébar & Martin 1989). Thus, hair identification in scat samples can increase species identification accuracy, as hairs are less damaged in the digestive process (Quadros & Monteiro-Filho 1998). However, this technique has limitations in the identification of feline hair, showing a high overlap in morphological characters between different species (Vanstreels et al. 2010, Rinaldi et al. 2015). This overlap occurs mainly when considering the morphological differences between small cats (Alberts et al. 2017).

Studies involving microscopic identification of hair spread around the world, bringing scientific and technical advances to the method, with the creation of keys and regional identification guides (Walllis 1993, Quadros & Monteiro-Filho 2010, Alberts et al. 2017). In the Neotropics, there has been an increasing number of studies involving the mammalian capillary microstructure (Rinaldi et al. 2015, Alberts et al. 2017, Tirelli et al. 2018). Consequently, the use of microscopic identification of hair in scat samples have increased in recent years (Rinaldi et al., 2015, Alberts et al., 2017, Kshettry et al., 2018, Tirelli et al., 2019). However, the application of this technique as a tool for the identification of both predators and prey species is still scant. Thus, the objective of this study was to use the technique of microscopic identification to identify hairs of predators and prey from scat samples of the two largest carnivores species in the Neotropics, jaguars (*Panthera onca* Linnaeus, 1758) and pumas (*Puma concolor* Linnaeus, 1771) from two different sites in Brazil, South America.

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Material and methods

Our study was part of two projects conducted from 2002 to 2005 in the Pantanal region and from 2012 to 2013 in the Atlantic Forest, Brazil, to investigate jaguar and puma food habits, respectively. Our first study site was a 150 km^2 working cattle ranch/wildlife reserve comprised of wetlands habitats, seasonally inundated grasslands and woodlands, in the southern region of Mato Grosso do Sul State (20º 05´S and 56º 36´W). Both jaguars and pumas occurred in this site. The second site was a 50 km^2 private area comprised of scattered fragments of Atlantic forest embedded in a matrix of agricultural land use interspersed with human habitats comprising on the northern region of Paraná State (23°35' S e 52°20' W). It is highly probable that only pumas occurred in this site. The closest known jaguar population is located at approximately 250 km in the Iguaçu National Park, and movement of jaguars towards our surveyed area is very unlikely due to the lack of forested habitat in between those areas.

1. *Scat analysis*

We collected scats opportunistically on trails and roads in both study sites. Only scats associated with jaguar and puma´s tracks or kills were included for the purpose of our analyses. Fifty (50) samples were randomly selected from a pool of samples from each study site (n = 149, Pantanal; n = 82, Atlantic Forest) and screened to test the method to identify prey and predator hairs through slides of cuticular impression and medullary preparation. We dried scats at 72°C for 24 h. After that, we immersed scats in water for 24-48 hours until soft. Then we washed and broke up scats with running water through a fine mesh sieve. Food contents were identified to families, genus, and species using macroscopic and microscopic characteristics of hair, nails, and hooves via comparison with reference material (Azevedo 2008).

A portion of each sample went through the screening process and the hair found was stored in containers and labeled. The hair was cleaned with ethyl alcohol and dried on absorbent paper. We prepared hairs and analyzed the medullary structures and cuticle scale patterns following Quadros and Monteiro-Filho (2006a) with some few adaptations. For the medullar preparation, we added hydrogen peroxide plus commercial ammonia solution and bleaching powder. Thus, in the case of thin hairs, we placed those in hydrogen peroxide 30 volumes plus commercial ammonia solution and bleaching powder for 90 minutes, so the hair color is washed and hair becomes transparent. Thick hairs of white-lipped and collared peccaires (Tayassu pecari Link, 1795; Pecari tajacu Linnaeus, 1758), capybara (Hydrochoerus hydrochaeris Linnaeus, 1766), paca (Cuniculus paca Linnaeus, 1766), and giant anteater (*Myrmecopha tridactyla* Linnaeus, 1758) were placed in 40 volumes hydrogen peroxide, commercial ammonia solution and bleaching powder for 100 minutes. Transverse cuts in the hair shield were made on thicker hairs, thus allowing better penetration of the solution used in the discoloration. After these procedures, the hair was washed in water and permanent slides were made using Entelan or the colorless enamel as mounting medium. For the observation of cuticle scale patterns, the already cleaned hairs were placed on a slide covered with colorless enamel or commercial base, which was previously dried between 15 and 20 minutes. The time varied in relation to the temperature and humidity of the place where the slides were prepared. After this procedure, hairs were placed on the slide and pressed between two wooden boards in a vise, thus obtaining the cuticular impression. The impressions of the hair on the enamel stayed at least 30 minutes drying and after this time the hair was removed from the slides. Predator's hairs were identified by the same procedures used to make slides for prey species. When predator hairs could not be identified by the cuticular and medullary patterns together, we used some macroscopic characteristics in the identification, such as shape, thickness, size, or bandage color. Feline hairs can be found together with hairs of its prey because of their self-cleaning behavior when the hair is ingested, thus allowing its identification in the scats (Eckstein & Hart 2000). Hairs that could not be identified as predator hair were also included in the study because scats were associated with predators' tracks or kills.

Cuticle scale patterns and medulla structure were visualized under a light microscope with magnifications of 100, 200, and 400x. Photomicrographs were obtained using the Image – Pro Plus software and camera attached to an Olympus BX51 microscope. For a better understanding of the variation in patterns found along the hairs, we took photos of the cuticular scales on the bulb (most proximal part of the hair), shaft (part between the bulb and hair shield), and shield (most distal end of the hair). For the medullary patterns, we took photos of mainly the shield region of each guard hair. The cuticular and medullary patterns cited for the studied species follow the nomenclature and definitions proposed by Quadros & Monteiro-Filho (2006a). To describe the patterns found, we used photomicrographs, descriptions and drawings of the works of Quadros & Monteiro-Filho (2006b). Subsequently, the images obtained from these slides were compared with a reference collection of hair microstructural images of fifty mammal species obtained from museums, the university Collections (LEVE - Laboratory of Vertebrate Ecology – University Federal of São João del Rei), keys contained in published articles and identification guides (Quadros & Monteiro-Filho 2010, Miranda et al. 2013).

1.2. *Data analysis*

We identified prey hairs according to six different categories: i. Hair microstructure (cuticle and medulla); ii. Hair microstructure (only medulla); iii. Hair microstructure (cuticle or medulla) and macrostructure (shape, thickness, size or colors); iv. Hair microstructure (cuticle or medulla) and hooves or nails; v. Hair macrostructure and hooves, and vi. Hair macrostructure (shape, thickness, size or colors). We identified predator´s hairs by only two categories: i. Hair microstructure (cuticle and medulla) and ii. Hair microstructure (cuticle or medulla) and macrostructure (shape, thickness, size or colors). As the cuticle has only low diagnostic power to differentiate hairs of pumas and jaguars, we associated the identification of cuticle and macrostructure of the hair with tracks and kills found together with the scats. We performed Chi-square goodness-of-fit tests using R (version 4.0.2) to examine the relation between the different categories of the technique of microscopic identification of hairs and the efficiency to identify items in the predators' scats (Octenjak et al. 2020).

Results

1.1. *Diet analysis by identification categories*

We found a total of 94 scat content items in the Pantanal samples. Of those, we identified 74, being 57 (77.0%) and 17 (23%) items of prey and predators, respectively. We found that the relation between categories of microscopic identification of hairs and the efficiency to identify items in predators´ hairs was significant, $\chi2$ (5, N = 57) = 130.26, $p < 0.001$. Hair microstructure (cuticle and medulla) was the most efficient category in identifying hairs of prey species. We identified predators' hairs in 17 (34%) of the Pantanal samples using category hair microstructure (cuticle or medulla) and macrostructure (n = 12 items, 70.6%) and hair microstructure (cuticle and medulla) (n = 5 items, 29.4%). We did not identify 20 (21.3%) of the total items using our described categories. For the Atlantic Forest samples, we found a total of 93 scat content items. Of those, we identify 84, being 61 (75.3%) of prey species, and 23 (24.7%) of predators' species (Table 1). We found that the relation between categories of microscopic identification of hairs and the efficiency to identify items in predators´ hairs was significant, $\chi2$ (5, N = 61) = 82.31, $p < 0.001$. As for the Pantanal samples, hair microstructure (cuticle and medulla) was the most efficient category in identifying hairs of prey species of the Atlantic Forest samples. We identified predators' hairs in 23 (46%) of the Atlantic Forest samples mainly using hair microstructure (cuticle and medulla) (n = 15 items, 65.2%) followed by hair microstructure (cuticle or medulla) and macrostructure (n = 8 items, 34.8%) categories. We did not identify 9 (9.7%) of total items from the described categories. For the Pantanal samples, we identified jaguar, domestic cattle, capybara, paca, *Mazama* genus and the families Tayassuidae and Muridae using more than one identification category (Table 2). For the Atlantic Forest, we identified capybara, paca, and the Tayassuidae and Muridae families using more than one identification category (Table 3).

1.2. Identification of mammals using microscopic and macroscopic characteristics of hair

We identified hairs of capybara macroscopically due to their dark brownish tone and large width-thickness. Microscopically, hairs were identified without a medulla in the shield and with a longitudinal division in the axis (Figure 1a). We observed the medullary pattern polygonal on the shaft (Figure 1b) and glandular on the shield (Figure 1c). The cuticle was transverse wavy on the bulb (Figure 1d) and transverse wavy with ornate edges on the shield (Figure 1e). Paca guard hairs were macroscopically identified due to their characteristic aculiform appearance presenting a clear difference in thickness between the short bulb and the marked hair shield. The microstructural patterns found in the medulla were polygonal on the shaft (Figure 1f) and glandular on the shield (Figure 1g). The cuticular pattern observed in paca was irregularly wavy on the bulb (Figure 1h).

Red brocket deer (*Mazama gouazoubira* Fischer, 1814) and gray brocket deer (*Mazama americana* Erxleben, 1777) were differentiated by the ornamentation of the most distal portion of the shield. In addition to these features, the gray brocket deer cuticular scale was smoother and more regular on the bulb than the red brocket deer, which was somewhat irregular in orientation and had slight ornamentation on the cuticle edge (Figure 1i). Through these cuticular characteristics and the polygonal pattern (Figure 1j), only the red brocket deer species was identified for jaguar samples. For puma samples, we identified deer species to the taxonomic genus level, because besides the occurrence of gray brocket deer and red brocket deer in the region, other species such as the Brazilian dwarf brocket deer (*Mazama nana* Hensel, 1872), the small red brocket deer (*Mazama bororo* Duarte, 1996), and the pampas deer (*Ozotocerus bezoarticus* Linnaeus, 1758) may occur in the state of Paraná (Duarte et al. 2012, IUCN 2020). The microstructural patterns of the latter two species have neither been yet described nor differentiated in the literature. In addition, reference collection of hair images showing microstructural patterns utilized in this study did not contain hair and photomicrographs of these species, thus precluding us from further comparisons and limiting our identification to genus level. The marsh deer (*Blastocerus dichotomus* Illiger, 1815) was identified by visual patterns of the guard hair as the reddish color and the wavy appearance on the shield. The microstructural patterns presented a polygonal pattern in the medulla (Figure 1k) and transverse wavy pattern in the cuticle (Figure 1l).

The two species of the genus Tayassuidae, the white-lipped peccary and the collared peccary could not be distinguished from each other by the cuticular and medullary patterns. For both species, the cuticle presented a transverse wavy pattern in the bulb (Figure 1m) and the medulla was cordonal in the shield (Figure 1n). In this case, the hair identification technique was effective in identifying the hair of wild pigs only at the family level.

We distinguished predators' hairs from their prey by cuticular and medullary patterns and macroscopic characteristics associated with the size, shape, thickness, and colors of hairs. For the jaguar, the medulla was trabecular, narrow, and with fringed edges (Figure 1o). For the puma, the medulla was trabecular, wide, and with fringed edges (Figure 1p). The cuticular pattern observed in both species was transverse wavy with smooth edges on the bulb (Figure 1q and 1r). In the samples from Paraná, we identified pumas' hairs associating cuticular and medullary patterns. In samples from Pantanal, we identified jaguar's hairs by

Table 1. Percentage of occurrence of prey items found in scat samples from the Pantanal of Mato Grosso do Sul State and Atlantic Forest of Paraná State, according to the identification of categories.

Categories of prey identification	Pantanal (N = 57)	Atlantic Forest (N = 61)
Hair microstructure (cuticle and medulla)	68.42 (39)	52.45 (32)
Hair microstructure (only medulla)	0	1.64 (1)
Hair microstructure (cuticle or medulla) and macrostructure	28.07 (16)	32.79 (20)
Hair microstructure (cuticle and medulla) and hooves or nails	0	6.56 (4)
Hair macrostructure and hooves	1.75 (1)	0
Hair macrostructure	1.75 (1)	6.56 (4)

Table 2. Proportion of prey and predator items of samples from the Pantanal according to categories of identification. N= number of scat content items.

Identified mammals	N	Hair microstructure (cuticle and medula)	Hair microstructure (cuticle or medula) and macrostructure	Hair macrostructure and hooves	Hair macrostructure
Ordem Carnivora					
N. nasua (Linnaeus, 1766)	2	100	-	-	-
P. onca (Linnaeus, 1758)	6	66.67	33.33	-	-
P. concolor (Linnaeus, 1771)	1	100		-	-
Felidae	10	-	100	-	-
Ordem Artiodactyla					
Mazama sp.	2	50	50	-	-
M. americana (Erxleben, 1777)	2	100		-	-
B. dichotomus (Illiger, 1815)	2	100		-	-
B. taurus (Linnaeus,1758)	4	50	50	-	-
Sus scrofa (Linnaeus, 1758)	1	-	-	1	-
Tayassuidae	8	75	25	-	
Ordem Rodentia					
C. paca (Linnaeus, 1758)	3	33.3	66.67	-	-
H. hydrochaeris (Linnaeus, 1766)	20	55	40	-	5
Dasyprocta sp.	2	100		-	-
Muridae	5	80	20	-	-
Ordem Pilosa					
T. tetradactyla (Linnaeus, 1758)	1	100	-	-	-
M. tridactyla (Linnaeus, 1758)	2	100	-	-	-
Ordem Didelphimorphia					
Didelphidae	2	100	-	-	-
Ordem Primates	1	100	-	-	-

cuticular and medullary patterns obtained together or associated with cuticular patterns, macroscopic characteristics and evidence found in the field such as tracks and kills.

Domestic cattle (*Bos taurus* Linnaeus,1758) was identified for presenting large amounts of white hair and the presence of leather in the samples. In addition to the macroscopic aspects of the hair, this specie was identified by the amorphous pattern of the medulla in the shield (Figure 1s) and transverse wavy cuticle in the bulb (Figure 1t). Tapir was microscopically identified by transverse wavy cuticle (Figure 1u) and medulla trabecular (Figure 1v) and the presence of a characteristic nail. Domestic pig (*Sus scrofa* Linnaeus, 1758) was also macroscopically identified by thick and black hairs and the presence of characteristic hoof. The cuticular impressions of the hair were uneven, precluding us to characterize the cuticular patterns.

Procyonids were identified by amorphous medullar patterns (Figure 2a) and irregular wavy cuticle pattern for the crab eating raccoon (*Procyon cancrivorus* Cuvier, 1798) (Figure 2b) and the coati (*N. nasua* Linnaeus, 1766) (Figure 2c). The genus *Dasyprocta* was identified by the alveolar medulla (Figure 2d) and transverse wavy cuticle with smooth edges in the bulb (Figure 2h). The southern tamandua (T. tetradactyla Linnaeus, 1758) was identified without medulla in the shield (Figure 2e) and with an ornate transverse wavy cuticle (Figure 2f). The giant anteater (M. tridactyla Linnaeus, 1758) was also identified without medulla on the shield, but with a smooth edge transverse wavy cuticle on the bulb (Figure 2g).

The orders Muridae and Didelphidae were identified with medulla alveolar (Figure 2i) and literary, respectively (Figure 2l), and both with petal cuticle (Figure 2j and Figure 2k). The order Primates was identified by the discontinuous uniseriate medullary pattern (Figure 2m-n) in the hair shield and cuticle with a transverse wavy pattern in the bulb (Figure 2o) and transverse wavy with ornamented edges in the shield (Figure 2p).

Discussion

In our study, most prey identification at species level was only possible when we associated the hair microstructural patterns with its macroscopic characteristics, such as shape, thickness, size or color. In addition, it was possible to diagnose predator's hair when present, using cuticular and medullary patterns together, associating cuticular or medullary patterns with hair macroscopic characteristics. Although the technique was efficient in identifying most species, some limitations in

Table 3. Proportion of prey and predator items of samples from the Atlantic Forest of Paraná according to the identification categories. N = number of items.

Identified mammals	N	Hair microstructure (cuticle and medula)	Hair microstructure (cuticle or medula) and macrostructure	Hair microstructure (only medulla)	Hair microstructure (cuticle and medula) and hooves or nails	Hair macrostructure
Order Carnivora						
N. nasua (Linnaeus, 1766)	2	-	-	-	100	-
P. cancrivorus (Cuvier, 1798)	2	100	-	-	-	-
P. concolor (Linnaeus, 1771)	15	100	-	-	-	-
Felidae	8	-	100	-	-	-
Order Artiodactyla						
Mazama sp.	6	100	-	-	-	-
Tayassuidae	3	75	-	25	-	-
Order Rodentia						
C. paca (Linnaeus, 1758)	18	55.56	44.44	-	-	-
H. hydrochaeris (Linnaeus, 1766)	20	30	50	-	-	20
Dasyprocta sp.	3	100	-	-	-	-
Muridae	2	50	50	-	-	-
Order Pilosa						
T. tetradactyla (Linnaeus, 1758)	1	100	-	-	-	-
Order Perissodactyla						
T. terrestris (young) (Linnaeus, 1758**)**	2	-	-	-	100	-
Ordem Didelphimorphia						
Didelphidae	1	-	100	-	-	-
Ordem Primates	1	100	-	-	-	-

obtaining microstructural patterns of hairs are important. For instance, hairs of capybara were identified using more categories than other species because of the low efficiency in clearly obtaining the medullary and cuticular patterns described in the literature for this species (Quadros & Monteiro-Filho 2010). Thus, one of the obtained patterns (cuticular or medullary) together with the hair macroscopic characters or even only these macroscopic characters of the hair, were necessary to identify the species. The polygonal medullary pattern for capybaras, not identified by Quadros & Monteiro-Filho (2010), corroborates the pattern found in other studies (Penna 2009).

It was not possible to differentiate species of Tayassuidae family as there is overlap of cuticle and medulla characteristics between collared peccary and white-lipped peccary. For this family, macroscopic identification together with medullary microestructure of the hair became important when it was not possible to obtain the characteristic cuticular pattern of the family. For species of the Tayassuidade family, the macrostructure of the hair should be used with caution. Although not the same, the shape, thickness, and color of the hair can be confused with these same characteristics of giant anteater hair. Thus, we believe that only the hair macrostructure is not recommended to diagnose the presence of one of the Tayassuidae species (or both) or to tell them apart from the giant anteater. Other techniques, such as morphometric analysis, cryosectioning technique, and cross-sections may be necessary to visualize possible differences (Felix et al. 2019, Mandoreba et al. 2019), but these techniques can be costly and unavailable for most field studies.

The presence of tapir hairs in one of the puma samples was important because of the uniqueness of this finding in puma´s diet. In places where pumas and tapir live in sympatry, predation of tapir by pumas is a rare event (Azevedo et al. 2016). Failure to identify tapirs by medullary patterns may be related to the type of hair in the sample. From the observed medullary pattern and the morphological aspect of the hair, it can be inferred that the hairs analyzed were probably under hairs, which are predominant in young individuals. Thus, it is likely the identification of other patterns than those cited in the literature, because those of young individuals (under hairs) are not commonly used for identification purposes (Quadros & Monteiro-Filho 2006a).

The order Primates was identified in both samples with very low relative frequency of occurrence. This result corroborates what has been found in other studies that also indicated that primates are not frequently consumed by jaguars (Azevedo 2008, Martins et al. 2008). The macroscopic appearance of hairs was not used in the identification,

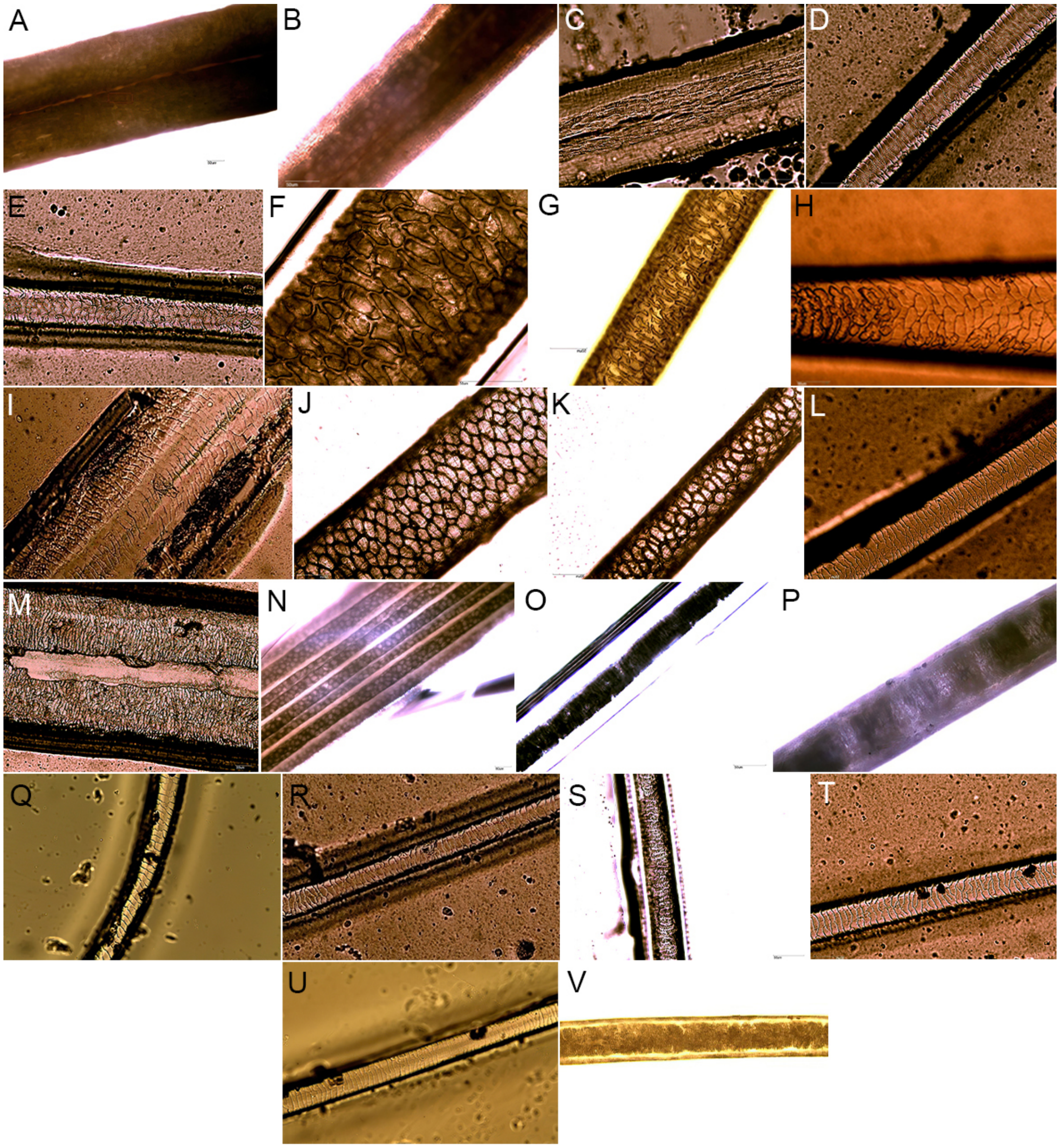

Figure 1: Microscopic identification of hairs of species, genus and orders of mammals using cuticular and medullary patterns. 1a-c medulla of Capybara (*H. hydrochaeris* -200x); 1d-e cuticle of Capybara (200x); 1f medulla of Paca (*C.paca*- 400x); 1g medulla of Paca (200x); 1h cuticle of Paca (200x); 1i cuticle of Red brocket deer (*Mazama gouazoubira* - 200x); 1j medulla of Red brocket deer (200x); 1k medulla of Marsh deer (*B. dichotomus*- 400x); 1l. cuticle of Marsh deer (200x); 1m cuticle of Tayassuidae (100x); 1n medulla of Tayassuidae (100x); 1o medulla of Puma (*Puma concolor* - 200x)*;* 1p medulla of Jaguar (*Panthera onca* - 200x) 1q cuticle of Puma (200X); 1r cuticle of Jaguar (200x); 1s medulla of *Bos taurus* (200x); 1t cuticle of *Bos taurus* (200x)*;* 1u cuticle of Tapir (*Tapirus terrestris* - 200x)*;* 1v medulla of Tapir (200x).

because the visual characteristics of these hairs indicated a wide possibility of potential prey species. Therefore, hairs from the order Primates were only identified through the microstructural patterns.

We should be cautions when analyzing and identifying the microstructures of guard hairs of the order Artiodactyla, as species are differentiated by subtle characteristics that are not easily recognized. Vázquez et al. (2000) studying hairs of red and gray brocket deer could not differentiate them based on the medullary and cuticular patterns of the guard hairs. However, studies that included analysis of hairs from artiodactyls from other parts of the world reported subtle differences between hair microstructures such as the borders´ ornamentation of the cuticle and the distance between the borders (De Marinis & Asprea 2006). Thus, the correct identification of these species requires clear cuticular patterns along with the characteristic polygonal medullary pattern.

The identification of feline hairs is also quite complicated, thus difficulting the differentiation of species. There are subtle differences among Brazilian feline hair microstructures but overlapping morphological patterns may render this technique inaccurate for scientific applications (Vanstreels et al. 2010, Rinaldi et al. 2015, Alberts et al. 2017). In this study, puma and jaguar medullary patterns were different across the ratio of medulla width to maximum hair width. In

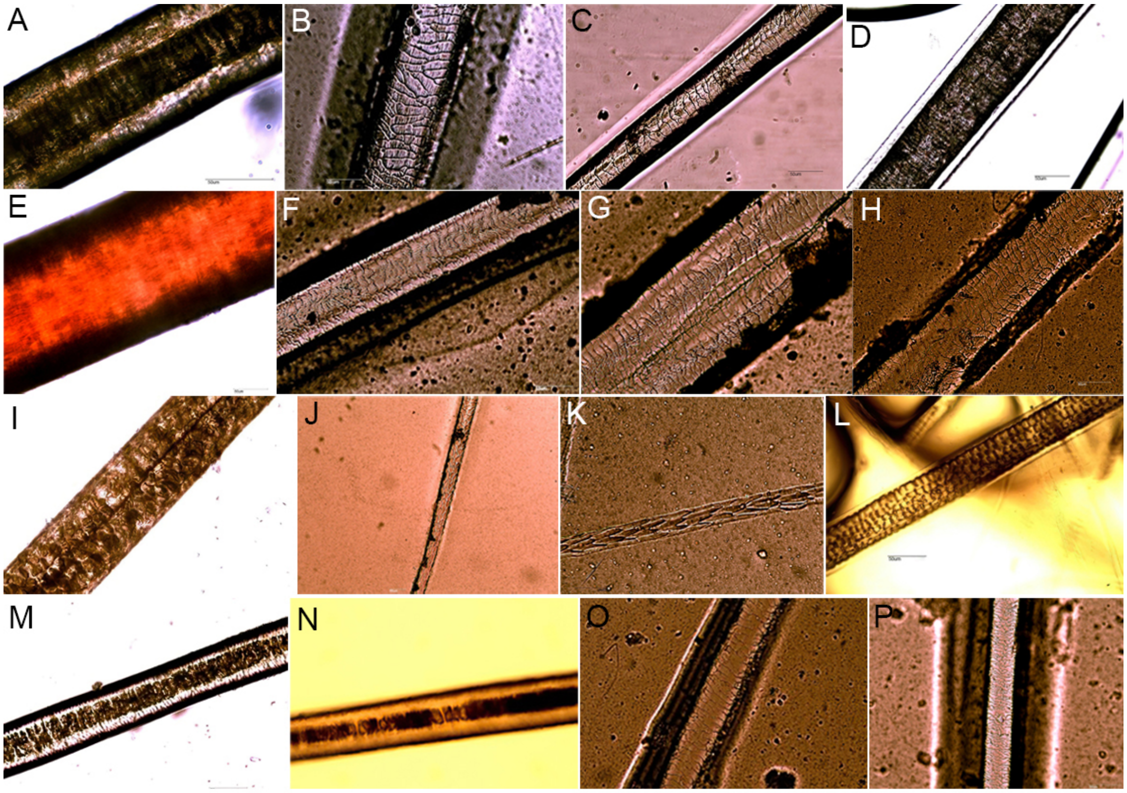

Figure 2: Microscopic identification of hairs of species, genus and orders of mammals using cuticular and medullary patterns (200x). 2a medulla of Procyonids (200x); 2b cuticle of Crab eating raccoon (*Procyon cancrivorus* - 200x); 2c cuticle of Coati (*N. nasua* - 200x); 2d. medulla of Dasyprocta (200x); 2e medulla of Southern tamandua (T. tetradactyla - 200x) and the Giant anteater (*M. tridactyla*); 2f cuticle of Southern tamandua (*T. tetradactyla*); 2g cuticle of Giant anteater (*M. tridactyla*); 2h; cuticle of *Dasyprocta* (200x); 2i medulla of Muridae (200x); 2j. cuticle of Muridae (100x); 2k. cuticle of Didelphidae (200x); 2l medulla of Didelphidae (200x); 2m-n medulla of Primates (200x); 2o-p cuticle of Primates (200x).

some samples, it was not possible to obtain the predator's medullary pattern, because of the small quantities of thin and small hairs. Thus, the use of diaphanizers can hinder visualization of the medullary pattern of the threads. In this case, we used the cuticular pattern wavy and transversal and macroscopic hair characteristics such as size, shape, thickness, and banding of colors. These characteristics indicated that hairs would belong only to a puma or a jaguar. For the Pantanal samples, we also found one sample containing hairs of puma, the other large predator that inhabits the surveyed site. This finding could indicate an event of intraguild predation (i.e. predation between two potential competing predator species, Arim & Marquet 2004) or that one of them may have eaten the recently dead carcass of the other one. In our study, we used tracks of adult individuals or recent dead carcasses associated with scats to minimize chances of collecting scats of small felid species.

Finally, procyonid species (crab eating raccoon and coati) were mainly identified and differentiated by medullary patterns, as both species had an irregular wavy cuticular pattern on the hair shaft. For coatis, the nails found in the samples became indispensable for identification when medullary patterns could not be visualized.

Conclusion

The microscopic hair identification technique using cuticular and medullary patterns was efficient to identify different taxonomic levels, with most identifications reaching the species level. However, the methodological protocol was not fully effective to obtain all microstructural hair patterns of the mammals presented in our samples. The overlapping of microstructural characteristics among guard hairs of different species and the presence of under hairs in the samples limited the hairs identifications to species levels. It is important that studies aiming to investigate dietary patterns of feline predators, combine the use of microscopic and macroscopic technique to improve the efficiency in the identification of hairs of prey and predator species.

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Author Contributions

Fernando Cesar Cascelli de Azevedo: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to manuscript preparation and critical revision. Fernanda Souza: Contribution to data analysis and interpretation; contribution to manuscript preparation.

Conflicts of interest

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

Ethics

The procedures described in this study have no conflict with the Brazilian Laws regarding the use of animals in scientific research.

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Article

Diversity of Cerambycidae (Insecta: Coleoptera) in the Cerrado of Central Brazil using a new type of bait

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Abstract: The Cerambycidae family (Insecta: Coleoptera) has approximately 38 thousand species. In Brazil, more than 4,300 species and 1,050 genera are registered, and despite the ecological and agricultural importance of this family, no study has yet been done in the Cerrado of the Distrito Federal (Brazil). The objective of this study was to evaluate the richness and abundance of Cerambycidae in the Cerrado area using two types of fruits (banana and pineapple), fermented with sugarcane juice, as bait and to verify whether the richness is influenced by climate variables. The work was carried out in an area of the cerrado *sensu stricto* at Água Limpa Farm in the Distrito Federal. Beetles were collected weekly using 40 bait traps with two types of fruits that remained in the field for 12 uninterrupted months (November 2013 to October 2014). The traps were installed 1.50 m above the ground level, distributed in four 80 m transects, and spaced 20 m apart. A total of 1,599 individuals, belonging to 13 genera and different 19 species, were collected. The main species were as follows: *Oxymerus basalis* (Dalman, 1823) representing 78.3%, *Retrachydes thoracicus thoracicus* (Olivier, 1790) representing 9.9%, and *Chydarteres bicolor* (Fabricius, 1787) representing 4.5% of the total specimens collected. There was a significant difference in richness and abundance of Cerambycidae among the baits evaluated, with the pineapple bait presenting the highest values. The greatest number of individuals and species occurred soon after the first rains, especially in November. Temporal variation was confirmed through Rayleigh's uniformity test, following the seasonality of the Cerrado, with the greatest number of individuals and species found in the rainy season. Temperature and humidity influenced the richness of cerambycid beetles. This is the first work carried out with pineapple fermented with sugarcane juice as bait to capture Cerambycidae, and this type of bait proved to be efficient for the collection of insects, comparable in efficiency to the synthetic baits that are normally used. All species collected were new distribution records for the Distrito Federal (Brazil).

Keywords: Fermented fruit; Climate variables; Baited trap; Longhorn beetles; New records.

Diversidade de Cerambycidae (Insecta: Coleoptera) no Cerrado do Brasil Central utilizando um novo tipo de isca

Resumo: A família Cerambycidae (Insecta: Coleoptera) possui aproximadamente 38 mil espécies. No Brasil são registradas mais de 4.300 espécies e 1.050 gêneros e, apesar da importância ecológica e agrícola desta família, ainda não foram realizados trabalhos no Cerrado do Distrito Federal. O objetivo deste trabalho foi avaliar a riqueza e abundância de Cerambycidae utilizando dois tipos de frutas (banana e abacaxi) fermentadas com caldo de cana em área de cerrado e verificar se a riqueza é influenciada pelas variáveis climáticas. O trabalho foi realizado em uma área de cerrado *sensu stricto* na Fazenda Água Limpa (FAL) em Brasília, Distrito Federal. A coleta de adultos de Cerambycidae foi realizada semanalmente usando 40 armadilhas iscadas com os dois tipos de frutas que ficaram em campo por 12 meses ininterruptos (novembro de 2013 a outubro de 2014). As armadilhas foram instaladas a

1,50 m do nível do solo, distribuídas em quatro transectos de 80 m e espaçadas 20 m entre si. Foram coletados 1.599 indivíduos, 13 gêneros e 19 espécies. As principais espécies foram *Oxymerus basalis* (Dalman, 1823) com 78,3% do total de espécimes coletado, *Retrachydes thoracicus thoracicus* (Olivier, 1790) com 9,9% e Chydarteres bicolor (Fabricius, 1787) com 4,5%. Houve diferença significativa na riqueza e abundância de Cerambycidae entre as iscas avaliadas, sendo a isca de abacaxi a que apresentou os maiores valores. O maior número de indivíduos e de espécies ocorre logo após as primeiras chuvas, principalmente no mês de novembro. A variação temporal foi confirmada através do teste de uniformidade de Rayleigh que acompanha a sazonalidade do Cerrado, com maior número de indivíduos e de espécies na estação chuvosa. A temperatura e umidade são as variáveis climáticas que influenciaram a riqueza de cerambicídeos. Este é o primeiro trabalho realizado com abacaxi fermentado com caldo de cana para captura de Cerambycidae, e este tipo de isca se mostrou eficiente para a coleta dos insetos, podendo ser comparado com as iscas sintéticas que normalmente são utilizadas. Todas as espécies coletadas são novos registros de distribuição para o Distrito Federal.

Palavras-chave: Fruta fermentada; Variáveis climáticas; Armadilha iscada; Serra pau; Novos registros.

Introduction

Cerambycidae (Insecta: Coleoptera) is a cosmopolitan family with more than 38,000 described species (Tavakilian & Chevillotte 2020); in the Neotropical Region, more than 8,000 species in 1,550 genera have been described (Monné 2020a). More than 800 species of Cerambycidae are registered for the Cerrado (Monné 2020b). They are phytophagous insects, with xylophagous larvae that feed on roots, logs, and branches of young rotting trees (Arnett et al. 2002, Marinoni et al. 2003) and adults that feed on wood, roots, leaves, pollen, sap, and fruits.

Adults are known as longhorn beetles because most have very long antennae (Galileo & Martins 2006), many times longer than their body length. The antennae serve as olfactory organs for locating host plants suitable for oviposition (Bezark & Monné 2013). They are known by the popular name of “serra pau” in Brazil because some species (eg Onciderini) have the habit of sawing the logs and branches of trees, for oviposition. This habit damages forests and agricultural areas, leading to the beetles being considered pests of perennial plants, such as fruit trees and forest species, where their larvae feed on logs, branches, and roots, forming galleries, with some species existing in their larval stage for as long as 10 years (Galileo & Martins 2006, Silva et al. 2016). Regarding their time of activity, some species are considered nocturnal and are found in their host plants, and others are diurnal, are attracted by flowers and act as pollinators (Bezark & Monné 2013).

There are hundreds of papers published in Brazil on the Cerambycidae family, addressing topics such as species diversity (Maia et al. 2003, Napp & Monné 2006, Souza & Silva 2012), genera review (Monné & Monné 2006), species seasonality (Marinoni & Ganho 2003), and ecological and behavioral aspects (Machado et al. 2006, Lemes et al. 2011). Despite some studies in the Goiás region (Canettieri & Garcia 2000) and in RPPN Santuário do Caraça, Minas Gerais (Monné & Monné 2017), few studies have been carried out in Central Brazil within the Cerrado biome.

The Cerrado is the second largest biome of Brazil, with about 2 million km² (Klink & Machado 2005, Prevedello & Carvalho 2006); it is considered a biodiversity hotspot and is an area of conservation priority (Myers et al. 2000, Mittermeier et al. 2005, Sano et al. 2010). This tropical savanna presents several types of phytophysiognomies, with a vegetation gradient that passes through herbaceous formations, grasses, shrubs, and forests, presenting differentiated microclimates and a variation in food resource availability.

It has two well-defined seasons: dry (April to September) and rainy (October to March), with an average annual precipitation of 1,500 mm and large intra-regional variations (Silva et al. 2008). This seasonality has a great influence on vegetation, decreasing or increasing resources, which generate alternation in the abundance of insects (Oliveira & Frizzas 2008, Becerra et al. 2009). In regions where the dry season is well defined, insect abundance tends to be smaller because it is synchronized with resource availability and climate (Wolda 1978, Pinheiro et al. 2002, Silva et al. 2011).

The biome presents a diversity of habitats that facilitates the distribution of organisms in different environments (Sano et al. 2010). However, it has constantly suffered from anthropogenic actions, such as severe losses and fragmentation of native areas due to agricultural activities (Klink & Machado 2005), which has led to reductions in ecosystem services provided by the Cerrado and its diverse inhabitants (Tilman et al. 2002). Despite the services that the insects provides, such as pollination, decomposition, and biological control of pests (Ramos et al. 2020), cerambycid beetles have been negatively affected by deforestation caused by timber extraction and agricultural activity (IUCN 2019). A study conducted in Australia showed that the Cerambycidae community is influenced by burning regimes, which promote habitat alteration; the study showed that burned areas had a greater richness of beetles than unburned areas, and that the abundance of beetles in areas burned triennially was greater than that in unburned areas (Eliott et al. 2019). The Cerrado is a biome closely linked to fire, and if the Cerambycidae community in Central Brazil follows the pattern found in Australia, it should also be affected by this factor.

Previous studies with Cerambycidae have used different sampling methods, the main ones being sweeping foliage, manual collections, sticky cards (Sakalian & Georgiev 2011), attraction to light (Canettieri & Garcia 2000), pheromone (Hanks et al. 2012), fermented bait (Schmeelk et al. 2016), malaise trap (Maia et al. 2003), bait traps with synthesized chemical compounds (Wong & Hanks 2016), and, in some cases, flight interception traps and pitfalls (Bezark & Monné 2013).

The bait traps have proven to be a very efficient sampling method for Cerambycidae (Schmeelk et al. 2016). However, there are a variety of bait types that can be used. Studies using alcohol and chemical compound mixes (Fernandes et al. 2010, Alisson et al. 2019), and fermented material mixes containing fungi, wood, banana, sugar, molasses, and beer (Schmeelk et al. 2016, Wong & Hanks 2016) as baits have already been performed. In Brazil, studies have mainly used sugarcane molasses as fermented baits, with 20% sugarcane molasses (Garcia 1987) and 10% honey (Santos et al. 2014), to test their attractiveness to borer (Garcia & Nakano 1984) and cerambycid beetles. The use of sugarcane molasses or sugarcane juice mixed with ripe fruit has also been implemented for the collection of other Coleoptera families (Pacheco et al. 2006, Jákl 2009, Orozco 2012, Evangelista Neto et al. 2017) as well as for other orders of insects (Zacca & Bravo 2012).

The objectives of this study were to evaluate the richness and abundance of Cerambycidae using two types of fruits (banana and pineapple) fermented with sugarcane juice as baits in an area of cerrado *sensu stricto* and to verify whether the richness of Cerambycidae is influenced by climatic variables (temperature, humidity, and precipitation). Our hypothesis is that although most fermented baits use bananas in their composition, pineapple fermentation produces more ethanol than banana fermentation, suggesting a higher sugar concentration (Hajar et al. 2012); therefore, with the use of sugarcane juice to enhance fruit fermentation, it is expected that the combination of sugarcane juice with pineapple will present more fermentation and will be more attractive to cerambycid beetles than the combination of sugarcane juice and banana. It is also expected that with the alternation of the dry and rainy season, the availability of fruits and flowers as well as other resources will vary, regulating the populations of these insects and causing seasonality in this group of organisms.

Material and Methods

1. *Study area*

The study was conducted at the Água Limpa Farm (FAL) belonging to the University of Brasília - UnB, Distrito Federal (Brazil). The FAL is a part of the Protection Area (APA) of the “Gama” and “Cabeça do Veado” basins (15º56’ to 15º59’ S and 47º55’ to 47º58’ W, 1,096 m) comprising an area of 4,500 ha. The Cerrado biome has an environmental heterogeneity that favors the diversified occurrence of phytophysiognomies. Consequently, both floristic and faunistic diversity are very high and include endemic plant and animal species (PELD 2018). The study was carried out in an area of cerrado *sensu stricto*, a phytophysiognomy characteristic of the biome, presenting low, tortuous plants—with thick bark logs and rigid leaves—that range from 1.5 to 5 m in height, demonstrating adaptations to the dry season during which fires usually occur, representing 70% of the biome (Felfili & Silva Júnior 2005).

Climatic data, including temperature, humidity, and precipitation, were obtained throughout the study period through the AgroClimatic Bulletin provided by the Faculty of Agronomy and Veterinary Medicine of the University of Brasília (FAV/UnB).

2. Cerambycidae sampling

The sampling of adults was carried out weekly for 12 months, from November 2013 to October 2014, using 40 bait traps that remained uninterrupted in the field. The traps consisted of a 2-L cylindrical plastic bottle with three 8 × 8 cm side windows located 10 cm above the base. Baits (150 mL), either consisting of banana (variety Dwarf Cavendish) or pineapple fermented for 48h in sugarcane juice, were placed in each trap, according to Rodrigues et al. (2013) and Puker et al. (2014). Banana baits were used in half of the traps and pineapple baits in the other half. The traps were placed in trees, about 1.5 m above ground level, distributed along four 80 m transects and spaced 20 m apart. Each transect was marked at a minimum of 20 m from the point of entry of the experimental area to avoid possible margin effects and traps were placed thereafter.

After collection, the insects were taken to the Coleoptera Biology and Ecology Laboratory of the University of Brasilia, and the cerambycid beetles were separated from the other insects. Species were identified by one of the authors (MAM, National Museum/Federal University of Rio de Janeiro). Vouchers of the collected material were deposited in the Entomological Collection of the Department of Zoology of the University of Brasilia (DZUB).

3. Data analysis

The experimental design had pseudoreplication. Therefore, Rayleigh’s uniformity test, which analyzes the functional relationship of variables under the concept of dimensional homogeneity (Mendoza 1994), was used to analyze temporal variation data. Circular analysis was used to evaluate the abundance and richness of Cerambycidae in different months of the year. To compare the abundance and richness of Cerambycidae with regard to the bait types used, a t-test was performed. To verify if the richness was influenced by climatic variables (temperature, humidity, and precipitation), Kruskal-Wallis and Mann-Whitney tests were performed with Bonferroni correction. To verify the sampling effort efficiency in relation to the survey and the diversity of Cerambycidae, species rarefaction curves were constructed based on the number of samples and number of individuals, using the Chao 1 index and 1,000 randomizations. All analyses were performed using Past 3.20 (Hammer et al. 2001) and Oriana (Kovach 2011).

Results

A total of 1,599 specimens, with 19 species belonging to 13 genera and five tribes, were collected. The species with the highest abundance were as follows: *Oxymerus basalis* (Dalman, 1823) comprising 78.3% of the total specimens collected, *Retrachydes thoracicus thoracicus* (Olivier, 1790) comprising 9.9%, and *Chydarteres bicolor* (Fabricius, 1787) comprising 4.5%.

In the pineapple bait traps, 1,013 individuals, consisting of 18 species belonging to 13 genera, were collected. In the banana bait traps, 586 individuals, consisting of 11 species belonging to six genera, were collected. There were significant differences in the richness ($t = 3.7422$; $p < 0.05$) and abundance ($t = 1.7237$; $p < 0.0005$) of Cerambycidae between the two types of baits evaluated. Ten different species were collected from the two types of baits, and the species *Coleoxestia waterhousei* (Gounelle, 1909); *Drychateres bilineatus* (Olivier, 1795); *Eburodacrys crassimana* Gounelle, 1909; *Eurysthea hirta* (Kirby, 1818); *Juiaparus mexicanus* (Thomson, 1861); *Lissonotus spadiceus* Dalman, 1823; *Oxymerus aculeatus aculeatus* Dupont, 1838; and *Plocaederus confusus* Martins & Monné, 2002, were collected only in the pineapple bait, whereas one species, *Sphallotrichus setosus* (Germar, 1823), was collected only in the banana bait (Table 1).

Table 1. Species and abundance of Cerambycidae collected in baited traps with fermented fruits in the dry and rainy seasons at Água Limpa Farm in the cerrado *sensu stricto* in Brasília-DF, Brazil, from November 2013 to October 2014.

Species	Tribe	Pineapple	Banana	Dry	Rainy
Chydarteres bicolor (Fabricius, 1787)	Trachyderini	49	23	-	72
Chydarteres dimidiatus dimidiatus (Fabricius, 1787)	Trachyderini	03	02	-	05
Chydarteres octoliwneatus (Thunberg, 1822)	Trachyderini	07	02	-	09
Chydarteres striatus striatus (Fabricius, 1787)	Trachyderini	02	02	-	04
Coleoxestia waterhousei (Gounelle, 1909)	Cerambycini	02	-	-	02
Dorcacerus barbatus (Olivier, 1790)	Trachyderini	10	08	-	18
Drychateres bilineatus (Olivier, 1795)	Trachyderini	01	-	-	01
Eburodacrys crassimana Gounelle, 1909	Eburiini	02	-	-	02
Eurysthea hirta (Kirby, 1818)	Elaphidiini	01	-	01	-
Juiaparus mexicanus (Thomson, 1861)	Cerambycini	02	-	-	02
Lissonotus spadiceus Dalman, 1823	Lissonotini	01	-	-	01
Oxymerus aculeatus aculeatus Dupont, 1838	Trachyderini	01	-	01	-
Oxymerus basalis (Dalman, 1823)	Trachyderini	776	476	-	1,252
Oxymerus luteus luteus (Voet, 1778)	Trachyderini	01	01	-	02
Plocaederus confusus Martins & Monné, 2002	Cerambycini	02	-	-	02
Retrachydes thoracicus thoracicus (Olivier, 1790)	Trachyderini	116	39	84	71
Sphallotrichus sericeotomentosus Fragoso, 1995	Cerambycini	01	01	-	02
Sphallotrichus setosus (Germar, 1823)	Cerambycini	-	01	-	01
Trachyderes succinctus succinctus (Linnaeus, 1758)	Trachyderini	36	31	01	66
Total	5	1,013	586	87	1,512

Cerambycid beetles were collected during all months of the year. The highest abundance (1,171 individuals) and richness (14 species) were observed in November, while the lowest abundance (one individual) and richness (one species) were observed in September (Figure 1). In the rainy season, 1,512 individuals (94.5% of the total), comprising 17 species, were collected. In the dry season, 87 individuals (5.4% of the total), comprising four species, were collected (Table 1). With the exception of *R. thoracicus thoracicus*, which was collected throughout the year, most species were collected during the rainy season.

April represents the period of transition between the rainy and dry seasons, and showed a small peak in abundance. During this period, 62 individuals, comprising two species, were collected. *Eurysthea hirta* and *O. aculeatus aculeatus* were collected only in the dry season. Circular data analysis showed similarity in the richness and abundance of Cerambycidae and that there was a seasonal distribution for the family, with abundance and richness peaks occurring in November (Figure 2). Abundance presented a well-defined peak in November, whereas richness was more evenly distributed throughout the year, but still showed higher concentration in the rainy season (Figure 2). Rayleigh's uniformity test showed that there was significant temporal variation in the abundance and richness of Cerambycidae ($P < 0.0001$).

It was observed that climate variables were correlated with the richness of Cerambycidae, but only temperature and humidity had statistical significance on richness (Table 2). Species rarefaction curves, based on the number of samples and the number of individuals observed, indicated that the sampling effort would need to be greater to better characterize the local community of Cerambycidae attracted by fermented fruit baits (Figure 3).

We registered the following species for the first time for the Distrito Federal (Brazil): *Chydarteres bicolor*, *Chydarteres dimidiatus dimidiatus* (Fabricius, 1787), *Chydarteres octolineatus* (Thunberg, 1822), *Chydarteres striatus striatus* (Fabricius, 1787), *Coleoxestia waterhousei*, *Dorcacerus barbatus* (Olivier, 1790), *Drychateres bilineatus*, *Eburodacrys crassimana*, *Eurysthea hirta*, *Juiaparus mexicanus*, *Lissonotus spadiceus*, *Oxymerus aculeatus aculeatus*, *Oxymerus basalis*, *Oxymerus luteus luteus* (Voet, 1778), *Plocaederus confusus*, *Retrachydes thoracicus thoracicus*, *Sphallotrichus sericeotomentosus* Fragoso, 1995, *Sphallotrichus setosus*, and *Trachyderes succinctus succinctus* (Linnaeus, 1758).

Discussion

In Brazil, the Cerambycidae family is represented by more than 1,050 genera and 4,300 species (Monné 2020a). In the present study, 13 genera and 19 species were collected in the cerrado *sensu stricto*, an area with the characteristic phytophysiognomy of the Cerrado biome. The diversity found in this study was low in comparison to other studies that have been done with the family in other biomes (Maia et al. 2003, Souza & Silva 2012, Ferreira & Rocha 2015, Monné & Monné 2017). A possible explanation for the low diversity found in the study, since more than 800 species are reported for the biome, is that we use only one type of trap. Other studies use more than one type of trap such as malaise, light trap, flight interception, trap with pheromones at different heights (Canettieri & Garcia 2000, Maia et al. 2003, Bezark & Monné 2013, Wong & Hanks 2016, Barros et al. 2020). Although, another

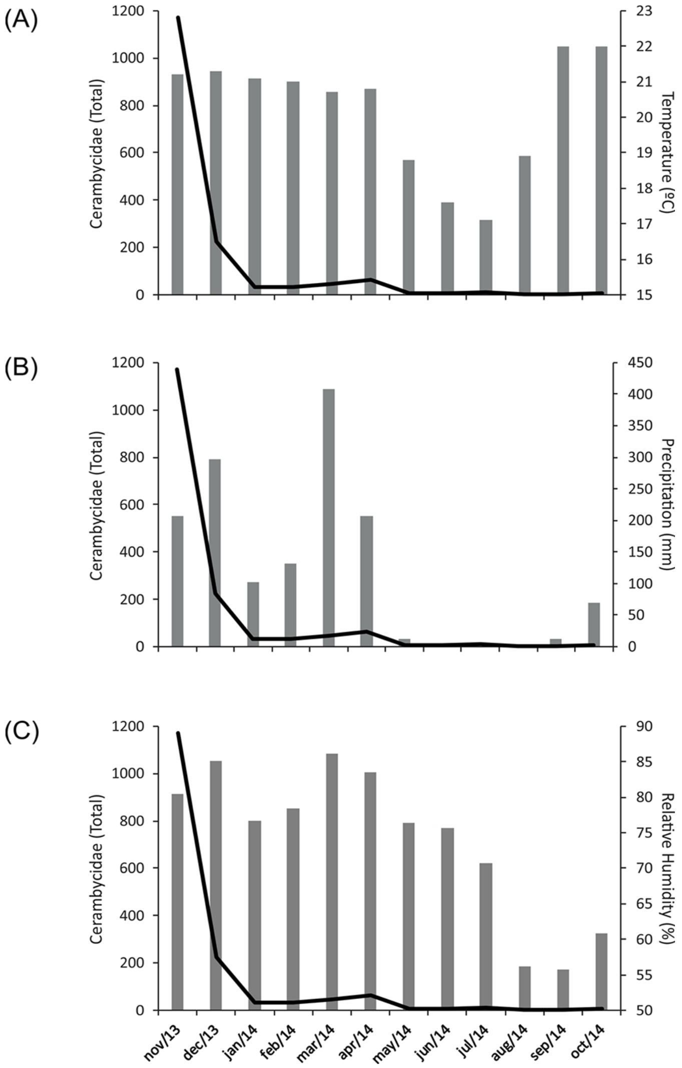

Figure 1. Average temperature (°C), precipitation (mm), relative humidity (%), and abundance of Cerambycidae collected in baited traps with fermented fruits at Água Limpa Farm in Brasília-DF, Brazil, from November 2013 to October 2014.

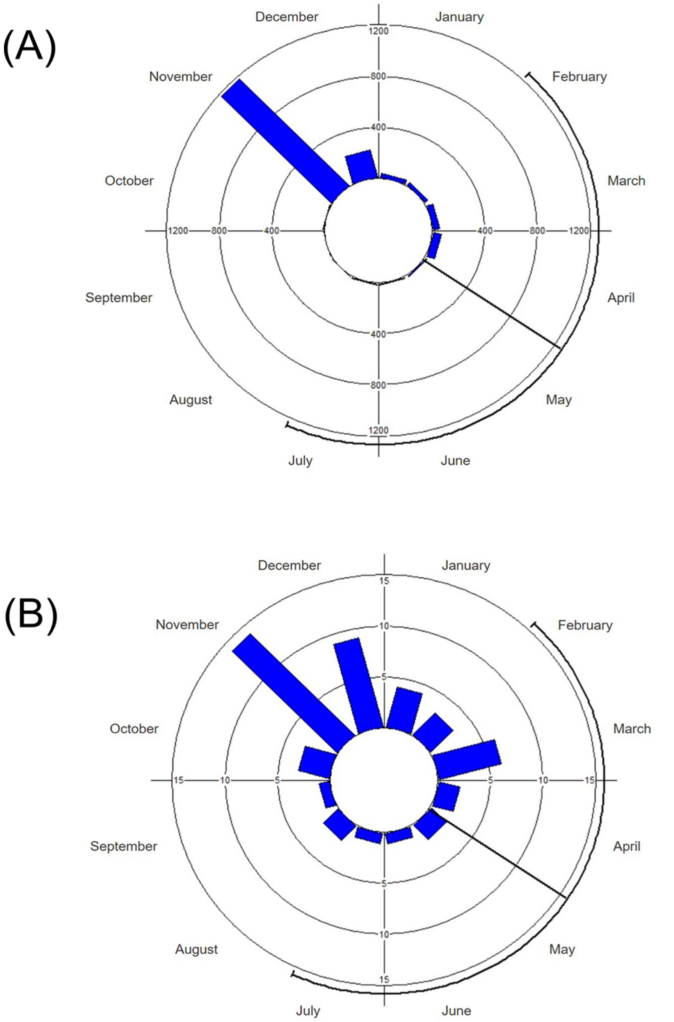

Figure 2. Circular analysis for (A) abundance and (B) richness of Cerambycidae collected in baited traps with fermented fruits at Água Limpa Farm in Brasília-DF, Brazil, from November 2013 to October 2014.

study carried out in the biome with bait traps has found only 13 species of Cerambycidae (Santos et al. 2014). The Cerrado has environmental heterogeneity which leads to diverse phytophysiognomies, presenting high floristic diversity and endemic plant species. The plants are low, tortuous, with thick bark logs and rigid leaves that can be from 1.5 to 5 m in height, showing adaptations to the dry season (Felfili & Silva Júnior 2005). Cerambycidae have a close relationship with their host plants and, despite the floristic diversity of the biome, many plant species may not be suitable hosts for Cerambycidae.

The diversity of Cerambycidae collected on pineapple bait differed statistically from banana bait in that 427 individuals and eight more species were collected on pineapple bait. Cerambicids are mostly collected with traps using alcohol-based baits, and greater abundance and richness was found when using the fermented pineapple bait. It is assumed that the fermentation of this fruit with sugarcane juice presents a greater volatilization of alcohols. In addition, pineapple possibly has higher amounts of sugars than banana, since in the fermentation of these two fruits, the alcoholic content of the banana (variety Dwarf Cavendish) is 0.035% (w/v), while that of the pineapple is 0.21% (w/v) (Hajar et al. 2012). Thus, pineapple has an alcohol content six times higher than banana, and this factor may have resulted in the greater attractiveness of the fermented pineapple when used as bait for the Cerambycidae community. This is the first work performed with fermented pineapple with sugarcane juice to capture Cerambycidae, and this type of bait proved to be efficient for the collection of insects and may be a promising bait for future studies with this group.

The sampled community follows the usual neotropical pattern, where there are a large number of rare and few dominant species (Antonelli et al. 2018). The two species that represented more than 90% of the collection were *O. basalis* and *R. thoracicus thoracicus*.

In this study, *O. basalis* dominated the assembly (representing 78.3% of the collected individuals), indicating that this is probably a species more adapted to the biome. The larvae of *O. basalis* make holes of about 1 mm in eucalyptus trees and can build galleries 50 cm in length inside the plant (Zanuncio et al. 2009). Besides eucalyptus, other genera of Myrtaceae occur in the Cerrado (Conceição & Aragão 2010), and these plants are possibly used as a resource by *O. basalis*, allowing their populations to establish themselves and reach expressive densities. Gottsberger (1988) reported *O. basalis* as a pest on *Annona coriacea* Mart. This species, in Brazil, has a wide distribution (Monné 2020a).

Table 2. Mann-Whitney test with Bonferroni correction for correlation between climatic variables and Cerambycidae richness for beetles collected with baited traps with fermented fruits at Água Limpa Farm in Brasília-DF, Brazil, from November 2013 to October 2014. The highlighted values represent a significant relationship between the variables.

Variables	Temperature	Precipitation	Humidity	Richness
Temperature	-	1	0.0002187	0.0002111
Precipitation	1	-	1	0.05092
Humidity	0.0002187	1	-	0.0002119
Richness	0.0002111	0.05092	0.0002129	-

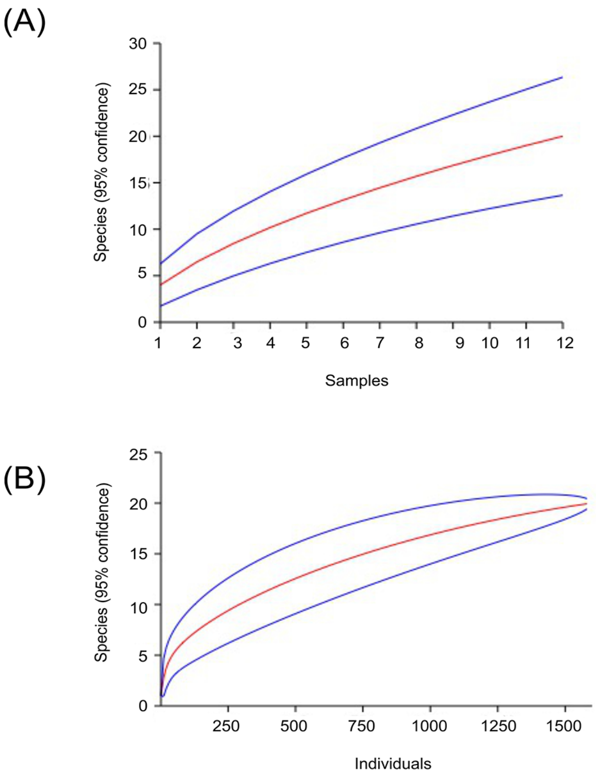

Figure 3. Rarefaction curves of Cerambycidae species collected in baited traps with fermented fruits at Água Limpa Farm in Brasília-DF, Brazil, from November 2013 to October 2014. Bars below and above the curves indicate standard deviations. (A) Curve created based on monthly samples collected for 12 months and (B) Curve created based on the collected 1,599 individuals.

Retrachydes thoracicus was the second most collected species (9.9%), and the only species found during all the year. It has several records of host plants (Monné 2020a) and is considered a pest of plants of the Fabaceae (Costa et al. 2019), Moraceae, Ulmaceae (Di Iorio 1997), and Salicaceae (Machado et al. 2012) families, and its larvae damage the species *Litchi chinensis* Sonn. (Casari & Albertoni 2017). This species occurs in Bolivia, Paraguay, Argentina, Uruguay and Brazil, in the northeastern (except Sergipe), midwestern (except Distrito Federal), southeastern, and southern regions (Monné 2020a, b).

In this study five tribes of Cerambycidae were found, Trachyderini being the most abundant tribe, representing 99.2% of the total collected (Table 1). Representatives of this tribe are very attracted by fermented baits, which may explain the result of this study. However, it is worth noting that the abundance of Trachyderini depends on the place of collection and the bait used, in other areas of Cerrado we found species of distinct genera, being 39 species of Trachyderini reported for the Cerrado biome. The species collected belong to the subfamily Cerambycinae, something expected since the traps with fermented baits normally are used to collect diurnal species. The other subfamilies of Cerambycidae, except Lepturinae, are usually nocturnal, therefore not attracted by this type of trap.

The temporal variation in the abundance and richness of Cerambycidae is directly related to the climatic characteristics of the Cerrado, with the largest number of individuals and species occurring in the rainy season (Figure 3). Rainfall distribution is a factor that influences changes in temperature and relative humidity, and indirectly affects plant growth, the main food resource of herbivorous insects (Oliveira & Frizzas 2008, Silva et al. 2011). The biome has two well-defined climatic seasons, with the rainy season that occurs from October to March, where most adult insect populations are concentrated (Silva et al. 2011), a fact also verified for the Cerambycidae family in the Cerrado, which presented 94.5% of individuals and 90% of collected species in the rainy season. The greater diversity (abundance and richness) of adults of Cerambycidae in the rainy season is probably due to the greater availability of food (leaves, fruits, and flowers) in the Cerrado during this period.

Seasonal distribution was observed for the family in the Cerrado, with peak abundance and richness in November, i.e., in the first half of the rainy season. The beginning of the rains is considered the trigger for the increase in population density; other studies conducted with Coleoptera in the Cerrado have found the same pattern, for example, in the families Cetoniidae (Evangelista Neto et al. 2017), Melolonthidae (Oliveira & Frizzas 2017), and Scarabaeidae (Frizzas et al. 2020).

Although precipitation is one of the most important abiotic factors, temperature and humidity were shown to have significant effects on the richness of Cerambycidae (Table 2). Temperature is one of the climatic variables that most influences the activity of many species of insects, determining the development rates of immature animals and the reproductive activity of adults.

Besides having observed a low richness (19 species), it was also observed that the rarefaction curves did not reach the asymptote, indicating the need for further study in the area. Other studies, performed with pheromone baits and fermented baits, obtained a higher abundance (six-folds as high compared to our study) of cerambycid beetles, in addition to demonstrating that traps at different heights could capture different species, as some species were captured at a specific height and were absent from other treatments (Schmeelk et al. 2016). Thus, this may be an explanation for the rarefaction curve not having reached the asymptote, since the present study used only fermented fruit as bait, and all traps were installed at the same height. Other types of bait and more kind of traps, in addition to having traps at different heights, will probably help obtain a more complete sampling of the Cerambycidae community.

Because it has distinct phytophysiognomies, the Cerrado presents a great diversity of habitats with varied vegetation coverage, moisture retention, food resources, and differentiated reproductive conditions. The species living in the Cerrado, a biome of high biodiversity and one with the most threatened species in the world (Mittermeyer et al. 2005), illustrate the possibility of knowing the organisms that compose it to assist in conservation policies and land use destinations. Considering that the present study was conducted only in the cerrado *sensu stricto*, there is a need for other studies to be carried out in other phytophysiognomies so that more species are registered for the Cerrado. It is important to consider different sampling methods because each group of insects presents variations in alimentary and behavioral habits and, as such, different types of traps can cover the community in a more complete way. On the other hand, sampling techniques should be standardized, since that will make it possible to quantify the richness and abundance of the community in the correct manner (Campos et al. 2000). Although there are few studies addressing fermented fruits in the capture of cerambycid beetles, in the existing studies, only the banana was used as a resource; in the present study, howeverd it was observed that the pineapple has a higher capture efficiency that could be compared with synthetic baits, demonstrating the importance of this type of bait.

All species collected in this study are new records for the Distrito Federal (Brazil). Despite the importance of the Cerambycidae family, there are few studies on this group of organisms in the Cerrado. Studies aimed at documenting and understanding the spatial patterns of biological diversity are of great importance in guiding conservation planning programs (Franklin 2009). This study is of great relevance in terms of public conservation policies, as they can potentially indicate priority sites for conservation, which is highly relevant for the Cerrado as it has suffered systematically from anthropogenic pressures, mainly in relation to deforestation and expansion of agricultural activities, in recent years.

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Author Contributions

Juliane Evangelista: contributed substantially to the concept and design of the study, data collection, data analysis and interpretation, and manuscript preparation, including critical revision and addition of intellectual content.

Marcus Vinicius Celani Rocha: contributed to the concept of the study, data collection, data analysis and interpretation, and preparation of the manuscript.

Marcela Laura Monné: contributed to the critical revision, data analysis and interpretation, and addition of intellectual content to the manuscript.

Miguel Angel Monné: contributed to the critical revision, data analysis and interpretation, and addition of intellectual content to the manuscript.

Marina Regina Frizzas: contributed substantially to the concept and design of the study, data interpretation, and manuscript preparation, including critical revision and addition of intellectual content.

Conflicts of interest

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

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Article

Attraction to conspecifcs in *Rhinella icterica* and *R. ornata* tadpoles (Anura: Bufonidae)

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Abstract: Tadpoles are able to perceive and discriminate signals from environment and they may use this ability in behaviors and ecological processes. Recognition mechanisms may be involved in schooling by means of attraction among individuals, characterizing a social process. By means of laboratory experiments the present study investigated the presence or absence of attraction to conspecifics in tadpoles of *Rhinella icterica* and *R. ornata*, two sympatric species from the Atlantic Forest of southeastern Brazil. We collected eggs of these two species in the field and reared them in laboratory according to two different methods (isolated or in groups of siblings). Tadpoles were submitted to experiments of choice between conspecifics, heterospecifics, and an empty compartment. *Rhinella icterica* tadpoles preferred to associate with conspecifics rather than *R. ornata* tadpoles, and we verified this is an innate behavior. *Rhinella ornata* tadpoles failed to discriminate between conspecifics and *R. icterica* tadpoles. When submitted to choice between a group of tadpoles of the other species and an empty compartment, *R. icterica* tadpoles presented random distribution, while *R. ornata* tadpoles preferred to associate with heterospecifics. Our results indicate *R. icterica* tadpoles have preference to associate with conspecifics, while *R. ornata* tadpoles may school indiscriminately. This study contributes for a better understanding of larval anuran social behavior.

Keywords: *anuran larvae; behavior; communication; sympatric species; Atlantic Forest; schooling; southeastern Brazil.*

Atração por coespecíficos em girinos de *Rhinella icterica e R. ornata* (Anura: Bufonidae)

Resumo: Os girinos são capazes de perceber e discriminar sinais do ambiente e podem usar essa capacidade em comportamentos e processos ecológicos. Mecanismos de reconhecimento podem estar envolvidos na agregação por meio da atração entre indivíduos, caracterizando um processo social. O presente estudo investigou, por meio de experimentos de laboratório, a presença ou ausência de atração por coespecíficos em girinos de *Rhinella icterica* e *R. ornata*, duas espécies simpátricas, da Mata Atlântica do sudeste do Brasil. Coletamos ovos dessas duas espécies no campo e os cultivamos em laboratório de acordo com dois métodos diferentes (isolados ou em grupo de irmãos). Os girinos foram submetidos a experimentos de escolha entre coespecíficos, heteroespecíficos e compartimento vazio. Girinos de *Rhinella icterica* preferiram associar-se a coespecíficos do que com girinos de *R. ornata*, e verificamos que este é um comportamento inato. Os girinos de *Rhinella ornata* não conseguiram discriminar entre coespecíficos e girinos de *R. icterica.* Quando submetidos à escolha entre um grupo de girinos da outra espécie e um compartimento vazio, os girinos de *R. icterica* apresentaram distribuição aleatória, enquanto os girinos de *R. ornata* preferiram associar-se a girinos heteroespecíficos. Nossos resultados indicam que girinos de *R. icterica* preferem associar-se a coespecíficos, enquanto girinos de *R. ornata* podem agregar-se indiscriminadamente. Este estudo contribui para uma melhor compreensão do comportamento social de larvas de anuros.

Palavras-chave: *larvas de anuros; comportamento; comunicação, espécies simpátricas; Mata Atlântica; agregação, Sudeste do Brasil.*

Introduction

Recognition mechanisms (of relatives, conspecifics, mates, neighbours, rivals, prey or predators) are essential for survival, reproduction and social interactions between organisms (Sherman et al. 1997). Among anuran larvae, chemical communication plays a key role in several behaviors, such as communication between a female and its offspring (Kam & Yang 2002; Stynoski & Noble 2012), detection of alarm cues in prey-predator contexts (Hews 1988; Hokit & Blaustein 1995; Summey & Mathis 1998; Kiesecker et al. 1996, 1999), intraspecific competition (Glennemeier & Denver 2002), microhabitat and food discrimination (Pfening 1990; Gamboa et al. 1991; Hall et al.1995; Sontag et al. 2006), and recognition of predators (Petranka et al. 1987; Lawler 1989; Kiesecker et al. 1996), conspecifics (Leu et al. 2013; Chapman et al. 2015; Raven et al. 2017), and kin (Blaustein & O'Hara, 1982; Waldman 1986; Rajput et al. 2014; Pizzatto et al. 2016; Raven et al. 2017).

In tadpoles, recognition abilities based on chemical signals are developed during the embryonic phase or shortly after hatching (Waldman 1981, 1882; Blaustein & O'Hara 1982; Hepper & Waldman 1992) and may persist after metamorphosis (Blaustein et al.1984; Waldman 1989; Graves et al. 1993; Flowers and Graves 1997). Due to vulnerability to predators at this stage, some species have developed strategies such as metamorphosis synchronization and aggregation (Pulliam & Caraco 1984; Hews, 1988). In this case, spatial aggregation may be based primarily on conspecific attraction rather than mediated by environmental factors (Graves et al. 1993). Non-social groups are formed in response to attractive environmental characteristics (e.g., feeding microhabitats and temperature gradients), while social groups are formed from attraction between individuals (Wassersug 1973; Hoff et al.1999). Thus, communication characterizes social behavior (Townsend et al. 2003).

In this context, the present study sought to analyse, through laboratory experiments, the presence or absence of attraction to conspecifics in tadpoles of two congeneric species (*Rhinella icterica* and *R. ornata*) that occur in sympatry and show a wide distribution in the Atlantic Forest of southeastern Brazil. These species are able to school during larval phase (Eterovick 2000; Simon 2010).

These species belong to the family Bufonidae, which is composed by 53 genera and 629 species (Frost 2020). In the genus *Rhinella* Fitzinger, 1826, there are 92 species distributed from southern United States to southern South America (Frost 2019). *Rhinella icterica* is included in *Rhinella marina* Group (Maciel et al. 2010), while *R. ornata* belongs to the *Rhinella crucifer* Group (Baldissera Jr. et al. 2004).

Reproductive isolation between *R. icterica* and *R. ornata* may not be effective, due to sympatric occurrence, overlapping of reproductive sites and seasons of reproduction and close relationship between them (Bertoluci 1998; Bertoluci & Rodrigues 2002; Abrunhosa et al. 2006), enabling interspecific amplexes and the occurrence of non-viable hybrids (Haddad & Cardoso 1990). The same habitat is shared by these two tadpole species of different kinship degrees and development stages, thus, an intra and interspecific communication system would has an important adaptive value for them.

Until now, only one study has sought to analyze attraction to conspecifcs in Brazilian tadpoles (*Rhinella marina*; Raven et al. 2017), but this was done with invasive populations in the Australian territory. Thus, the present study contributes to an important knowledge gap of Brazilian tadpoles' behavior.

Therefore, we aimed to answer, for each species, the following questions: (1) do tadpoles prefer to associate with conspecifics rather than heterospecifics? (2) Does prior social experience (isolation or group rearing) influence recognition mechanism?

Material and Methods

1. *Egg collection*

We collected eggs from two spawns of *R. icterica* and two spawns of *R. ornata*, between 24 July and 14 August 2017, encompassing the reproductive season of the target species (Bertoluci & Rodrigues 2002). We identified spawns based on size and arrangement of eggs within the gelatinous strings: smaller eggs arranged in a single string in *Rhinella ornata*, larger eggs arranged in a double string in *R. icterica* (Simon 2010). We transported eggs to laboratory in plastic pots containing local water.

We collected eggs in the Estação Biológica de Boracéia (EBB), a well-preserved Atlantic Forest reserve of Serra do Mar in São Paulo state (23˚38' S, 45˚52' W). EBB has an area of 16,450 ha and is located at altitudes around 900 m a.s.l. The area is covered by Dense Ombrophylous Forest, where the presence of palm trees, ferns and giant bamboos is common (Travassos & Camargo 1958; Heyer et al.1990; Bertoluci & Rodrigues 2002). Rainy season runs from September to March, and temperature can reach 24˚C in the hottest months (September to March) and 5˚C in the colder months (April to August) (Bertoluci & Rodrigues 2002).

2. *Rearing methods*

We conditioned eggs of two spawns (spawn A and spawn B) of each species, in laboratory, according to two different methods. They were separated from the rest of the embryos in the spawn between stages 16 and 18 (Gosner 1960).

At the 1st rearing method (group of siblings), we placed 300 eggs of each spawn of *R. icterica* in two different 50L opaque containers and we did the same with eggs of *R. ornata*. At the 2nd method (isolation rearing), we placed 50 eggs from spawn A, of each species, each egg in a 0.5L opaque container, in 50 different containers.

We maintained eggs and tadpoles at room temperature and natural photoperiod. Tadpoles were fed once daily with ornamental fish food in large quantities so that food was always available. Aeration pumps for aquariums provided oxygenation. We changed the water in each container twice a week to keep the environment clean. After the metamorphosis, we released juveniles at the egg collection sites.

3. *Experiments*

Tests were carried out in four plastic containers (100 × 15 × 10 cm), filled with spring water (Fig. 1). At each end of a container we adapted a plastic net with a mesh of 0.5 mm, delimiting the stimulus groups areas (20 tadpoles in each group). The central part of each container was marked with a permanent pen, dividing it into three equal-sized compartments.

We performed the tests between 25 August and 27 September 2017, during daytime, between 08:00 a.m. and 6:00 p.m. Tested tadpoles were between stages 25 and 38 (Gosner 1960).

We placed test tadpoles in the center of each container and submitted to choice tests between two groups (Fig. 1) (as in Blaustein & O'Hara 1982; Cornell et al. 1989; Leu et al. 2013; Rajput et al. 2014; Pizzatto et

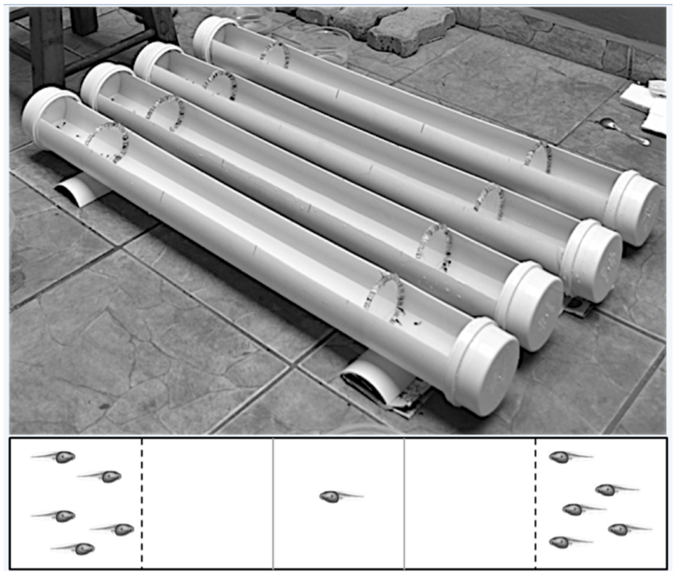

Figure 1. Test arena. Stimulus groups (20 tadpoles in each group) are represented in the right and left extremities, and test tadpole is represented in central area. Dashed line represents a net, which delimits stimulus groups areas, but allows chemical and visual stimuli flow to central region. Vertical gray lines represent demarcation of areas close to each stimulus group. Each division is 20 cm long, totalling 100 cm of arena total length.

al. 2016). We filmed the experiments (Kodak z990 camera), so researcher presence did not influence tadpole behavior. After 10 minutes of test tadpole acclimation, we filmed each experiment for 29 minutes (1740 s), and then we measured the time each tadpole remained in the compartment next to each stimulus group. Each tadpole was tested only once and, after each test, containers were cleaned and the water changed. At each test we turned the containers to 90° and inverted the side of each stimulus group. Each experiment was replicated 32 times. Four replicates were filmed at a time. All procedures were repeated for both species.

We organized the experiments as follows:

Control: all tadpoles (test tadpoles and stimulus groups) from the same spawn and reared together in one container. With this experiment we were able to analyse if there were errors in experimental design. The expected was that test tadpoles had no preference for association with one of the two groups.

Experiment 1: test tadpole from spawn B reared according to 1st rearing method. Stimulus group 1 formed by tadpoles of spawn A (conspecifics tadpoles, but not siblings of test tadpole). Stimulus group 2 formed by tadpoles of the other studied species (heterospecifics). With this, we sought to analyse if there was preference of association with conspecifics by the species tested.

Experiment 2: test tadpoles from spawn A reared according to the 2nd rearing method (in isolation). Stimulus group 1 formed by tadpoles of spawn B, reared according to 1st rearing method. Stimulus group 2 formed by heterospecific tadpoles. With this, we sought to analyse if the rearing method in total isolation would influence association choice by tested tadpoles with one of the stimulus groups.

Experiment 3: test tadpole from spawn A, reared according to the 1st rearing method. Stimulus group 1 formed by heterospecific tadpoles. Stimulus group 2 empty (tadpoles absence). With this experiment we sought to analyse sociability of tested tadpoles (preference for grouping or isolation), and attraction or repulsion by tested tadpoles to heterospecific tadpoles.

4. Data analyses

We performed statistical analyses with R platform (R Core Team 2017). We consider level of significance $\alpha = 0.05$. Tests were two-tailed. Confidence level used was 95%. For each experiment, data considered in the statistical tests were the differences between the time spent by the test tadpole in the compartments located near stimulus groups 1 and 2 (T1– T2). We verified if data of each experiment corresponded to normal distribution by Shapiro-Wilk test (H_o = data correspond to normal distribution). To data that reached the normal distribution we applied paired t-test, due to dependence of samples, since two measurements were made for the same individual in each experiment (paired data). Non-parametric alternative to t-paired test is Wilcoxon signed-rank test, which was applied to data that did not reach normal distribution. With the tests, we were able to analyse whether the difference between time spent by test tadpoles near each of two stimulus groups was statistically significant. The null hypothesis was that mean (or median) of the differences is null (H_o: $\Delta = 0$), while the alternative hypothesis was that mean (or median) of the differences is not null (H1: $\Delta \neq 0$). When the null hypothesis was rejected, we used binomial test to verify whether the number of tadpoles that spent the most time near a stimulus group was statistically significant in relation to total number of replicates.

Data obtained in experiments Control, 1 and 3 with *Rhinella icterica* tadpoles and in experiments Control, 1 and 3 with *R. ornata* tadpoles corresponded to normal distribution (Shapiro-Wilk test; Table 2 and 3); in these cases, we used paired t-test to detect differences between time spent by test tadpoles next to each stimulus group. However, in experiments 2 with *R. icterica* data did not correspond to normal distribution (Shapiro-Wilk test; Tables 1 and 2), so we used Wilcoxon test.

Results

The differences between time spent by test tadpoles close to each stimulus group, as well as the mean of the differences (value used in the Paired t-test) and pseudomedian differences (value used in the Wilcoxon test), when negative, indicate a longer permanence time of test tadpoles close to stimulus group 2, whereas, when positive, they indicate a longer permanence time of the tadpoles test near to stimulus group 1 (Tables 2 and 3, Figs. 2 and 3). In general, data obtained with experiments varied more for *R. ornata* than for *R. icterica* (Figs. 2 and 3).

In experiments Control and 3 with *R. icterica* and in experiments Control and 1 with *R. ornata*, the mean differences did not differ statistically from hypothetical mean obtained by paired t-test (Tables 2 and 3). Therefore, for these experiments, we did not reject the null hypothesis and concluded that there was no statistically significant difference between time spent by tadpoles next to each stimulus group (H_o: $\Delta = 0$).

In experiment 1 with *R. icterica* and in experiment 3 with *R. ornata*, the mean differences differed statistically from hypothetical mean obtained by paired t-test (Tables 2 and 3). Similarly, in Experiment 2 with *R. icterica*, the pseudomedian differences differed statistically from the hypothetical median by Wilcoxon test (Tables 2 and 3). Therefore, for these experiments, we rejected the null hypothesis and concluded that there was a statistically significant difference between time spent by tadpoles next to each stimulus group (H1: $\Delta \neq 0$). In these experiments, we confirmed, with the binomial test, that the number of tadpoles that showed preference for group 1 was significant in relation to the total number of replicates (Tables 2 and 3).

Table 1. Synthesis of experiments and control. In the first rearing method, tadpoles were reared with its siblings, and in the second method each tadpole was reared in isolation. The test tadpoles were submitted to choice between two stimulus groups of conspecifcs, heterospecifics or empty compartment.

Experiment	Test tadpole	Stimulus Group 1	Stimulus Group 2
Control	1st rearing method	Conspecifcs	Conspecifics
1	1st rearing method	Conspecifcs	Heterospecifics
2	2nd rearing method	Conspecifcs	Heterospecifics
3	1st rearing method	Heterospecifics	Empty

Table 2. Statistical tests results for each experiment with *Rhinella icterica* tadpoles. When P < 0,05, there is statistically significant difference between time spent by tadpoles next to each stimulus group. df = degrees of freedom; CI = confidence interval.

Exper.	Shapiro-Wilk test	Paired-t test (t) or Wilcoxon (V)	Mean of differences	Pseudomedian of differences	CI (95%)		Binomial test
					Inf. Limit	Sup. Limit	
Control	W = 0.97 *P* = 0.58	T = -0.98; df = 31; *P* = 0.33	-159.68	-	-490.68	171.3	
1	W = 0.97 *P* = 0.53	T = 4,89; df = 31; *P* < 0.001	430.93	-	251.49	610.38	N = 24 *P* < 0.001
2	W = 0.92 *P* = 0.02	V = 384; df = 31; *P* = 0.02	-	176	31.99	325.99	N = 22 *P* < 0.001
3	W = 0.98 *P* = 0.91	T = 0.57; df = 31; *P* = 0.57	55.68	-	-142.55	253.93	

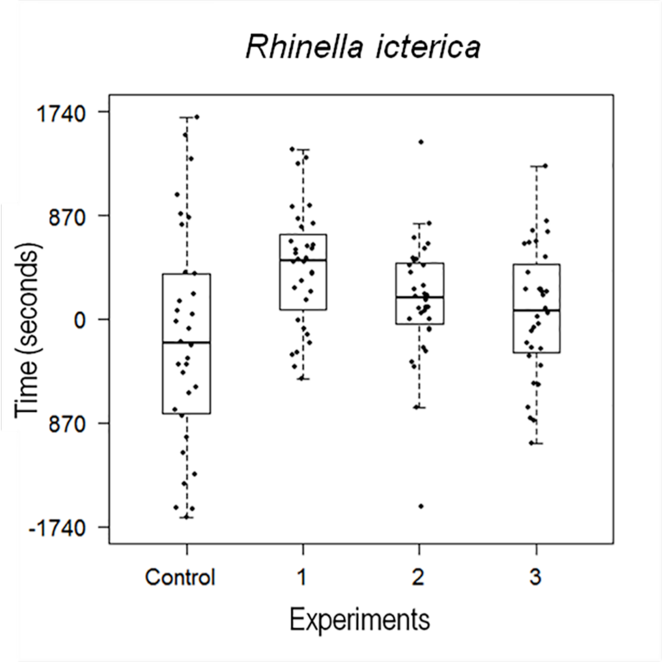

Figure 2. Boxplot of data resulting from the experiments with *Rhinella icterica* tadpoles. Each point represents the difference between time spent by tested tadpole, in each trial, close to stimulus groups 1 and 2.

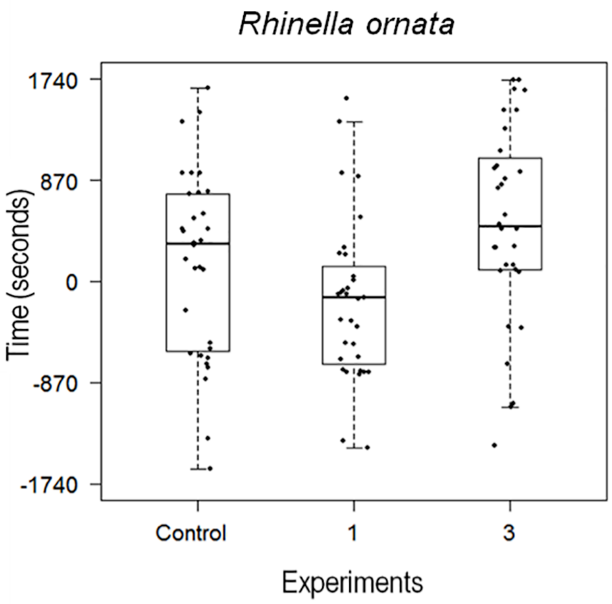

Figure 3. Boxplot of data resulting from the experiments with *Rhinella ornata* tadpoles. Each point represents the difference between time spent by tested tadpole, in each trial, close to stimulus groups 1 and 2.

Unfortunately, we made a mistake when choosing test tadpole in Experiment 2 for *Rhinella ornata*, and we realized in time to not use these data, but was not possible to repeat the experiment. Therefore, we were unable to determine whether isolation rearing would influence attraction to conspecifcs in tadpoles of *R. ornata.*

Discussion

Rhinella icterica tadpoles demonstrated a strong attraction to conspecifics. This result suggests that these tadpoles possess the ability to discriminate between conspecifics and heterospecifics. We could conclude

Table 3. Statistical tests results for each experiment with *Rhinella ornata* tadpoles. When P < 0,05 there is statistically significant difference between time spent by tadpoles next to each stimulus group. df = degrees of freedom; CI = confidence interval.

Exper.	Shapiro-Wilk test	Paired-t test (t)	Mean of differences	CI (95%)		Binomial test
				Inferior Limit	Superior Limit	
Control	W = 0.96 *P* = 0.47	T = 1.08; df = 31; *P* = 0.28	154.37	-135.44	444.19	-
1	W = 0.94 *P* = 0.10	T = -1.41, df = 31 *P*= 0.16	-174.12	-425.26	77.01	-
3	W = 0.95 *P* = 0.20	T = 3.32; df = 31; *P* = 0.02	496.5	192.14	800.85	N= 25 *P*< 0.001

that this attraction behavior is innate, since tadpoles reared in isolation, from the embryonic stage, also demonstrated preference to associate with conspecific tadpoles. The preference to conspecifics is a result of attraction to conspecifics rather than repulsion to heterospecifics, because tadpoles of *R. icterica* have been randomly allocated when submitted to a choice between heterospecifics and an empty compartment.

Rhinella marina tadpoles, that belong to the same phylogenetic group as *R. icterica* (*Rhinella marina* Group; Maciel et al. 2010), when submitted to choice tests between a non-siblings group and an empty compartment, showed no attraction for conspecific when these were non-siblings (Raven et al. 2017). However, *R. marina* tadpoles diminish activity and avoid chemical cues of injured conspecifics, characterizing a recognition and discrimination process, but this does not lead to aggregation, which is formed in response to environmental factors such as light, temperature and habitat structural complexity (Hagman & Shine 2008; Raven et al. 2017). There are different reactions of another bufonid tadpoles to injured conspecifics cues: *Anaxyrus boreas* also exhibit repulsion to these signals, but with increased activity (Hews & Blaustein 1985), while *Sclerophrys danielae* answer to these cues aggregating (Spieler & Linsenmair 1997).

Sontag et al. (2006) demonstrated that tadpoles of *Anaxyrus americanus* recognize conspecifics signals to find food sources and even to discriminate food quality. Similarly, tadpoles of *R. marina* have a stronger attraction to conspecific cues feeding than to cues directly from food (Raven et al. 2017).

Conspecific recognition mechanisms can be used in cannibalism context. Crossland & Shine (2011) found that *R. marina* tadpoles are attracted by chemical cues from conspecific eggs and they eat them. This behavior is not a result of indiscriminate foraging, as these tadpoles rarely eat eggs of other species present in the same habitat (Crossland & Shine 2010). This type of cannibalism can provide an important nutrition source for tadpoles and reduce future intraspecific competition (Crossland et al. 2011). In addition, by finding conspecific eggs, *R. marina* tadpoles can release chemicals that suppress the development of embryos (Crossland & Shine 2012, Clarke et al. 2015).

Crossland et al. (2011) have demonstrated that the substances involved in attraction process of *R. marina* tadpoles by conspecific eggs are toxins used in defence against predators, known as bufadienolids. Thus, it is possible that these substances are involved in attraction to conspecifics in tadpoles of *R. icterica* and other bufonids. These examples of behaviors based on attraction to conspecific cues in species genetically related to *R. icterica* suggest that attraction to conspecifics in this species may be related to similar behaviors. However, further studies are needed to verify if this attraction to conspecific is related with some recognition mechanism.

We found a notable difference between *R. icterica* and *R. ornata* tadpoles in relation to attraction to conspecifics. *Rhinella ornata* tadpoles did not prefer any of the groups, formed by either conspecifics or heterospecifics, indicating that attraction to conspecifics in this species may not occur. *Rhinella ornata* tadpoles spent more time near heterospecifics than the empty compartment, which suggests that *R. ornata* tadpoles prefer to associate with tadpoles of another species than to be isolated.

Some species of tadpoles exhibit schooling behavior (e.g., Beiswenger 1975, 1977; Wassersug et al. 1981; Eterovick 2000; Heursel & Haddad 2002). Functions and benefits attributed to this behavior include predation rate decrease—due to predator confusion and aposematism enhancement—, foraging rate increase, and greater efficiency in thermoregulation (Watt et al.1997; Hoff et al. 1999; Eterovick 2000; Hero et al. 2001). However, when resources are limited, some costs of group formation may be evident, such as increasing competition, cannibalism and predation, as well as increasing disease and inbreeding susceptibility (Hamilton and May 1977; Bateson 1983; Shykoff & Schmid-Hempel 1991; Pfening et al.1993; Goater 1994). Spatial attraction of tadpoles of *R. ornata* by tadpoles groups of *R. icterica*, as well as the non-repulsion of tadpoles of *R. icterica* to tadpoles groups of *R. ornata* tadpole, suggests that spatial interaction between these tadpoles in the natural environment does not involve large competition costs.

Some authors have attempted to explain the absence of discrimination among siblings by tadpoles under laboratory conditions as a consequence of lack of stimuli to reproduce the aggregation behavior (Blaustein & Waldman 1992). The aggregation formation, with relatives or not, may be dependent on the balance between the costs and benefits of this behavior (Hamilton 1964). Both, recognition processes and aggregation can vary within the same species depending on some factors, such as presence and density of predators (Wrona & Dixon 1991; Fitzgeral 1992; Watt et al. 1997) differences in tadpole diet (Gamboa et al. 1991; Pfenning 1990), development stage (Rautio et al. 1991; Blaustein & Waldman 1992; Nicieza 1999), and resource distribution and temperature variation (Hokit & Blaustein 1997). *Lithobates sylvaticus* tadpoles, for example, demonstrated kin recognition and attraction in laboratory experiments, but in natural environments they demonstrated both attraction and repulsion to kin in different ponds (Waldman 1984; Halverson et al. 2006). We were not able to consider the variable stage of development in our study, because of the rapid development and the great variation of development of tadpoles from the same spawn. This research opens a pathway for further studies analyse each variable that may be related to attraction to conspecifics in tadpoles of these two bufonids.

In this study, when opting for laboratory tests, we sought to avoid much of these variables, which could influence the results. Thus, our results suggest that attraction to conspecific may be a important factor for aggregation or association with other tadpoles in *R. icterica*, while tadpoles of *R. ornata* may aggregate indiscriminately or due to other variables than attraction to conspecifics. The behavior differences between two ecologically and genetically closely related species indicate that there may be no generalizations in anuran larvae behavior.

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Author Contributions

Alexandre Polettini Neto. 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.

Jaime Bertoluci. 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.

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

Taxonomic notes on the typology of four species of genus *Notodiaptomus* Kiefer, 1936 (Copepoda: Calanoida: Diaptomidae)

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Abstract: During a taxonomic study of the genus *Notodiaptomus* Kiefer 1936, taxonomic materials were located for *N. iheringi, N. spinuliferus, N. henseni* and *N. deitersi*, and all had their status investigated. Inconsistencies were found and are reported in this note, from the original details to the current typological situation. Nomenclatural conformations and new types are specified. These reinforce the taxonomic validity of the species, recently redescribed in other research. Thus in this note the neotype to *N. iheringi, N. spinuliferus,* and *N. henseni*, and provide a topotype to *N. deitersi* are defined.
Keywords: *Neotropical Copepods; Typification; Taxonomic stability; Nomenclature Zoological Code.*

Nota taxonômica sobre a tipologia de quatro espécies de *Notodiaptomus* Kiefer, 1936 (Copepoda: Calanoida: Diaptomidae)

Resumo: Durante o estudo taxonômico do gênero *Notodiaptomus* Kiefer (1936), *N. iheringi*, *N. spinuliferus, N. henseni* e *N. deitersi* tiveram seus materiais taxonômicos localizados e investigados. Inconsistências foram observadas e são relatadas nesta anotação, desde os detalhamentos originais até a atual situação tipológica. Conformações nomenclaturais e novos tipos são especificados e reforçam a validade taxonômica destas espécies, neótipos para *N. iheringi, N. spinuliferus, N. henseni* e topótipo para *N. deitersi.*
Palavras-chave: *Copépodes neotropicais; Tipificação; Estabilidade taxonômica; Código de nomenclatura zoológica.*

Introduction

Notodiaptomus is a genus of Copepoda Edwards, 1840 originally proposed by Kiefer (1936). A species group from "*Nordestinus* complex" of the genus *Diaptomus* (*sensu lato*) formed the initial basis for the formation of this genus (Wright, 1935), originally with 11 and, currently, 43 valid taxa (WoRMS 2020).

Amongst these, *Notodiaptomus iheringi* (Wright, 1935), *Notodiaptomus spinuliferus* Dussart and Matsumura-Tundisi in Dussart, 1985, *Notodiaptomus henseni* (Dahl, 1894) and *Notodiaptomus deitersi* (Poppe, 1891) were described, respectively, for the northeast, southeast, midwest and northern areas of Brazil. *Notodiaptomus iheringi*, *N. henseni* and *N. deitersi* were recently redescribed by Santos-Silva et al. (2015), who established taxonomic limits, but without resolving problems associated with type specimens. *Notodiaptomus spinuliferus*, which has gained a heterogeneous description over time (Dussart and Matsumura-Tundisi 1986; Paggi 2001; Matsumura-Tundisi 2008), has nonconformities in relation to the original description and a missing holotype, all of which weakens its taxonomic validity and status.

As part of a widest taxonomic revision of the genus *Notodiaptomus* these species were tracked through the literature, and located at the Museum of the Carcinological Collection of Zoology from the University of São Paulo, and the collection of the Invertebrate Zoology Department from the Smithsonian Institution National Museum Natural History, Washington D.C. (NMNH), where their type-series were accessed and examined. Specimens of *N. iheringi* and the type-material of *N. spinuliferus* were found, but not those of *N. henseni* or *N. deitersi*, although these were reported as being in this collection (Santos-Silva et al. 2015). All located specimens were analyzed. Those said to be in the collection, but not found, were tracked via the original literature. Finally, four species were found to have typological inconsistencies and required typological adaptation, because they possess some level of damage to their stored material-type.

Accordingly, we provide here definitions of the *N. iheringi, N. spinuliferus, N. henseni* and *N. deitersi* type-series. Additionally, we provide comments on the state of preservation, storage and disposal of the analyzed type-material present in of the visited taxonomic collections.

Materials and Methods

Specimens analyzed in this study came from the Carcinological Collection of the Museum of Zoology of the University of São Paulo (MZUSP) and the collection of the Invertebrate Zoology Department of the Smithsonian Institution National Museum Natural History, Washington D.C. (NMNH). The state of preservation and conditions of the preserved material were investigated using the original taxonomic records and applying the norms of the International Code of Zoological Nomenclature (ICZN 2012). To assist analysis, a Leica MZ12.5 trinocular optical stereomicroscope and a Leica CTR5000 10x-100x trinocular optical microscope with attached Leica DFC450 camera and Leica Application Software (LAS) were used for images and micrographic measurements of specimens, all accessed with prior authorization from the MZUSP taxonomic collections curator. Permanent slide dissection and assembly procedures followed Kihara & Rocha (2009): lactophenol as medium, whole coverslips (24x24 mm) on self-adhesive plastic wedges and Entellan® Rapid Micro-Mounting Medium. For wet preservation, 70% alcohol was either deployed or renewed. All material examined, produced or added (i.e., "Additional Material") in this study continues to form part of the initially accessed taxonomic collection, *N. spinuliferus, N. henseni* and *N. deitersi* in the Museum of Zoology at the University of São Paulo (São Paulo, Brazil), and *N. iheringi* in the Smithsonian Institution National Museum Natural History (Washington DC, USA).

Results

Based on the analyzes carried out in the scientific collection of the MZUSP, on focus to the species *N. deitersi*, *N. iheringi*, *N. henseni*, and *N. spinuliferus*, the descriptive characterization of the respective type-material becomes updated as follows:

Notodiaptomus deitersi (Poppe, 1891). **Assigned collection:** MZUSP. **Deposition label:** Topotype. **Access code informed:** 12.823. **Deposit details:** 20♂♂ and 20♀♀ (in alcohol 70%), adults, on 30.X.1996, collected by V.P. da Silva. **Situation:** not existent. **Resolution employed:** supplementation of material [by this designation]. **Additional material information**: BRAZIL: MATO GROSSO. Baía Pedra Branca (15°27'44"S 60°06'44"O), Vila Bela da Santíssima Trindade [431 km from Cuiabá]. Specimens of adult colleted by V. P. da Silva, on 30.X.1996. **Additional material label:** Topotype. 20♂♂, undissected, preserved wet (MZUSP 40395) and 20♀♀, undissected, preserved wet (MZUSP 40395). Confirmed by E. N. Santos-Silva, on 01.III.2015: ♂♂ body of 1098 micrometers in mean length (N=20), variation between 1060 and 1166 micrometers; ♀♀ body of 1148 micrometros mean length (N=20), variation between 1113 and 1176 micrometers, both excluding caudal setae. **Deposited in:** Carcinological Collection of the Museum of Zoology of the University of São Paulo (MZUSP), São Paulo, Brazil. **Ecology:** organism typical of ponds, lakes and bays environments.

Notodiaptomus iheringi (Wright, 1935). **Assigned collection:** MZUSP. **Deposition label:** non-type material. **Access code informed:** (a) MZUSP 6192, (b) MZUSP 6192 and (c) MZUSP 6193. **Deposit details:** 4♂♂ adult (a), 3♀♀ adult (b), and 4 juveniles [sex not provided] (c), all in alcohol, un-dissected. **Situation:** destroyed exemplars [except juveniles]. **Resolution employed:** Neotype designation from material from the locality-type existing in the MNHN [by this designation]. **Additional material information**: BRAZIL, PARAÍBA. Açude Puxinanã (*Puxinanã Reservoir*), vila de Puxinanã, close to Campina Grande [type-locality]. Adult specimes colleted and determined by S. Wright, no date, deposited in the Smithsonian Institution National Museum Natural History (MNHN). **Additional material label:** Neotype. 1♂, un-dissected, preserved wet (USNM 1548280). Topotype. 1♂, un-dissected and 2♀♀, un-dissected (USNM 79542). Confirmed by C. WALTER, 04.XI.2019: neotype 1100 micrometers excluding caudal setae, prosoma 800 micrometers. **Deposited in:** Invertebrate Zoology Department of the Smithsonian Institution National Museum Natural History (NMNH), Washington D.C. **Ecology:** an organism typical of limnetic environments, encountered in reservoirs, lakes, rivers and streams.

Notodiaptomus henseni (Dahl, 1894). **Assigned collection:** MZUSP. **Deposition label:** Neotype. **Access code informed:** none. **Deposit details:** 1♂ adult, in alcohol, un-dissected. **Situation:** nonexistent. **Resolution employed:** supplementation of material [by this designation]. **Additional material information**: BRAZIL: PARÁ. Igarapé Uruazinho [*Uruazinho stream*] (0°04'03"S 47°08'56"O), Maiauatá, rio Tocantins waterfall region. Specimen of an adult semaphoronte colletad and determined by M. N. Cipólli & M. A Juliano de Carvalho, on 27.VIII.1970. **Additional material label:** Neotype. 1♂, undissected, preserved in alcohol 70% (MZUSP 40393). Topotype. 10♀♀, by this designation, undissected, preserved wet (MZUSP 40394), 10♂♂, un-dissected, preserved in alcohol 70% (MZUSP 40394). Confirmed by E. N. Santos-Silva, on 01.III.2015: neotype 1208 micrometers excluding caudal setae, prosoma 890 micrometers. **Deposited in:** Carcinological Collection of the Museum of Zoology of the University of São Paulo (MZUSP), São Paulo, Brazil. **Ecology:** organism typical of river mouths, reservoirs, marginal lagoons, lakes, and streams environments.

Notodiaptomus spinuliferus Dussart and Matsumura-Tundisi in Dussart, 1985. **Assigned collection:** MZUSP. **Deposition label:** (a) holotype and (b) paratypes. **Access code informed:** (a) MZUSP 6969, (b1) MZUSP 6970, and (b2) MZUSP 6971. **Deposit details:** (a) 1♂ adult, un-dissected, permanently mounted on slide [one]; (b1) 1♂ adult, un-dissected, permanently mounted on slide [one], and (b2) 2♂♂ and 5♀♀, un-dissected, preserved in alcohol 70%. |Material collected and determined by Dussart & Matsumura-Tundisi, on 14.III.1979. **Situation:** holotype missed [broken slide], and paratypes viable paratypes for exam. **Resolution employed:** supplementation of material [by this designation]. **Additional material information**: BRAZIL: SÃO PAULO. Ilha Solteira reservoir (20°17'58"S 51°08'32"O), Paraná river, frontier between the states of São Paulo and Mato Grosso do Sul. Specimens adult collected and determined by Dussart & Matsumura-Tundisi, on 14.III.1979 [same type-locality]. **Additional material label:** Neotype. 1♀ adult, undissected, preserved in alcohol 70% (MZUSP 40049). Paratype. 1♂ adult, un-dissected, permanent mount (MZUSP 6970); 1♂ adult, dissected, mounted on 5 permanent slides (MZUSP 6971), 1♀ adult, dissected, mounted on 4 permanent slides (MZUSP 6971), 1♂ and 3♀♀, adult, un-dissected, preserved in alcohol 70% (MZUSP 6971). Confirmed by L. J. O. Geraldes-Primeiro, on 05.IX.2019: neotype body 1260 micrometers (excluding caudal setae), prosoma 1001 micrometers; male 1079 micrometers (N=3) excluding caudal setae, prosoma 761 micrometers (N=3), smaller and finer than the female; female body 1231 micrometers (N=4) excluding caudal setae, prosoma 998 micrometers (N=4). **Deposited in:** Carcinological Collection of the Museum of Zoology of the University of São Paulo (MZUSP), São Paulo, Brazil. **Ecology:** an organism typical inhabitant of standing waters.

Discussion

Morphological examination in *N. iheringi, N. spinuliferus, N. henseni,* and *N. deitersi* revealed related inconsistencies to the original records of the types-specimens of these species (i.e. typological records), some of which were non-existent, disappered, or danified.

The specimens of *Notodiaptomus iheringi* were the first examined and 11 individuals assigned to the taxon were found to be partially or completely damaged. Adult male semaphoronte specimens were the only ones for which morphometric measurements were possible, though these had damaged morphologies that made them inappropriate for detailed examination. Examination allowed us to determine that the current preservation status of the material was the result of an unsuccessful attempted at alcohol rehydration.

Notodiaptomus iheringi was described by Wright in 1935, initially from *Diaptomus sensu lato*, with an unspecified holotype and non-specified location, probably with non-existent type material (Reid 1991). Kiefer (1936) in creating *Notodiaptomus* transferred several taxa to this genus, which has since then undergone some repositioning (Dussart 1985; Rolla et al. 1990) to achieve the present status. Reid (1985) provided a redescription to the species from material from sites in Rio de Janeiro state, Lagoa da Saudade (21°38'17"S 41°11'06"O), precisely. It is likely the material of the MZUSP collections accessed and analysed during the current study, especially the females, from which we could identify only preserved organic residues.

Through the work of Reid (1991), we tracked the existence of material from the species type locality, in the NMNH, under code 79542. Santos-Silva et al. (2015) informed that from this material it would be specified neotype of the taxon, which we verified never to have occurred. Thus, due to the inexistence of the type material, a poor condition in the existing MZUSP specimens, and the existence of specimens from the type locality originally to the taxon, we designate the neotype of the specie (USNM 1548280), from of the material existing at the NMNH' biological collection.

The current records, therefor, satisfy the conditions required by article 75, recommendation 75 of the ICZN (2012), which defines these conditions as necessary presuppositions for typological designation, which is hereby assigned to fix and give greater stability to the *N. iheringi.*The inconsistencies observed in *Notodiaptomus spinuliferus* were of different sort and concerned the disappearance of the type-specimen and specific non-observance of the taxonomic availability criteria stated in the nomenclatural code.

Notodiaptomus spinuliferus was described by Dussart (1985), with attributed authority to Dussart & Matsumura-Tundisi in this report. However, even if the specified assignment is as given in Dussart "*cf. Dussart & Matsumura-Tundisi, sous presse*", the description offered by Dussart (1985) meets the availability (article 11) and publication (article 8) criteria of the ICZN (2012), and therefore it is valid to consider it as the nomenclatural act for this taxon. There are several misquoted citations for the species authority record which, in compliance with ICZN Article 51.2 (51) recommendation 51E, should be cited invariably as *Notodiaptomus spinuliferus* Dussart & Matsumura-Tundisi in Dussart, 1985.

Regarding the typological analysis of the material deposited by Matsumura-Tundisi & Dussart at the MZUSP, the precarious preservation state in which the holotype was stored (on a permanent slide) (Figure 1)

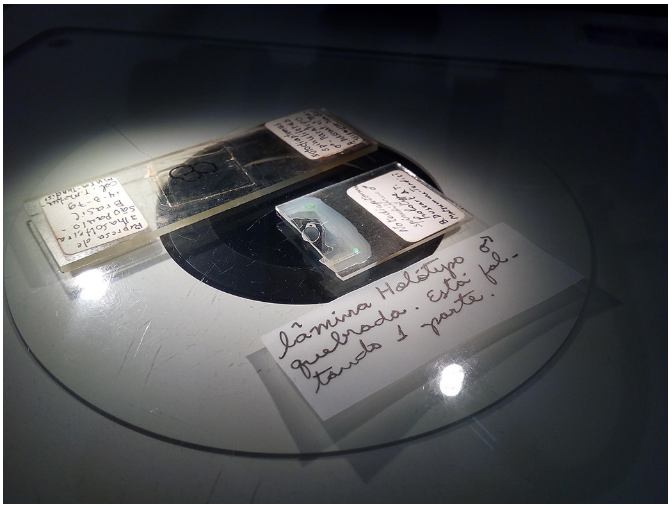

Figure 1. Type material of *Notodiaptomus spinuliferus*, condition of preservation of the holotype (MZUSP 6969) and paratype (MZUSP 6970).

makes it unsuitable for accurate and detailed morphological examination. The specimen in question would be a male and only the original slide, deposited in 1985, was found. This was fragmented and probably, because if this, with the type specimen missing. Of the eleven paratypes reported as being in existance (these being: one slide containing a male [MZUSP 6970], and five males and five females [MZUSP 6971]), only eight have been confirmed as still existant: one male (MZUSP 6970), two males (MZUSP 6971), and five females (MZUSP 6971). Of these, one male and one female were identified, dissected and mounted on nine permanent slides, five male-containing slides and four female-containing slides, remaining as the paratypes.

A female from the MZUSP 6971 lot was selected, examined and deposited as an undissected specimen in alcohol 70%, as the neotype MZUSP 40049. This designation was made in accordance with Art. 75 of ICZN (2012). All procedures for dissection and assembly of permanent slides followed the recommendations (Kihara & Rocha, 2009) for type material that are designed for future taxonomic examinations.

Notodiaptomus henseni and *N. deitersi* were taxa with type material reported by Santos-Silva et al. (2015) to be housed in the MZUSP. However, these accessions appear non-existent, and therefore could not be examined. The type locality for *N. henseni* was not specified clearly in Dahl (1894), but Santos-Silva et al. (2015) provided evidence that makes the mouth of the Tocantins river, Pará state, the most likely original site. Thus, a specimen was selected for designation as a neotype of the species (MZUSP 40393) and added topotypes to the MZUSP collection, all them derived from the material collected at the above-mentioned location, during the Permanent Amazon Expedition (PAE) in 1970, and located in the National Amazon Research Institute's Plankton Laboratory collection.

For *N. deitersi*, 20 males and 20 females under code MZUSP 12.823 indicated by Santos-Silva et al. (2015) as topotype of the species could not be located. This is the type species of the genus *Notodiaptomus* and was intended by Santos-Silva et al. (1999) and Santos-Silva et al. (2015) to provide taxonomic stability. However, through the current contribution, we additional and specified taxon material for the MZUSP, which now that conforms to the information originally specified to paratypes of the taxon (Santos-Silva et al., 1999).

Therefore, we concluded that the typological situation of the investigated taxa is properly stabilized. Thus, all species possess existente type-material, localized, and defined as evidence of their morphological patterns. This grants the necessary nomenclatural validity for the taxa, and it is essential for further accuracy morphological investigations.

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Author Contributions

Luis José de Oliveira Geraldes-Primeiro: Responsible for the development of the study, for taxonomic examinations, acquisition and interpretation of data, writing of this proposal, and submission of this manuscript.

Joana D'Arc de Jesus Pinto: As curator of the type-material at carcinological collection of the Museum of Zoology of São Paulo, the co-author was responsible in obtaining the material examined in this study. Futhermore, she was directly responsible for the records and catalogue-updates of the specimens examined and added to the scientific collection.

Raimundo Erasmo Souza Farias: The co-author participated in the interpretation of the analysis of the data obtained and in the writing of the paper. He was a key member of the intellectual consistency of this study and relevant to the critical review of all content.

Edinaldo Nelson dos Santos-Silva: The co-author was decisive in obtaining part of the data and directly examined one of the species presented in this study. Futhermore, he was participating in the critical review and development of intellectual content.

Conflicts of Interest

The authors declare that have no conflict of interest that might constitute an embarrassment to the publication of this article.

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Spatio-temporal variation of *Mocis latipes* (Guenée, 1852) (Lepidoptera: Erebidae) populations in Brazil according to meteorological factors

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Abstract: *Mocis latipes* (Guenée, 1852) (Lepidoptera: Erebidae) has been recognized as a major owlet caterpillar associated to the herbivory of gramineaceous plants across the American continent. During outbreaks, the caterpillars are capable of completely consuming preferred hosts (grasses) and, when these hosts are destroyed, they can move to adjacent non-grass plants and cause similar damage. Meteorological variable such as temperature and humidity are described as factors that affect the development and abundance of *M. latipes*. This paper aimed to describe and compare the spatial and temporal distribution of *M. latipes* in different locations in Brazil and to evaluate the influence of meteorological variables on the temporal range. A total of 12 locations were evaluated, in each collection point light traps were installed near cultivated areas. In order to understand the influence of meteorological variables on the abundance of *M. latipes*, the data were analyzed using a Generalized Linear Model according to Poisson regression. A linear regression was also used to verify the relation between the abundance and the latitude. A total of 1,985 moths were collected. The highest collections were in Amazon and Cerrado biomes. Results show that abundance was inversely related to increasing latitude and Poisson regression analysis indicated that the main meteorological variables were significantly related to abundance at each site. This study shows that due to the high preference for gramineas and the high temperature requirements (30°C), *M. latipes* is an important species in hot regions and regions with hight humidity. Furthermore, even in higher latitudes, in subtropical areas, during summer months, populations can Rapidly growth being able to cause economic damages.

Keywords: *light trap; owlet moth pest; populational variations; striped grassworm.*

Variações espaço-temporais das popupações de *Mocis latipes* (Guenée, 1852) (Lepidoptera: Erebidae) no Brasil de acordo com fatores meteorológicos.

Resumo: *Mocis latipes* (Guenée, 1852) (Lepidoptera: Erebidae) tem sido reconhecida como uma das principais espécies consumidoras de gramíneas em todo o continente americano. Durante os surtos, as lagartas são capazes de consumir completamente os hospedeiros preferenciais (gramíneas) e, quando os hospedeiros preferenciais são destruídos, podem mover-se para plantas adjacentes não gramíneas e causar danos semelhantes. Variáveis meteorológicas, como temperatura e umidade, são conhecidas por afetar o desenvolvimento e a abundância de suas populações. Este trabalho teve como objetivo descrever e comparar a distribuição espaço-temporal de *M. latipes* em diferentes localidades do Brasil e avaliar a influência de variáveis meteorológicas sobre suas variações temporais. Foram avaliados 12 locais, em cada ponto de coleta foram instaladas armadilhas luminosas próximas às áreas de cultivo. Para entender a influência das variáveis meteorológicas na abundância de *M. latipes*, os dados foram analisados pelo Modelo Linear Generalizado, empregando a regressão de Poisson. Uma regressão linear também foi utilizada para verificar a relação entre a abundância e a latitude. Um total de 1.985 mariposas foram coletadas. Os maiores números de indivíduos foram coletados nos biomas Amazônia e Cerrado. Os resultados mostram que a abundância de mariposas está inversamente relacionada ao aumento da latitude. A análise de regressão de Poisson indicou que as principais variáveis meteorológicas foram significativamente relacionadas à abundância em cada local. Este estudo mostra que devido à preferência por gramíneas em estado vegetativo e às altas exigências de temperatura (30°C), *M. latipes* é uma espécie importante em regiões quentes, especialmente nas épocas de maior umidade. Além disso, mesmo em latitudes elevadas, durante os meses de verão, as populações podem aumentar rapidamente, podendo causar danos econômicos.

Palavras-chave: *armadilha luminosa; noctuoides-praga; variações populacionais; curuquerê-dos-capinzais.*

Introduction

Mocis latipes (Guenée, 1852) (Lepidoptera: Erebidae) is an important grass pest that occurs throughout the Americas, from Canada to Argentina, Chile and Uruguay (Bethune 1869, Biezanko et al. 1957, Barth 1958, Angulo & Jana-Sáenz 1983, Pastrana 2004, Wagner et al. 2011, Alves et al. 2019). Due to the fact that *M. latipes* is widely distributed, it is common for it to receive different names across its range of distribution. For example, in North America it is called as "striped grassworm" and "striped grass looper" (Genung & Allen Jr. 1974, Reinert 1975, Koehler et al. 1977); in Central America, "falso medidor de los pastos" and "Guinea-grass moth " (Fennah 1947, Calderón et al. 1981); in Brazil "curuquerê-dos capinzais" and "lagarta-dos-capinzais" (Pigatti & Mello 1960, Cavalcante 1977, Silva et al. 1991, Correia et al. 1999), in Colombia "gusano agrimensor" (Alvarez-R & Sanchez-G 1981) and in Argentina "gusano cuarteador" (Costilla et al. 1973, Hayward 1960, Salvatore & Willink 2004, Acosta et al. 2005).

Although some authors consider *M. latipes* as a polyphagous pest (e.g. Fonseca & Autuori 1932, Bissell 1940, Biezanko et al. 1957, 1974, Hayward 1960, Labrador 1964, Kimball 1965, Silva et al. 1968, Costilla et al. 1973, Ware 1973, Bertels 1975, Kleyla et al. 1979, Pastrana 2004, Formentini et al. 2015), the main host plants of *M. latipes* are grasses. Ogunwolu & Habeck (1975) has shown that the first to fourth instar larvae are unable to complete their life cycle in non-grassy plants. Despite that, *M. latipes* can be considered a polyphagous species because it has also been collected from non-grassy plants (Ogunwolu & Habeck 1975) or, after caterpillars completely consume grasses or when selective herbicides are used, they are able to migrate and defoliate other crops (Capinera 2005).

The importance of *M. latipes* as a pest is highlighted due to reports of population outbreaks (e.g. Watson 1933, Pugliese 1954, Capriles & Ferrer 1973, Reinert 1975, Minno & Snyder 2008) and its extensive damage to the main cultivated Poaceae, including grains, such as corn, rice, sorghum and wheat (Bodkin 1914, Hempel 1914, 1920a, 1920b, Costa 1944a, 1944b, Fonseca 1944, Bertels & Rocha 1950, Dinther 1955, Falanghe & Dias Netto 1961, Hseih 1979, Cruz 1991, Cruz & Santos 1983, Ferreira 1984, Silva & Carvalho 1986, Páliz-Sánchez & Mendonza-Mora 1999, Pitre et al. 1999, Vergara et al. 2001, Hickel et al. 2018), forage crops (Vickery 1924, Lopes 1955, 1961, Labrador 1964, Strayer 1971, Koehler et al. 1977, Calderón et al. 1981, 1982, Costa et al. 1983, Silvain 1984, Silvain & Dauthuille 1985, Miret 1986, Gibbs 1990, Milán et al. 1990, Silva et al. 1994, Jiménez et al. 1997, Teixeira & Townsend 1997, Correia et al. 1999, Piedra & Carrillo 1999, Sánchez Soto & Ortiz Garcia 1999, Alarcón et al. 2004, Fazolin et al. 2009), and sugar cane (Dine 1913, Wolcott 1921, Holloway 1933, Reiniger 1946, Queiroz 1965, Mendonça Filho 1972, Costilla et al. 1973, Guagliumi 1973, Mahadeo 1977, Planalsucar 1982, Salvatore & Willink 2004, Acosta et al. 2005, Salvatore et al. 2009, Marquez 2013).

Usually, *M. latipes* is reported as a cyclic pest (e.g. Calderón et al. 1981, Saunders et al. 1998) whose abundance is influenced by environmental factors such as humidity, precipitation and temperature (Calderón et al. 1982, Ferreira & Parra 1985, Gibbs 1990). Therefore, under favorable weather conditions and food availability, their populations increase rapidly.

Temperature and humidity are the main environmental factors that influence occurrence, distribution and complete development of *M. latipes* biological cycle. Therefore, the imbalance of these conditions directly interferes in population size (Bertels 1970, Bernardi et al. 2011). Understanding spatial and temporal distribution of pest species is important to reduce risks of Outbreaks and to develop sustainable management control (Pedigo & Rice 2009). There is a lack of studies on population dynamics of *M. latipes* in different regions in Brazil. Population outbreaks are known to occur early in the rainy season, especially in the Amazon (Teixeira & Townsend 1997, Fazolin et al. 2009). However, the factors that cause these occurrences are still unknown. Thus, this paper reports the influence of meteorological variables and latitude on spatio-temporal abundance of *M. latipes* in different regions in Brazil.

Material and Methods

1. *Moth sampling*

Mocis latipes moths were captured in systematized collections at 12 regions in Brazil (Table 1), being those regions representatives of the main Brazilian biomes and on a latitudinal range (2° North to 31° South). At each collection point, a light trap (Light Trap - Bio Controle®), adapted to Pennsylvania model (Frost 1957), was placed on a pole 3 meters from the ground. Collections were made once a month, from July 2015 to June 2016, during each novilunium repeated for five nights (repetitions). Collection methodology followed Specht et al. (2005) and Piovesan et al. (2018). Traps were placed in areas surrounded by several agricultural crops, especially cotton, soybean, corn and pastures (Table 1). Corn was the only crop presented in all regions.

2. *Mocis latipes identification*

Moths were identified by comparing collected specimens against reference material from the Coleção Entomológica da Embrapa Cerrados (CPAC) and bibliographic resources (Brou Jr., 2004). After

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Table 1. Geographic location, municipality, state, biome, climate (Köppen-Geiger according Beck et al., 2018) and main annual cultures, C - cotton, M - maize, S - Soybean, and P - pastures, of each light trap collecting point.

	Latitude	Longitude	Altitude (m)	Municipality	State	Biome	Climate	Cultures
1	2.695597	-61.005028	87	Alto Alegre	Roraima - RR	Amazon	Aw	C, M, S
2	-2.695596	-54.570650	114	Mojuí dos Campos	Pará - PA	Amazon	Am	M, S
3	-10.032536	-67.626908	183	Rio Branco	Acre - AC	Amazon	Af	M, S
4	-10.519042	-48.2933306	262	Porto Nacional	Tocantins - TO	Savanna	Aw	M, S
5	-11.867083	-55.600608	362	Sinop	Mato Grosso - MT	Amazon	Aw	C, M, S
6	-12.078417	-45.869111	782	Luís Eduardo Magalhães	Bahia - BA	Savanna	BSh	C, M, S
7	-15.606811	-47.745125	1169	Planaltina	Distrito Federal - DF	Savanna	Aw	M, S
8	-19.662519	-47.960878	784	Uberaba	Minas Gerais - MG	Savanna	Aw	M, S
9	-20.753231	-41.489800	120	Alegre	Espírito Santo - ES	Atlantic Forest	Cwa	M, P
10	-23.189694	-51.171861	545	Londrina	Paraná - PR	Atlantic Forest	Cfa	M, S
11	-28.230742	-52.403625	671	Passo Fundo	Rio Grande do Sul - RS	Atlantic Forest	Cfa	M, S
12	-31.351372	-54.020142	232	Bagé	Rio Grande do Sul - RS	Pampa	Cfa	M, S, P

identification, the specimens were stored in glass containers with 92.8GL ethyl alcohol, labeled with date and collecting location. Specimens were kept as voucher material in the CPAC collection and the specific abundance data were tabulated in a spreadsheet for analysis.

3. *Meteorological data*

The meteorological variables considered were the monthly average of the maximum and minimum temperatures (°C), relative humidity (%) and cumulative precipitation (mm^3/m^2/month). The data were obtained from Embrapa Cerrados, Embrapa Agrossilvipastoril, Capixaba Institute for Research, Technical Assistance and Rural Extension - Incaper and National Institute of Meteorology - INMET. Except for data obtained from INMET, all other data were collected at meteorological stations near the sample units.

4. *Statistical analysis*

To evaluate the relationship between the abundance of *M. latipes* and the latitude (Decimal Degrees - DD) used the generalized linear model (GLM) according to Poisson regression with eleven collections points established in the Southern Hemisphere (Table 1). In the same way, as abundance data considers moth count, a GLM Poisson regression was used in order to determine the conjunct influence of meteorological variables on the expected abundance of *M. latipes* at each collecting points. The analysis includes the estimates and respective significance, and the standard errors of the estimate for each collecting point and a general estimate with all points, except for Londrina, Passo Fundo and Bagé, due to the non-significance of the coefficients of the variables when studied individually. The analysis was performed on R version 3.4.0 (R Development Core Team 2017).

Results

In total, 1,985 moths of *M. latipes* were collected with greater abundance concentrated in the lower latitudes, in the Amazon and Cerrado biomes. Almost half of the insects (42.11%) were collected in Alto Alegre-RR, followed by Rio Branco-AC (13.55%) and Mojuí dos Campos-PA (11.33%) (Table 2; Figure 1). Moths were found in all sampled locations, most of the year and in 92 collections (63.88%) from the 144 collected. Disregarding the southernmost locations (Bagé, Passo Fundo and Londrina), where it is cold during the winter time, *M. latipes* were collected in 84 out of 108 events, which corresponds to more than ¾ (77.78%) of the collections.

The inverse relationship between latitude and abundance of *M. latipes* shown in Figure 1 is statistically confirmed by Poisson regression analysis (Figure 2) whose coefficients were Akaike Information Criterion - AIC 321.780; Intercept: estimate 6.194, SD 0.055, z value 113.100 ($p > 0.001$) and Latitude: estimate 0.115, SD 0.004, z value 25.930 ($p > 0.001$).

The monthly occurrence data (Table 2) indicated that, despite occurring in practically the whole year, *M. latipes* populations varied differently in each location.

Poisson regression analysis indicated for most locations that the conjunct of accumulated monthly precipitation, the monthly averages of minimum and maximum temperature and the monthly average

Table 2. Monthly abundancy of *Mocis latipes* moths collected with light traps, during five nights per novilune.

Locality	**2016**						**2017**						**Total**	**Percent**
	Jul	**Aug**	**Sep**	**Oct**	**Nov**	**Dez**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**		**(%)**
Alto Alegre - RR	144	531	103	1	0	2	6	2	14	16	17	0	836	42.116
Mojuí dos Campos - PA	3	1	3	64	2	14	66	8	4	3	10	47	225	11.335
Rio Branco - AC	1	1	7	3	12	3	2	2	62	109	39	28	269	13.552
Porto Nacional - TO	3	0	0	12	17	9	4	4	32	67	7	2	157	7.909
Sinop - MT	1	1	2	3	2	2	2	0	0	0	168	8	189	9.521
Luís Eduardo Magalhães - BA	0	0	1	1	33	41	25	2	12	8	8	2	133	6.700
Planaltina - DF	0	1	0	0	22	77	1	2	0	1	3	3	110	5.542
Uberaba - MG	0	0	1	0	0	6	0	1	1	3	18	2	32	1.612
Alegre - ES	0	2	1	0	1	4	0	0	2	0	0	2	12	0.605
Londrina - PR	0	1	0	0	0	0	1	0	0	0	14	1	17	0.856
Passo Fundo - RS	0	0	0	1	0	0	1	2	0	0	0	0	4	0.202
Bagé - RS	0	0	0	0	0	0	0	0	1	0	0	0	1	0.050
Total	152	538	118	85	89	158	108	23	128	207	284	95	1985	100

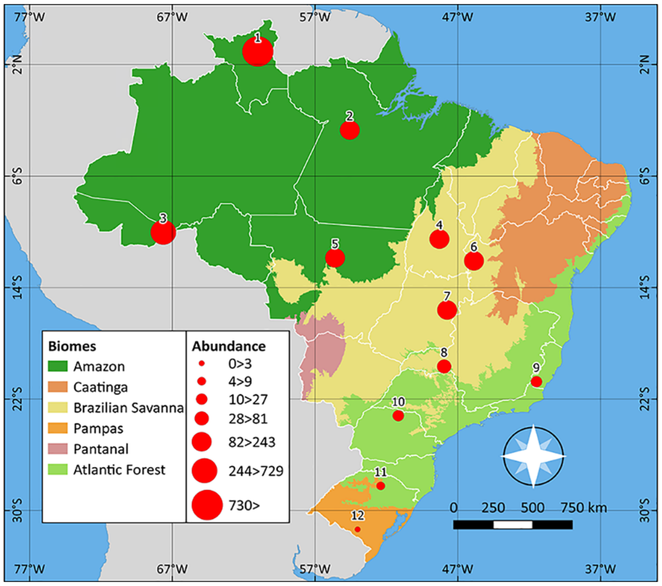

Figure 1. Variation in number (red circles) of *Mocis latipes* sampled in 12 localities of Brazil (July 2015 – June 2016). For a complete description of localities and dates, see Tables 1-2.

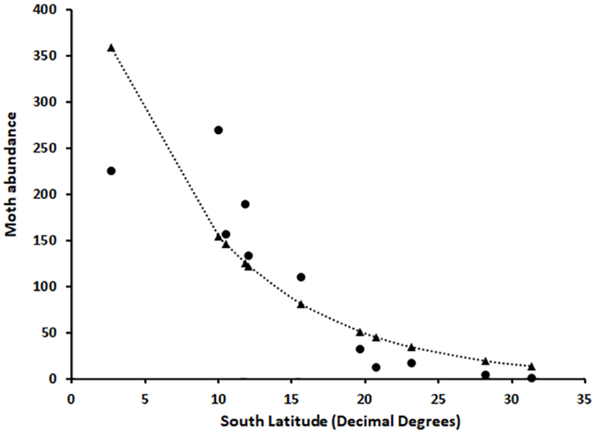

Figure 2. Representation of the observed (circles) and estimated by Poisson regression analysis (triangles) abundance of *Mocis latipes* in each collection point, according South Latitude.

of relative humidity positively influenced abundance (Table 3). As observed between monthly abundance (Table 2), the relations between the number of moths and meteorological variables varied between locations (Table 3).

Discussion

The occurrence of *M. latipes* throughout Brazil (Figure 1) was expected due to the wide distribution previously mentioned in the bibliography (ex. Reinert 1975, Carvalho 1976, Saunders et al. 1998, Bentancourt & Scatoni 2006, Wagner et al. 2011). The simultaneous occurrence of this species in such a wide and diverse territory is correlated with several biological aspects attributed to insects presenting great geographic distribution that includes: great capacity for dispersion and migration (Ferguson et al. 1991, Brou Jr. 2004, Krauel et al. 2018, Alves et al. 2019), short life cycle with multivoltinism associated with high fertility (ex. Labrador 1964, Reinert 1975, Cruz & Santos 1983, Ferreira & Parra 1985, Silva & Carvalho 1986, Silva et al. 1991, Piedra & Carrilo 1999, Wagner et al. 2011), and polyphagy, which allows food to be obtained anywhere and at any time. In this last aspect, *M. latipes* is better classified as an oligophytophage because it develops preferentially in grasses (Ogunwolu & Habeck 1975) despite consuming other vegetables especially at the end of larval development (Hayward 1960, Labrador 1964, Kimball 1965, Silva et al. 1968, Costilla et al. 1973, Biezanko et al. 1974, Kleyla et al. 1979, Pastrana 2004, Formentini et al. 2015). Despite being an oligophagous, *M. latipes* has in its favor that grasses are vegetables that have a relatively preserved physical and chemical structure throughout evolutionary history (Kellogg 2001). Thus, this determines that *M. latipes*, like most insects associated with grasses can develop satisfactorily in most species (Tscharntke & Greiler 1995). Moreover, both annual and perennial grasses are found in almost all environments throughout the year, except for closed forest areas, deserts or very cold regions (Strömberg 2011, Dixon et al. 2014). In addition, cultivated grasses often become invasive, having additional

Table 3 . Results (AIC - Akaike Information Criterion, Intercept values - Estimate (with significance) and Standard Error -SE) obtained by the Poisson regression analysis between the monthly abundance of *Mocis latipes* moths collected in each location and the main meteorological variables (Precipitation accumulated in the month; Max. Temp - Average of monthly maximum temperature; Min. Temp - Average monthly minimum temperature and RH - average monthly relative humidity).

	Intercept	**Precipitation**	**Max. Temp**	**Min. Temp.**	**RH**
General+ (AIC 4770.80)					
Estimate	-8.898***	0.001***	0.035ns	0.274***	0.058***
SE	0.884	0.000	0.029	0.022	0.004
Alto Alegre (AIC 188.35)					
Estimate	34.591***	0.001ns	-0.101ns	-2.522***	0.388***
SE	3.891	0.001	0.099	0.224	0.022
Mojuí dos Campos (AIC 210.390)					
Estimate	204.286***	0.002ns	-1.811***	-3.048*	-0.873***
SE	36.823	0.003	0.274	1.206	0.100
Rio Branco (AIC 375.380)					
Estimate	47.646***	-0.002**	-1.204***	0.472***	-0.185***
SE	8.005	0.001	0.177	0.086	0.0417
Porto Nacional (AIC 102.820)					
Estimate	-41.221***	-0.008***	0.350ns	0.764***	0.218***
SE	9.221	0.002	0.216	0.209	0.030
Sinop (AIC 167.480)					
Estimate	-70.738***	-0.047***	1.028***	1.343***	0.264***
SE	8.706	0.005	0.214	.0156	0.028
Luís Eduardo Magalhães (AIC 94.159)					
Estimate	-42.095***	0.004***	0.088ns	1.537***	0.097***
SE	8.214	0.001	0.247	0.400	0.022
Planaltina (AIC 247.470)					
Estimate	12.475**	0.002ns	-0.609***	0.106ns	0.060**
SE	4.686	0.001	0.158	0.173	0.0197
Uberaba (AIC 73.275)					
Estimate	-102.700**	0.001ns	3.218**	-2.711**	0.767**
SE	36.710	0.003	1.205	0.965	0.244
Alegre (AIC 30.520)					
Estimate	-38.255*	0.014**	1.509*	-1.558*	0.254*
SE	17.735	0.005	0.682	0.615	0.116
Londrina (22.787)					
Estimate	-523.314ns	0.088ns	16.264ns	-13.989ns	3.752ns
SE	475.690	0.063	15.000	12.609	3.348
Passo Fundo (20.082)					
Estimate	-18.697ns	0.032ns	-0.067ns	1.417ns	-0.139ns
SE	48.558	0.038	2.287	3.657	0.697
Bagé (AIC 12.00)					
Estimate	-223.100ns	0.191ns	13.460ns	-15.570ns	0.942ns
SE	2.182e+06	6.739e+02	6.244e+04	5.915e+04	2.018e+04

amounts of nutrients in their tissues and less physical or chemical defense structures (Tamiru et al. 2015).

The large number of specimens collected in five nights of a single month (531) in Alto Alegre - RR (Table 2) refers to the large population numbers of the species that, if under favorable environmental conditions and food availability, will determine the occurrence of outbreaks (Bodkin 1914, Watson 1933, Fennah 1947, Labrador 1964, Capriles & Ferrer 1973, Costilla et al. 1973, Hseih 1979, Calderón et al. 1981, Silvain 1984, Silvain & Dauthuille 1985, Gibbs 1990, Jiménez et al. 1997, Correia et al. 1999, Sánchez Soto & Ortiz Garcia 1999, Minno & Snyder 2008) but mainly due to its relation with higher temperatures in tropical and equatorial areas, since the optimal development temperature is 30°C (Ferreira & Parra 1985).

This relation between greater abundance and higher temperature is in line with the significance of the negative regression between latitude and abundance found in this study (Figure 2). Indeed, despite the wide range of occurrences in the American continent, the largest number of studies that emphasize the importance of *M. latipes* as a pest refers to areas located in lower latitudes or that have climates characterized by high temperatures, especially in Central American countries and the

Caribbean (e.g. Bodkin 1914, Fennah 1947, Labrador 1964, Capriles & Ferrer 1973, Hseih 1979, Calderón et al. 1981, 1982, Silvain 1984, Silvain & Dauthuille 1985, Gibbs 1990, Jiménez et al. 1997, Teixeira & Townsend 1997, Correia et al. 1999, Sánchez Soto & Ortiz Garcia 1999, Minno & Snyder 2008). Considering the specific area of this study the greatest importance of *M. latipes* as a pest is highlighted by several studies in hot and humid regions, especially the Amazon (Silva et al. 1994, Teixeira & Townsend 1997, Fazolin et al. 2009), northeastern coast (Mendonça Filho 1972, Costa et al. 1983, Correia et al. 1999) and Southeast of Brazil (Hempel 1914, 1920a, 1920b, Fonseca 1944, Lopes 1955, 1961, Cavalcante 1977, Ferreira 1984, Cruz 1991).

Considering the higher latitudes sampled in this study (Table 2, Figure 1) it is important to note that even in places below the tropical line there are outbreaks with significant production losses, both in the Northern hemisphere (Vickery 1924, Watson 1933, Genung & Allen Jr. 1974, Ogunwolu & Habeck 1975, Reinert 1975, Koehler et al. 1977, Minno & Snyder 2008), and in the southern hemisphere, in Argentina (Costilla et al. 1973, Salvatore & Willink 2004, Acosta et al. 2005, Salvatore et al. 2009) and in Uruguay (Bentancourt & Scatoni 1996). However, the greater occurrence of *M. latipes* and outbreaks are conditioned to the hottest times of the year. This is due to the fact that, even in high latitudes, in the subtropic, during summer solstice temperatures can be high, conditioning a favorable environment to increasing the development of population, which may cause outbreaks in specific years (Cavalcanti et al. 2009). On the other hand, it should be considered, in addition to the photoperiod, that winter temperatures are close to or below the base development temperature of 13.7°C (Ferreira & Parra 1985). This determines that in addition to prolonging the life cycle, as reported in the Northern Hemisphere, to survive against adverse weather conditions, instars need to develop pupal diapause (to be confirmed in Brazil) or adults must migrate to regions where temperatures are higher, and return when local conditions become favorable to their development (ex. Ferguson et al. 1991, Brou Jr. 2004, Wagner et al. 2011).

The numbers presented in this study consist of a small sample represented by the moths caught in the range of the traps covers (estimated at 400m to *Spodoptera frugiperda* - Vilarinho et al. 2011) at just twelve points in Brazil. These samples represent only adults that survived the weather conditions and all natural enemies present during all stages and collection time. It is also not possible to associate adults with the host plants on which the caterpillars fed and developed. Despite all these limitations, the high number of adults of *M. latipes* collected in all locations (Figure 1, Table 2) highlights its importance as a key specimes in grass-insect interactions in most of Brazil (e.g. Costa 1944a, 1944b, Fonseca 1944, Pugliese 1954, Lopes 1955, 1961, Queiroz 1965, Mendonça Filho 1972, Cavalcante 1977, Planalsucar 1982, Costa et al. 1983, Ferreira 1984, Ferreira & Parra 1985, Cruz 1991, Silva et al. 1994, Teixeira & Townsend 1997, Correia et al. 1999, Fazolin et al. 2009), as well as in most countries of the American continent (e.g. Dine 1913, Bodkin 1914, Holloway 1933, Watson 1933, Fennah 1947, Dinther 1960, Guagliumi 1962, Labrador 1964, Capriles & Ferrer 1973, Costilla et al. 1973, Genung & Allen Jr. 1974, Ogunwolu & Habek 1975, Koehler et al. 1977, Mahadeo 1977, Hseih 1979, Calderón et al. 1981, 1982, Silvain 1984, Silvain & Dauthuille 1985, Miret 1986, Jiménez et al. 1997, Milán et al. 1990, Portillo et al. 1991, Saunders et al. 1998, Piedra & Carrilo 1999, Páliz-Sánchez & Mendoza-Mora 1999, Sánchez Soto & Ortiz García 1999, Pitre et al. 1999, Vergara et al. 2001, Brou Jr. 2004, Salvatore & Willink 2004, Acosta et al. 2005, Marquez 2013). Regarding the fact that it is one of the most predominant specimes, it is important to consider its role in natural ecosystems, especially in agroecosystems where there is a greater proportion or intensification of grass planting, including grains, forages, pastures and sugar cane (e.g. Labrador 1964, Koehler et al. 1977, Calderón et al. 1981, 1982, Costa et al. 1983, Cruz & Santos 1983, Ferreira 1984, Silvain 1984, Silvain & Dauthuille 1985, Miret 1986, Silva & Carvalho 1986, Gibbs 1990, Milán et al. 1990, Cruz 1991, Jiménez et al. 1997, Teixeira & Townsend 1997, Páliz-Sánchez & Mendonza-Mora 1999, Pitre et al. 1999, Vergara et al. 2001, Acosta et al. 2005, Salvatore et al. 2009, Marquez 2013, Hickel et al. 2018). Among the roles to be considered for this species, it is also considered a primary consumer, nutrient cycling promoter, food source for pests (including insects, birds and mammals), parasitoids and pathogens (Wagner et al. 2011).

In relation to the natural enemy organisms of *M. latipes*, there are studies about the relationship of a countless number of species associated with their natural biological control (predators, parasitoids and pathogens), in different stages of development, in different countries of the American Continent (e.g. Sauer 1946, Silva et al. 1968, Lopes 1969, Gonçalves & Gonçalves 1974a, 1974b, Genung et al. 1976, Guimarães 1977, De Santis 1979, 1989, Lourenção et al. 1982, Collins & Watson 1983, King & Saunders 1984, Hall 1985, Santos 1989, Rogers et al. 1990a, 1990b, Galán & Rodríguez 1991, Cave 1992, Rogers & Marti Jr. 1993, Whitaker Jr. et al. 2007, Rolfe et al. 2014). These studies mention that the action of several natural enemies is important when analyzing the abundance data of *M. latipes* in any area of its distribution. As shown for other owlet moths (Pereira et al. 2018), their population levels are maintained, at least most of the time, by the action of natural enemies. This might be related to the different population levels, between locations and in different months presented in this study (Table 2).

In relation to ecological balance promoted by the association and the presence of natural enemies, it is important to note that throughout the American continent *M. latipes* occurs in conjunction with other owlet moths associated with grasses, including congeneric species (Babayan et al. 1975, Ogunwolu & Habek 1975, Koehler et al. 1977, Brou Jr. 2004) and, specially, *S. frugiperda* (J.E. Smith) (e.g. Fonseca 1944, Pugliese 1954, Lopes 1955, 1961, Guagliumi 1962, Queiroz 1965, Mahadeo 1977, Hseih 1979, Calderón et al. 1981, Costa et al. 1983, Silvain 1984, Silvain & Dauthuille 1985, Portillo et al. 1991, Páliz-Sánchez & Mendoza-Mora 1999, Pitre et al. 1999, Sánchez Soto & Ortiz Garcia 1999, Fazolin et al. 2009) forming species complexes. *Spodoptera frugiperda* is a polyphagous species (Montezano et al. 2018) with high biotic potential (Montezano et al. 2019a, 2019b) and, as in this study (Table 2, fig. 1) it was collected in all the locations presented here (Piovesan et al. 2018). It is important that *M. latipes* and other owlet moths, including *S. frugiperda* share the same predators, pathogens, also egg, larvae and pupae parasitoids (Silva et al. 1968, Rogers & Marti Jr. 1993, Camera et al. 2010). This determines that the presence of *M. latipes*, even in low populations, allows the maintenance of populations of different groups of natural enemies in natural ecosystems and agroecosystems. Thus, the occurrence of immature *M. latipes* allows the maintenance of a wide range of natural enemies, it is essential for the maintenance of natural biological control over time, for the entire species complex.

The significant relationship between the abundance of *M. latipes* with at least one of the meteorological variables (Table 3), in practically all Brazilian territory except in the three most southern locations (Figure 1) reinforces the strong association between population variations and climatic conditions mentioned in several studies (e.g. Bertels 1970, Genung & Allen Jr. 1974, Babayan et al. 1975, Calderón et al. 1981, Gibbs 1990, Jiménez et al. 1997, Pitre et al. 1999, Salvatore & Willink 2004, Fazolin et al. 2009, Hickel et al. 2018). The relationship between population variations and climatic conditions, is evidenced by the large number of moths collected in rainy months (July, August and September), in Alto Alegre, RR, located in the Northern Hemisphere (Table 2) while all the other places are located in the Southern Hemisphere, these same months correspond to winter time (dry or cold), with very low population levels. Indeed, studies always relate the highest population levels with warm months, preferably during the rainy season in the Northern Hemisphere (Bodkin 1914, Vickery 1924, Watson 1933, Fennah 1947, Capriles & Ferrer 1973, Calderón et al. 1981, Gibbs 1990, Minno & Snyder 2008) as in the Southern Hemisphere (Lopes 1955, Costilla et al. 1973, Carvalho 1976, Lourenção et al. 1982, Teixeira & Townsend 1997, Correia et al. 1999, Salvatore & Willink 2004, Acosta et al. 2005).

Regarding the different numbers of moths collected each month, in each location (Table 2) besides the climatic characteristics (Cavalcanti et al. 2009), biogeographic and phytophysiognomic variations should also be considered (Heppner 1991). Although *M. latipes* is an oligophytophagous species with a preference for grasses (ex. Ogunwolu & Habeck 1975, Wagner et al. 2011), in each location the moths were able to choose to lay eggs on plants arranged as a space-time mosaic containing different native and/or cultivated species that served as food for their larvae.

The *M. latipes* abundance spatial variations described in this study, combined with the knowledge of its great dispersion capacity, including migration (ex. Barth 1958, Ferguson et al. 1991, Wagner et al. 2011, Alves et al. 2019) point to the need for studies related to the molecular characterization of populations in order to allow assessments of local populations, migration routes and/or gene flow as done for other owlet moths (e.g. Palma et al. 2015, Nagoshi et al. 2017). This information is extremely important to assess whether the occurrence of population outbreaks of this species is related only to the fast reproduction of local populations or whether it involves dispersion or migration events. Thus, it should be noted that even in Southmost areas, where the abundance of *M. latipes* was extremely low, there are several registers of its occurrence (ex. Tarragó et al. 1975, Link 1977, Specht & Corseuil 2002, Specht et al. 2004, 2005, Zenker et al. 2010) indicating that the species was present in more than 50% of weekly collections between July 1994 and June 1995 (Specht & Corseuil, 2002). Furthermore, further to the South, Bentancourt & Scatoni (2006) relate sporadic population outbreaks of this species in Uruguay, noting that, even at greater latitudes, the occurence of this species is relatively constant with increases in population linked to favorable conditions to its development. Especially in these places it is questioned whether the presence of the species is due to a local population or as a result of migration as described for the Northern Hemisphere (Brou Jr. 2004, Wagner et al. 2011).

The results of this study as well as most previous publications (ex. Carvalho 1976, Lourenção et al. 1982, Fazolin et al. 2009, Bentancourt & Scatoni 1996, Saunders et al. 1998, Wagner et al. 2011) indicates that *M. latipes*, besides having a wide distribution, it has low population levels during most of the year, with sporadic population outbreaks during favorable weather conditions, food availability and inadequate cultural management. Therefore, it must be considered that agricultural occupation has modified most of the ecosystems where the effect of seasonal variations on native host plants is minimized by cultivating grasses that serve as alternative hosts more resistant to drought in the savanna and to the cold in greater latitudes. These plants can serve as a green bridge between the most favorable seasons to the development of *M. latipes* and other insects (Favetti et al. 2017). Considering grasses as host plants, it must be taken into consideration that *M. latipes* has food available both in open native environments and in the most diverse combinations of forage, grain and sugar production. In addition to these plants directly related to production, other crops should be considered for soil protection and biomass production to be incorporated in crop rotation systems (Dias et al. 2016, Favetti et al. 2017), integrated crop-livestock systems (Vilela et al. 2011) and even when introduced grasses become pests occupying most ecosystems for most of the year (Minno & Snyder 2008).

Even though this study shows an inverse relationship between latitude and *M. latipes* abundance along with other meteorological variables, it should be considered that abiotic factors are conditional to species development, and not necessarily associated with the decrease of abundance. Therefore, additional hypotheses need to be tested considering specific abundance and environmental factors. One example is the high abundance of *M. latipes* in tropical areas, it can be related to favorable conditions, while that in subtropical regions only summer months present favorable conditions. On the other hand, areas presenting favorable conditions all year long also provide a high number of natural enemies. Thus, due to the large number of variables related to *M. latipes* itself (which includes developmental biology in different conditions, the ability to diapause, migrate, defend against natural enemies) and biotic factors associated with each location (availability of different host plants native or cultivated, presence and abundance of natural enemies, interspecific competition) cause the associations established between population levels, latitude and environmental factors to be explored continuously. A better understanding of these associations will allow a more accurate understanding of the effects of local environmental variations and global climate changes on insect populations, pests or not.

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Conflict of Interest

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

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Article

Effects of urbanization on the fauna of Odonata on the coast of southern Brazil

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Abstract: Urbanization significantly increases the rates of environmental disturbance, being one of the main causes of habitat loss and biodiversity. The growing trend of converting the natural landscape into areas for real estate speculation in the coastal region of the southernmost part of Brazil is a current concern, as the region is home to unique ecosystems, such as dunes, wetlands and large brackish lagoons. As they are organisms sensitive to environmental changes, variations in the structure of Odonata communities are used as indicators of habitat quality reflecting the human impact on the environment. Here we assessed how the Odonata community is affected by the growing urbanization around natural ponds on the coast of the state of Rio Grande do Sul, testing the hypothesis that the increase in the percentage of urbanization negatively influences the Odonata community, following the same pattern found for other groups of invertebrates. The collections took place in 28 coastal ponds, which were classified as urbanized and non-urbanized based on the surrounding ground cover. Anisoptera's richness, abundance and composition were influenced by urbanization, but the same was not found for Zygoptera. The analysis of indicator species specifies three species associated with non-urbanized areas: *Erythrodiplax* sp.1, *Erythemis credula* and *Telebasis corallina.* Our study highlights the importance of Odonata as organisms that indicate environmental integrity and reinforces the need for urban planning strategies that favor the conservation and maintenance of the environments affected by urbanization.

Keywords: Anisoptera; aquatic conservation; aquatic insects; urban ecology; ponds; Zygoptera.

Efeitos da urbanização sobre a fauna de Odonata no litoral sul do Brasil

Resumo: A urbanização aumenta significativamente as taxas de perturbação ambiental, sendo está uma das principais causas da perda de habitat e biodiversidade. A tendência crescente de conversão da paisagem natural em áreas para especulação imobiliária na região costeira do extremo sul do Brasil é uma preocupação atual, pois a região abriga ecossistemas únicos, como dunas, áreas úmidas e grandes lagoas salobras. Como são organismos sensíveis às alterações ambientais, as variações na estrutura das comunidades de Odonata são utilizadas como indicadores de qualidade do habitat refletindo o impacto humano no ambiente. Nós avaliamos como a comunidade de Odonata é afetada pela crescente urbanização em torno de lagoas naturais no litoral do estado do Rio Grande do Sul, testando a hipótese de que o aumento do percentual de urbanização influencia negativamente a comunidade de Odonata, seguindo o mesmo padrão encontrado para outros grupos de invertebrados. As coletas ocorreram em 28 lagoas costeiras, que foram classificadas como urbanizadas e não urbanizadas com base na cobertura do solo no entorno. A riqueza, abundância e composição de Anisoptera foram influenciadas pela urbanização, mas o mesmo não foi encontrado para Zygoptera. A análise de espécies indicadoras especifica três espécies associadas a áreas não urbanizadas: *Erythrodiplax* sp.1, *Erythemis credula* e *Telebasis corallina*. Nosso estudo destaca a importância dos Odonata como organismos indicadores de integridade ambiental e reforça a necessidade de estratégias de planejamento urbano que favoreçam a conservação e manutenção dos ambientes afetados pela urbanização.

Palavras-chave: Anisoptera; conservação aquática; insetos aquáticos; ecologia urbana; lagoas; Zygoptera.

Introduction

Urban areas are cultural spaces with a high density of people. They have extensive impermeable surfaces that are occupied by infrastructure, forming a dynamic mosaic. In this context, the process of landscape change for the establishment of urban areas is called urbanization (Muzón et al. 2019). Urbanization has significantly increased the rates of environmental disturbance, this being one of the main causes of habitat and biodiversity loss (Czech et al. 2000, McDonald et al. 2008). The construction of buildings and roads leads to great changes in the natural landscapes, destroying and homogenizing habitats. Urbanization is the cause of some of the greatest local extinctions (McKinney 2002), reducing species richness and abundance of certain taxa (McKinney 2008, Buczkowski & Richmond 2012). However, the urban environment might harbor some endangered species and can also promote the diversity of some species adapted to the conditions imposed by urbanization as it reduces the richness of native species (McKinney & Lockwood 1999).

In southern Brazil, the coastal region follows a national tendency of the urbanization, where there is a valuing coastal area, mainly because of political and economic reasons (Moraes 2007). Urban development on the coast began in the 1980s and became more intense as acquiring properties became easier (Strohaecker 2007). The coastal zone of Rio Grande do Sul harbors rare ecosystems, such as wetlands and brackish lagoons, which are ecosystems with great environmental vulnerability (Strohaecker 2007). In the face of the extension of the impacts caused by urban development, it becomes increasingly essential to understand the effects of urbanization on natural habitats.

According to Muzón et al. (2019), almost all known orders of aquatic insects can inhabit freshwater ecosystems in urban areas. However, urbanization is a complex process. Several studies describe the effects of urbanization on richness and abundance of different groups such as birds (Chace & Walsh 2006), mammals (Tait et al. 2005), reptiles (Barret & Guyer 2008), amphibians (Hamer & McDonnell 2008) and several groups of land arthropods (McIntyre et al. 2001), showing that the effects of urbanization can vary according to taxon. However, most studies show a negative effect associated with urbanization, which can be explained mostly by habitat loss and/or degradation of the remaining habitat (McKinney 2008).

One of the most representative orders of aquatic insects is Odonata. These insects are found in a variety of water bodies, from rivers and streams to lakes and temporary ponds (Corbet 1999), being frequently mentioned as indicators of habitat quality because, through variations in their community structure, they can reflect the human impact on the environment (Clark & Samways 1996, Callisto et al. 2001, Ferreira-Peruquetti & De Marco 2002, Oliveira-Junior et al. 2015, Renner et al. 2016, Renner et al. 2018, Renner et al. 2019).

Odonata are among the best-known insect groups in the world (Kalkman et al. 2008). Odonata are estimated to comprise 6322 species in the world (Schorr & Paulson 2019), and the Neotropical region harbors the second highest diversity, with more than 1700 species (Von Ellenrieder 2009). About 750 species are known to occur in Brazil (Olaya 2019), and, in the state of Rio Grande do Sul, a survey indicated the presence of 182 species (Dalzochio et al. 2018a).

Some studies aimed to evaluate the effect of urbanization on the Odonata community. They suggest that the changes occurring in the process, both in the landscape scale (Samways & Steytler 1996) and the physical and chemical parameters of water (Corbet 1999), may negatively affect the diversity pattern of this order. However, the impact is different depending on the species (Monteiro-Júnior et al. 2014). Zygoptera species, in general, are more sensitive to environmental disturbances, as they have narrower niches and less dispersion capacity, when compared to anisopterans (Monteiro-Júnior et al. 2015, Corbet 1999). Thus, some species of Zygoptera may be less tolerant to urban lentic environments, consequently presenting less diversity (Prescott & Eason 2018). In contrast, Anisoptera species are favored in this type of environment, since they are more generalist and have greater dispersion capacity (Corbet 1999, Goertzen & Suhling 2013). The two suborders also have different thermoregulatory requirements, and in open landscapes, such as wetlands, there is a predominance of Anisoptera species (Juen et al. 2014).

In this context, our work seeks to answer the following questions: 1) How does percent urbanization affects species richness, abundance and composition in the coastal plain of Rio Grande do Sul? 2) Are there species that can be considered indicators of urbanized or non-urbanized environments?

We expected that the increase in percent urbanization will have a negative influence on the Odonata community in this region, following the same pattern found for several invertebrate groups. However, some species may be more tolerant of this variable.

Material and Methods

1. *Study area*

The study was conducted in natural ponds in the municipalities of the coastal plain of the state of Rio Grande do Sul. The state of Rio Grande do Sul is located in the southern portion of Brazil (27º04’ - 33º45’ S; 49º42’ - 57º38’ W) (Figure 1) with an area of about 282.000 km². The coastal plain is characterized by a sedimentary plain consisting of dunes, ponds and lagoons (Strohaecker 2007). According to the delimitation established by the Program of Coastal Management (GERCO-RS) of the State Program of Environmental Protection (FEPAM), the northern coast has an extension of 120 km of coastal line and a surface area of 3700 km². The dominant climate is subtropical, belonging, according to Köppen’s classification, to the Cfa type (Kuinchtner & Buriol 2001), having well-defined four seasons with a mean temperature of 15 °C in winter and 27 °C in summer. The terrain has a mean altitude of 40 and 50 meters and the annual precipitation varies between 1500 and 1700 mm (Rossato 2011).

2. *Sample design*

We sampled 28 ponds, respecting a minimum distance of 1000 m between them. In this context, we delimited a radius of 1 km from the center of the sampling pond and, using the tool ruler to calculate area and the most recent images available in the software Google Earth Pro™, we calculated the percentage of the areas with built structures, such as roads, houses and buildings. Thus, the study included 15 non-urbanized ponds and 13 urbanized. Ponds with urbanization percentage points below 20% were considered non-urbanized and those with more than 20% of urbanization were considered urbanized. The area of the sampled pond varied from 0.0027 to 3.96 hectares (Figure 1). The ponds were sampled twice between November 2016 and March 2018,

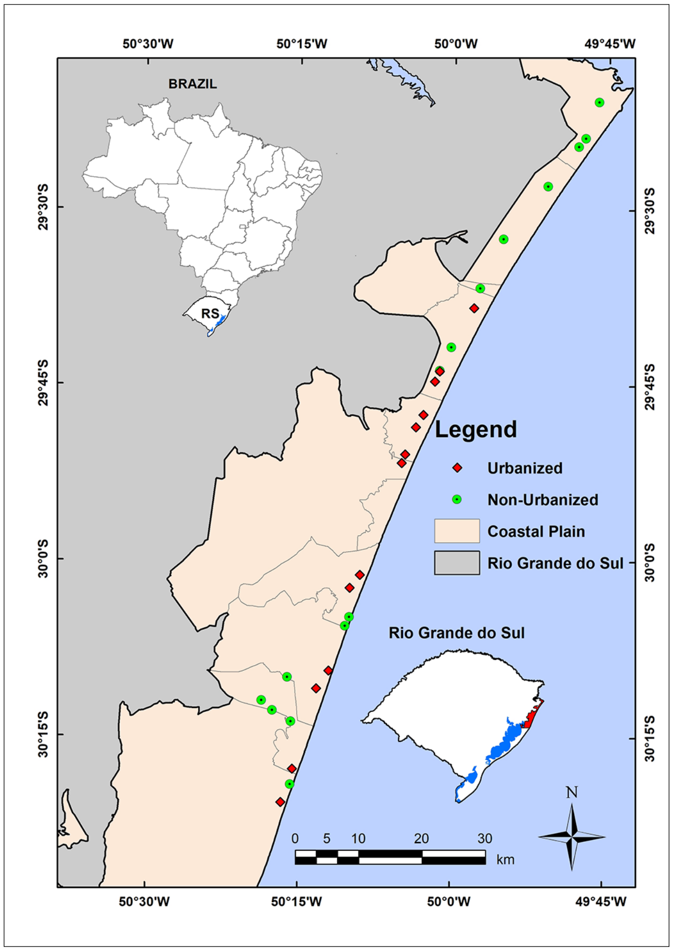

Figure 1. Location of the sampled areas on the northern coast of the state of Rio Grande do Sul.

except in winter due to the absence of activity of Odonata adults, the two samples per pond were added.

3. *Collection of biological material*

The study was based on the collection of adults. Specimens were collected using aerial insect nets by a team consisting of three researchers and a sampling effort of 40 minutes per locality. Collections were performed solely on sunny days, between 10:00 and 16:00 hours. Captured specimens were immediately fixed in 96% ethanol and preserved in glass flasks that were identified with collection date and location for later determination in the laboratory.

Species determination was conducted in the Laboratory of Ecology and Evolution of UNIVATES with the aid of a stereomicroscope and identification keys for Odonata adults of the Neotropical region: Garrison et al. (2006); Garrison et al. (2010), Heckman (2008), Heckman (2006), Lencioni (2005) and Lencioni (2006). The specimens will be housed in the invertebrate collection of the Natural History Museum of UNIVATES (MCNU).

4. *Data analysis*

Species (S) richness was considered as the number of species, while abundance was considered as the total number of individuals. The analyses were conducted separately for each suborder of Odonata, as they are biologically and ecologically distinct (Dutra & De Marco 2015). All statistics routines were conducted in the statistical program R project (R Core Team 2019).

Due to the possibility of containing a greater number of perches in the non-urbanized area, since its surrounding vegetation is preserved, we have developed a coverage-based rarefaction analysis, according to Chao et al. (2014). We used package rareNMtests and rarefaction. individual function (Cayuela & Gotelli 2014).

To determine the relation of urbanization to richness and abundance for each suborder, we elaborated a Generalized Linear Mixed Model (GLMM). We used the percentage of urbanization of each sampled site as an explanatory variable and as a random variable the log area (m^2) of the ponds. These analyses were conducted using the package lme4 (Bates et al. 2015) and the function glmer. For both suborders, richness followed a negative binomial distribution and abundance followed a Poisson distribution.

To evaluate whether urbanized and non-urbanized ponds were similar regarding species composition, we used the abundance data matrix transformed into Hellinger. First, we conducted a dispersion analysis (PERMDISP), to understand how homogeneous the samples were within the treatments, using the betadisper function. Next, we conducted an One-Way Permanova using Bray-Curtis dissimilarity index and 9999 permutations, with the adonis2 function. To represent the results found at Permanova, an NMDS was created, using the vegan package and the metaMDS function. Both functions are from the Vegan package (Oksanen et al. 2019).

With the purpose of knowing which species could serve as indicators in environments with and without urbanization influence, we obtained information through the analysis of indicator species (INDVAL – Dufrene & Legendre 1997). This index is calculated estimating the specificity (whether the species shows an association with certain habitat types, occurring only in certain environments or conditions) and fidelity (whether the species is invariably present under a certain environmental condition). This analysis was elaborated with the aid of the package labdsv 1.5.0 for R project (Roberts 2012). To complement the INDVAL analysis, we applied the Multinomial Species Classification Method (Chazdon et al. 2011), which uses a multinomial model based on estimated species relative abundance to classify species as generalists and specialists in two distinct habitats, with the package vegan, function clamtest.

Results

The areas of the analyzed ponds ranging from 26 m^2 to 39,79 m^2, with an average of 3,47 m^2. The richness varied from 3 spp to 13 spp, with an average of 8 spp. The abundance ranged from 9 individuals to 55 individuals, with an average of 29 individuals (Table 1). A total of 820 individuals were collected, in 38 species and 17 genera, with *Orthemis schmidti* Buchholz, 1950 being a new record for the state. Anisoptera was the most abundant suborder with 462 individuals belonging to 11 genera and two families, Libellulidae and Gomphidae. Zygoptera had 358 individuals distributed into 6 genera and two families, Coenagrionidae and Lestidae (Table 2).

The most abundant species of Anisoptera were *Erythrodiplax paraguayensis* (Förster, 1904) (n = 124) and *Erythrodiplax* sp.1(n = 102), which represented 49% of the total number of individuals of this suborder that were collected in this study. The most abundant

Table 1. Sampling locations: coordinates, pond area, percentage of urbanization, classification of the environment, richness e abundance.

Sites	Coordinate	Pond (m²)	Urbanization (%)	Environment	Richness	Abundance
1	S 29º51'42.1" W 50º04'59.8"	1031	51	Urbanized	5	25
2	S 29º50'57.3" W 50º04'40.8"	490.37	40	Urbanized	3	10
3	S 29º48'38.5" W 50º03'38.9"	556.94	53	Urbanized	9	22
4	S 29º47'34.1" W 50º02'55.5"	173.69	40	Urbanized	9	23
5	S 29º44'44.1" W 50º01'48.51"	191.85	55	Urbanized	9	15
6	S 30°02'22" W 50°10'01"	35.09	59	Urbanized	11	48
7	S 29º38'26.83" W 49º58'2.48"	8185.37	44	Urbanized	5	9
8	S 29°43'50.9" W 50°01'20.5"	6077.6	31	Urbanized	8	31
9	S 30°01'16" W 50°09'01"	113.26	98	Urbanized	5	25
10	S 30°10'57" W 50°13'14"	1478.92	37	Urbanized	9	36
11	S 30°09'26" W 50°12'03"	1249.13	46	Urbanized	8	55
12	S 30°20'40.8" W 50°16'40.3"	26.21	75	Urbanized	6	12
13	S 30°17'49.95" W 50°15'33.9"	49.22	59	Urbanized	8	16
14	S 30°13'46.6" W 50°15'41.3"	39785.76	9	Non-urbanized	6	35
15	S 29°36'45.5" W 49°57'25.3"	2597.74	3	Non-urbanized	7	30
16	S 29º41'48.2" W 50º00'13.3"	4254.79	14	Non-urbanized	12	55
17	S 29º32'32.7" W 49º55'10.6"	2973.77	4	Non-urbanized	9	34
18	S 29º28'00.5" W 49º50'52.8"	3883.59	6	Non-urbanized	9	43
19	S 29º24'37.5" W 49º47'55.5"	10258.92	7	Non-urbanized	5	28
20	S 29º23'53.9" W 49º47'14.3"	2258.8	16	Non-urbanized	8	24
21	S 29º20'48.3" W 49º45'57.8"	1367.8	17	Non-urbanized	7	15
22	S 29º43'47.6" W 50º01'19.4"	816.97	20	Non-urbanized	7	21
23	S 30°05'37" W 50°10'26"	2006.09	2	Non-urbanized	9	27
24	S 30°04'51" W 50°10'01"	859.77	18	Non-urbanized	5	13
25	S 30°12'50,1" W 50°17'31,7"	625.63	1	Non-urbanized	9	40
26	S 30°19'08,5" W 50°15'43,8"	4077.11	1	Non-urbanized	9	35
27	S 30°11'59" W 50°18'36"	1018.66	7	Non-urbanized	13	47
28	S 30°01'60" W 50°16'05"	846.61	14	Non-urbanized	8	46

species of Zygoptera were *Ischnura fluviatilis* Selys, 1876 (n = 217) and *I. capreolus* (Hagen, 1861) (n = 89), which made up 85 % of the specimens in this suborder.

The coverage-based rarefaction analysis indicates the same percentage of coverage for both urbanized and non-urbanized areas (Figure 2). Although the coverage sample value was lower than 0,6, this curve indicates that there is no bias caused by possible higher numbers of perches in non-urbanized areas.

The mean richness of Anisoptera per point was 5.16 species, while that of Zygoptera was 2.60. The GLMM showed that urbanization does not influence the richness of Anisoptera (Z=-1.32; p=0.187 neither Zygoptera (Z=-0.071; p = 0.943). The mean abundance of Anisoptera per point was 16.5 individuals, while that of Zygoptera was 12.78. The GLMM showed that urbanization has a negative influence on the abundance of Anisoptera (Z= -2.82; p = 0.004) (Figure 3) but does not influence the abundance of Zygoptera (Z = -0.251; p = 0.802).

The dispersion analysis (PERMDISP) did not indicate heterogeneity in the samples of Zygoptera (F = 0.152; p = 0.699) or Anisoptera (F = 2.65; p = 0.115). The PerMANOVA, indicated significant differences in Anisoptera species composition between urbanized and non-urbanized environments ($F_{1;27}$ = 3.235; p = 0.001), while the composition of Zygoptera was not statistically different between the studied environments ($F_{1;27}$ = 0.3774; p = 0.821) (Figure 4).

The indicator species analysis (INDVAL) indicated only three species associated with non-urbanized areas: *Erythrodiplax* sp.1 (INDVAL = 0.861; p = 0.005), *Erythemis credula* (Hagen, 1861) (INDVAL = 0.789; p = 0.005) and *Telebasis corallina* (Selys, 1876) (INDVAL = 0.632; p = 0.025). These three species were classified as “non-specialists” for non-urbanized areas by the Multinomial Species Classification Method. Although without significant values, the species *Brachymesia furcata* (Hagen, 1861), *Erythrodiplax fusca* (Rambur, 1842), *Erythrodiplax latimaculata* Ris, 1911, *Erythrodiplax umbrata* (Linnaeus, 1758), *Lestes paulistus* Calvert, 1909, *Libellula herculea* Karsch, 1889, *Micrathyria stawiarskii* Santos, 1953, *Orthemis schmidti* Buchholz, 1950, *Progomphus intricatus* Hagen in Selys, 1858, *Tramea abdominalis* (Rambur, 1842) and *Tramea cophysa* Hagen, 1867 were found exclusively in non-urbanized areas, while *Acanthagrion gracile* (Rambur, 1842), *Acanthagrion lancea* Selys, 1876, *Erythemis attala*

Table 2. Species collected in the study sites on the northern coast of Rio Grande do Sul, Brazil, between November 2016 and March 2018

Species and families	**Urbanized**	**Non-urbanized**	**Total**
Gomphidae			
Progomphus intricatus Hagen, 1858	0	1	1
Libellulidae			
Brachymesia furcata (Hagen, 1861)	0	1	1
Erythrodiplax sp.1	13	89	102
Erythrodiplax sp.2	13	4	17
Erythemis attala (Selys, 1857)	1	0	1
Erythemis credula (Hagen, 1861)	4	26	30
Erythemis peruviana (Rambur, 1842)	2	9	11
Erythrodiplax atroterminata Ris,1911	15	7	22
Erythrodiplax fusca (Calvert,1895)	0	1	1
Erythrodiplax latimaculata Ris, 1911	0	2	2
Erythrodiplax media Borror, 1942	39	55	94
Erythrodiplax melanorubra Borror, 1942	4	0	4
Erythrodiplax nigricans (Rambur, 1842)	2	0	2
Erythrodiplax paraguayensis (Förster, 1905)	41	83	124
Erythrodiplax umbrata (Linnaeus, 1758)	0	1	1
Libellula herculea (Selys, 1857)	0	1	1
Micrathyria catenata Calvert, 1909	2	0	2
Micrathyria spuria (Selys, 1900)	2	1	3
Micrathyria stawiarskii Santos, 1953	0	2	2
Nephepeltia flavifrons (Karsch, 1889)	12	5	17
Orthemis ambinigra Calvert, 1909	1	2	3
Orthemis discolor (Burmeister, 1839)	1	0	1
Orthemis schmidti Buchholz, 1950*	0	3	3
Pantala flavencens (Fabricius, 1798)	4	2	6
Perithemis mooma Kirby, 1889	2	5	7
Tramea abdominalis (Rambur, 1842)	0	1	1
Tramea binotata (Rambur, 1842)	1	0	1
Tramea cophysa Hagen, 1867	0	2	2
Coenagrionidae			
Acanthagrion gracile (Rambur, 1842)	1	0	1
Acanthagrion lancea Selys, 1876	3	0	3
Argia sp.	0	2	2
Homeoura chelifera (Selys, 1876)	13	10	23
Ischnura capreolus (Hagen, 1861)	44	45	89
Ischnura fluviatilis (Selys, 1876)	103	114	217
Telebasis corallina	0	17	17
Telebasis willinkii Fraser, 1948	2	2	4
Lestidae			
Lestes paulistus Calvert, 1909	0	1	1
Lestes undulatus Say, 1840	1	0	1
Total	326	494	820

* First record for the State of Rio Grande do Sul

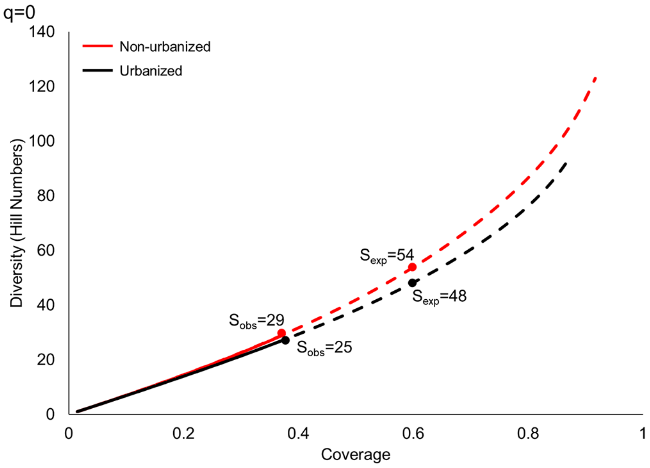

Figure 2. Coverage-based rarefaction analysis (urbanized and non-urbanized area).

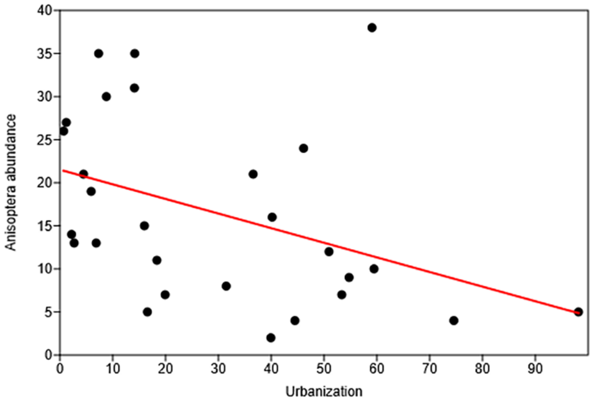

Figure 3. Generalized linear model (GLMM) for abundance Anisoptera.

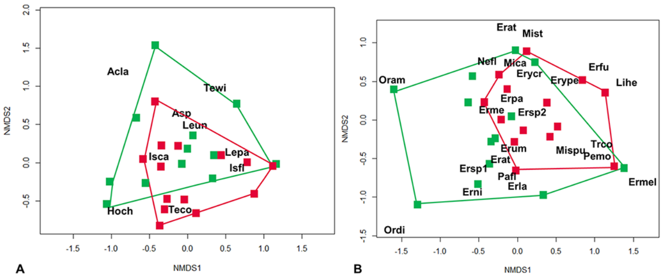

Figure 4. NMDS representing the composition of Zygoptera (A) and Anisoptera (B) species in urbanized (green) and non-urbanized (red) ponds.

(Selys, 1857)*, Erythrodiplax melanoruba* Borror, 1942*, Erythrodiplax nigricans* (Rambur, 1842)*, Lestes undulatus* Say, 1840*, Micrathyria catenata* Calvert, 1909*, Orthemis discolor* (Burmeister, 1839) *and Tramea binotata* (Rambur, 1842). were found exclusively in urbanized areas (Table 3).

Discussion

The expansion of urban areas and the consequent loss and fragmentation of habitats, is one of the most significant environmental impacts on natural landscapes (Villalobos-Jiménez et al. 2016). Despite the importance of studies that assess the impact of urbanization on aquatic habitats, there are few studies on the use of ponds by species of aquatic insects, regarding they use to environmental integrity (Willigalla & Fartmann 2012). Adequate pond management can be an important factor in conservation strategies (Chien et al. 2019), and ponds, even small and in urban areas, can work as a reservoir for several species, as well as present similar species richness and aquatic invertebrate families when compared to non-urban ponds, although there are clear differences in the composition of communities (Hill et al. 2016).

Species of the order Odonata are excellent indicators of environmental quality, and species composition, followed by diversity and taxonomic distinctness, are the parameters that best respond to environmental disturbance gradients (Miguel et al. 2017). In general, studies with Odonata that consider urbanization gradients demonstrate that species richness increases along a gradient from the center of a city to the rural area and is significantly highest in rural areas (Willigalla & Fartmann 2012). Studies in the Cerrado biome, in open areas, indicate that zygopteran species richness decreased as habitat integrity decreased, with the opposite pattern being observed for the anisopterans (Carvalho et al. 2013).

Our results showed that urbanization is a predictor variable for the structuring of the Odonata community on the coast of Rio Grande do Sul, influencing abundance, richness and composition of the suborder Anisoptera. Species of Zygoptera seem to be influenced by other factors since the variable urbanization had no significance for the analyzed metrics.

This is the first study that characterized the Odonata fauna on the coastal area of Rio Grande do Sul. The 38 species found correspond to 21% of the total known for this state (Dalzochio et al. 2018a). The most species were lentic species typical of wetland environments, which are characteristic of the sampled region. The model explained that the richness and abundance of Anisoptera were negatively influenced by the urbanized areas, but there was no variation in richness and abundance of Zygoptera. Most likely these results are related to the ecological needs of these suborders. In this context, species of Zygoptera have a higher ecological diversity and some species can be considered eurytopic (Samways & Steytler 1996). Therefore, they are not subject to significant changes in their richness and abundance in urbanized ecosystems. However, specialist species can also find refuge in urbanized areas (Harabiš & Dolný 2015). As analyzed by Monteiro-Junior et al. (2014), habitats heavily degraded by urbanization lead to a loss in the number of species of both suborders.

The landscape around the sampled areas (e. g. buildings and paved roads) is a predictor variable for the species richness and abundance patterns (Jeanmougin 2014) since the landscape surrounding the aquatic environments is fundamental for the foraging and maturation of Odonata species (Bried & Ervin 2006). Generally, the increase in urbanization has a negative effect on the diversity of these animals. However, there are more generalist species that are able to live in human-disturbed habitats (Clark & Samways 1996).

Table 3. Indicator species (INDVAL) and their respective classification by Multinomial Species Classification Method (CLAM). Values in bold are statistically significant for INDVAL.

Species	Urbanized	Non-urbanized	INDVAL	P-value	CLAM
Acanthagrion gracile	1	0	0.277	0.405	Too rare
Acanthagrion lancea	1	0	0.392	0.180	Too rare
Argia sp.	0	1	0.365	0.435	Too rare
Brachymesia furcata	0	1	0.258	1.000	Too rare
Erythemis attala	1	0	0.277	0.435	Too rare
Erythemis credula	0	1	0.789	0.005	Specialist of Non-urbanized sites
Erythemis peruviana	1	1	0.327	NA	Generalist
Erythrodiplax atroterminata	1	1	0.598	NA	Generalist
Erythrodiplax fusca	0	1	0.258	1.000	Too rare
Erythrodiplax latimaculata	0	1	0.258	1.000	Too rare
Erythrodiplax media	1	1	0.866	NA	Generalist
Erythrodiplax melanorubra	1	0	0.277	0.510	Too rare
Erythrodiplax nigricans	1	0	0.392	0.210	Too rare
Erythrodiplax paraguayensis	1	1	0.964	NA	Generalist
Erythrodiplax sp.1	0	1	0.861	0.005	Specialist of Non-urbanized sites
Erythrodiplax sp.2	1	0	0.674	0.105	Specialist of urbanized sites
Erythrodiplax umbrata	0	1	0.258	1.000	Too rare
Homeoura chelifera	1	1	0.598	NA	Generalist
Ischnura capreolus	1	1	0.866	NA	Generalist
Ischnura fluviatilis	1	1	0.982	NA	Generalist
Lestes paulistus	0	1	0.258	1.000	Too rare
Lestes undulatus	1	0	0.277	0.525	Too rare
Libellula hercúlea	0	1	0.258	1.000	Too rare
Micrathyria catenata	1	0	0.392	0.205	Too rare
Micrathyria spuria	1	0	0.328	0.620	Too rare
Micrathyria stawiarskii	0	1	0.258	1.000	Too rare
Nephepeltia flavifrons	1	1	0.500	NA	Generalist
Orthemis ambinigra	1	1	0.267	NA	Too rare
Orthemis discolor	1	0	0.277	0.495	Too rare
Orthemis schmidti	0	1	0.258	1.000	Too rare
Pantala flavencens	1	1	0.423	NA	Too rare
Perithemis mooma	1	1	0.463	NA	Too rare
Progomphus intricatus	0	1	0.258	1.000	Too rare
Telebasis corallina	0	1	0.632	0.025	Specialist of non-urbanized sites
Telebasis willinkii	1	1	0.267	NA	Too rare
Tramea abdominalis	0	1	0.258	1.000	Too rare
Tramea binotata	1	0	0.277	0.495	Too rare
Tramea cophysa	0	1	0.365	0.415	Too rare

The changes in land use and land cover from the urbanization process in the region are recent, beginning in the 1990s (1995), and correspond to the fourth cycle of occupation of the northern coast of Rio Grande do Sul (Lopes et al. 2018). Therefore, it is likely that it is not yet possible to measure the process of adaptation of Odonata species (Goertzen & Suhling 2019). Additionally, the urbanization process in the tropics usually occurs rapidly, causing the loss of sensitive species (Monteiro-Júnior et al. 2014).

Regarding species composition, although Zygoptera was not influenced by urbanization, all landscape elements act in biological communities, which, in turn, interact to structure the whole environment (Turner & Gardner 2001). The dimension and number of certain landscape elements are crucial for the formation and continuity of specific communities (Bond & Parr 2010). In addition, target taxa, such as Odonata, respond to anthropogenic changes. Therefore, the species distribution and general diversity of these taxa are related to the landscape structure or to the variables of land use (Soares Filho 1998). The variable urbanization, in this case, may have limited the dispersion of certain species of Anisoptera, while not affecting other species, such as Zygoptera.

Studies in lagoons in North America and Europe found that the richness of Odonata increases or is not affected by urban processes (Craves & O'Brien 2013, Goertzen & Suhling 2013), which indicates that some species are likely becoming tolerant to urbanization. This can be the case of species found in urbanized environments: *Acanthagrion gracile*, *Acanthagrion lancea*, *Erythemis attala*, *Erythrodiplax melanorubra*, *Erythrodiplax nigricans*, *Lestes undulatus*, *Micrathyria catenata*, *Orthemis discolor* and *Tramea binotata*. Most species belonging to the genera *Acanthagrion*, *Erythemis*, *Erythrodiplax*, *Lestes*, *Micrathyria* and *Orthemis* are considered generalists (Dalzochio et al. 2018b). They have short life cycles and adapt very quickly to environmental changes, being found even in very hostile environments. Moreover, generalist species can explore the available resources due to reduced competition, resulting in the high abundance of tolerant species (Villalobos-Jiménez et al. 2016).

In this regard, one of the species that was pointed out as an indicator of non-urbanized environments, *Erythrodiplax* sp.1, was unexpected since most species in this genus are considered generalists (Dalzochio et al. 2018b). However, there are cases in which species of the genus *Erythrodiplax* were established as bioindicators, for example, in the Cerrado biome, were the genus indicated pasture environments and non-shaded areas (Dutra & De Marco 2015). The suggestion of the species *Telebasis corallina* and *Erythemis credula* as bioindicators may be associated with the presence of macrophytes or with the physical and chemical parameters of the sampled locations (Fulan et al. 2011). In this context, species of Zygoptera, such as *Telebasis corallina*, are more vulnerable to changes in landscape structure and vegetation cover and may become locally extinct (Monteiro-Júnior et al. 2015).

We observed that the richness and abundance of the species of the suborder Anisoptera was reduced with urban expansion through the construction of buildings, pavement of roads and high flow of people. In this sense, urban planning is necessary to ensure ecosystem maintenance and limit environmental degradation. However, due to the complexity of the human occupation in this region, more studies are necessary. Additionally, we suggest the use of Odonata to evaluate these environments. We hope that this study will help with measurements that aim to restore, conserve and maintain environments affected by urbanization.

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Author Contributions

Cléber Sganzerla: Contribution to data collection, data analysis and interpretation and manuscript preparation.

Marina Schmidt Dalzochio: Contribution to data collection, data analysis and interpretation and manuscript preparation.

Gabriel dos Santos Prass: Contribution to data collection and manuscript preparation.

Eduardo Périco: Contribution in the concept and design of the study, data analysis and interpretation and 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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Article

The first larval stage (Zoea I) description of the caridean shrimp *Ogyrides occidentalis* (Ortmann, 1893) (Alpheoidea: Ogyrididae) reveals congruence with taxonomic status

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Abstract: A complete and detailed description of the first zoeal stage of *Ogyrides occidentalis* is provided. Larvae were obtained in the laboratory from a female with embryos collected in Ubatuba, State of São Paulo, Brazil. The morphological characters are compared with previous description of the close related *O. alphaerostris*. Despite of some similarities (number of appendages, pleonites, and setae on the majority of appendages) substantial differences were found between the two species, as the size of larvae and rostrum and segmentation of some structures (antenna exopod, first maxilliped coxa and basis). However, these differences must be interpreted carefully because larval description of *O. alphaerostris* was conducted before the proposed standardization for decapod larval morphology descriptions. The present larval description furnished additional information to corroborate the recent resurrection of *O. occidentalis* and will be useful for future comparative and ecological research.

Keywords: *Caridea; Decapoda; larval morphology; post-hatching development.*

Descrição do primeiro estágio larval (Zoea I) do camarão carídeo *Ogyrides occidentalis* (Ortmann, 1893) (Alpheoidea: Ogyrididae) revela congruência com o status taxonômico

Resumo: Foi realizada uma descrição completa e detalhada da primeira zoea de *Ogyrides occidentalis*. As larvas foram obtidas em laboratório a partir de uma fêmea com embriões coletada em Ubatuba, estado de São Paulo, Brasil. Os caracteres morfológicos são comparados com a descrição anterior da espécie proximamente relacionada *O. alphaerostris*. Apesar de algumas semelhanças (número de apêndices, somitos abdominais e cerdas na maioria dos apêndices) foram encontradas diferenças substanciais entre as duas espécies, como o tamanho das larvas e do rostro, e segmentação de algumas estruturas (exopodito da antena e entre coxa e base do primeiro maxilípede). No entanto, essas diferenças devem ser interpretadas com cuidado porque a descrição larval de *O. alphaerostris* foi realizada antes da padronização proposta para as descrições da morfologia larval de decápodes. A presente descrição larval forneceu informações adicionais para corroborar a recente ressurreição de *O. occidentalis* e será útil para futuros estudos ecológicos e comparativos.

Palavras-chave: Caridea; Decapoda; desenvolvimento pós-embrionário; morfologia larval.

Introduction

The genus *Ogyrides* Stebbing, 1914 is the only representative of the caridean family Ogyrididae. Considering the recently resurrection of *Ogyrides occidentalis* (Ortmann, 1893) (Terossi & Mantelatto 2020), this genus comprises 13 valid species distributed along tropical and subtropical coasts around the world (De Grave & Fransen 2011, Ayón-Parente & Salgado-Barragán 2013, De Grave et al. 2020, WoRMS 2020).

Three species of *Ogyrides* have been recorded from Brazilian waters: *O. hayi* Williams, 1981 in the State of Ceará by Pachelle et al. (2016); *O. alphaerostris* (Kingsley, 1880), an apparently amphi-American species and the occurrence in Brazil is uncertain (Williams 1981, Wicksten & Méndez 1988, Hendrickx 1993, Ayón-Parente & Salgado-Barragán 2013, Almeida et al. 2013, Terossi & Mantelatto 2020); and *O. occidentalis* trawled in waters up to 52 m deep, with occurrence in Pará, Ceará, Bahia, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, Rio Grande do Sul (Christoffersen 1979, Almeida et al. 2013, Terossi & Mantelatto 2020). These three species, together with *O. tarazonai* Wicksten & Méndez, 1988 and *O. wickstenae* Ayón-Parente & Salgado-Barragán, 2013, both from the eastern tropical Pacific (Wicksten & Méndez 1988, Ayón-Parente & Salgado-Barragán 2013), account for five species with occurrence in American waters.

Larval descriptions of ogyridid shrimps are scanty. An incomplete description was accomplished for *O. delli* Yaldwyn, 1971 by Packer (1985), with only data on dorsal view of cephalothorax and the maxilla of the fourth zoea from New Zealand. The single detailed and complete description available is about *O. limicola* Williams, 1955 from Virginia, USA by Sandifer (1974). However, according to De Grave & Fransen (2011), *O. limicola* is currently considered a junior synonym of *O. alphaerostris*. Thus, the larval descriptions of *O. limicola* (Sandifer 1974) actually refers to *O. alphaerostris,* a species from the Western Atlantic (Terossi & Mantelatto 2020).

The obtention of some fresh zoea I hatched under laboratory conditions from a parental female of the recently resurrected *O. occidentalis* lead us to hypothesize that larval characters could differ from those of *O. alphaerostris* described as *O. limicola* by Sandifer (1974). In this context, the objective of the present study was to describe the morphology of the first larval stage (zoea I) of *O. occidentalis*, and to compare its morphology with the single detailed description of the same larval stage of a congener (*O. alphaerostris*). A detailed larval description is essential for future comparisons to help the understanding about the phylogenetic relationships of the representatives of the genus, as well as the family Ogyrididae.

Material and Methods

One female with embryos of *O. occidentalis* was collected at Ubatuba, State of São Paulo, Brazil (23º26′13″S, 45º04′4″W), in August 2013. The collections were made at a depth of approximately 5 m, using a shrimp-fishing boat equipped with an otter-trawl net (mesh size 20 mm and 18 mm in the cod end) for trawling. The female with embryos was transported alive to the Laboratory of Biology of Marine and Freshwater Shrimps (LABCAM) and maintained in a 2-liter container with seawater from the sampling site and some biogenic debris (leaves, sticks and shells) for shelter, until the larval hatchings. Newly hatched larvae were conserved in a mixture (1:1) of 70% ethyl alcohol and glycerin.

Tissue sample was taken from the parental female for molecular analysis of partial fragments of the ribosomal rRNA, 16S rRNA, gene to confirm the species identification (GenBank Accession number MT365660; see details of methodology in Terossi & Mantelatto 2020). The carapace length (CL, mm) of 10 larvae was measured as the maximum length from the posterior margin of the ocular orbit to the posterior margin of the carapace. Total length (TL) is given as the distance from the tip of the rostrum to the posterior margin of the telson, excluding setae. Appendages were dissected under a Zeiss™ Stemi 200C trinocular stereomicroscope, and drawings and measurements were made using a Leica™ DM750 microscope equipped with a *camera lucida*. Larval description and setal counts are based on the recommendations of Clark et al. (1998) and updated by Clark & Cuesta (2015); we followed the setal terminology proposed by Garm et al. (2004) and Landeira et al. (2009). Six larvae were dissected for detailed examination and description. The long terminal plumose natatory setae on exopods of maxillipeds were drawn truncated.

Voucher of the spent parental female and respective larvae were deposited at the Crustacean Collection of the Biology Department of FFCLRP, University of São Paulo, Brazil (CCDB/FFCLRP/USP, access number: CCDB 6131).

Results

The parental female has a CL of 5.3 mm. Due to the low number of hatched larvae (16), only the zoea I of *O. occidentalis* was described and illustrated. Morphological differences between description of *O. alphaerostris* accomplished by Sandifer (1974) as *O. limicola* and the present description of *O. occidentalis* are listed in Table I.

Ogyrides occidentalis (Ortmann, 1893)

Zoea I (Figs. 1a-f; 2a-f)

Dimensions: CL = 0.358 ± 0.001 mm; TL = 1.285 ± 0.01 mm (n = 10).

Carapace (Figs. 1a-c): elongated with an acute spine in the pterygostomian region; rostrum slender, without setae, slightly overreaching the extremity of the eyes; eyes sessile.

Antennule (Figs. 1a, b, d): peduncle unsegmented; inner flagellum with 4 terminal aesthetascs and 1 terminal plumose seta; outer flagellum as a long plumose seta.

Antenna (Figs. 1a, b, e): peduncle unsegmented, with a terminal inner spine near endopod; endopod unsegmented, with two rows of 5-6 spines in the mediodistal region and 1 plumose seta in the medial region; exopod (antennal scale) unsegmented, with 8 plumose setae on inner side, and 1 plumose plus 3 short simple setae on the outer side.

Mandibles (not illustrated): incisor and molar process developed; palp absent.

Maxillule (Fig. 2a): coxal endite with 7 plumodenticulate setae (5 long and 2 short); basial endite with 5 stout plumodenticulate setae; endopod with 3 subterminal (2 plumoserrate and 1 minute simple) and 2 terminal plumoserrate setae; exopodal seta absent.

Maxilla (Fig. 2b): coxal endite bilobed with 9 marginal setae (3 sparsely plumose, 5 plumose with short setules and 1 serrate) on proximal lobe and 4 setae (1 sparsely plumose, 2 plumose and 1 serrate) on distal lobe; basial endite bilobed with 5 setae (3 sparsely plumose, 1

Table 1. Morphological comparison (mainly differences) between the first zoeal stage (zoea I) of *Ogyrides occidentalis* (Ortmann, 1893) and *O. alphaerostris* (Kingsley, 1880).

Source	Sandifer (1974)	Present study
Species	*O. alphaerostris*	*O. occidentalis*
Locality	Virginia, U.S.A.	São Paulo, Brazil
Type locality	Virginia, U.S.A.	Pará, Brazil
Characteristics		
Total length (mm)	2.03 (1.74-2.11)	1.285 ± 0.01
Rostrum	Not reaching the extremity of the eyes	Slightly overreaching the extremity of the eyes
Antenna (exopod)	3-segmented; 8 ps + 2 ss	Unsegmented; 9 ps + 3 ss
Maxillule (endopod)	4 s (strong)	5 s (4 strong + 1 small ss)
Maxilla (coxal endite proximal lobe)	7-9 s	9 s
Maxilla (basial endite)	4-5 s on each lobe	5 s on each lobe
1st Maxilliped (coxa and basis)	Fused	Not fused
2nd Maxilliped (coxa)	†	1 s
2nd Maxilliped (basis)	2-3 longer s + 3-4 ss	2 longer s + 4 ss
3rd Maxilliped (coxa)	†	0 s
3rd Maxilliped (basis)	2-3 s	3 s
Pleon (setae on fourth pleonite)	absent	present

Abbreviations: s, setae; ps, plumose setae; ss, simple setae; (†) no data.

simple and 1 hardy plumose) on proximal lobe and 5 setae (4 sparsely plumose and 1 hardy plumose) on distal lobe; endopod 5-lobed, with 3 (2 sparsely plumose and 1 hardy plumose), 2 (1 sparsely plumose and 1 hardy plumose), 1 hardy plumose, 1 hardy plumose, and 2 (1 hardy and 1 plumose) setae, respectively; exopod (scaphognathite) margin with 5 plumose setae; microtrichia on margins of endopod and exopod as illustrated.

First maxilliped (Fig. 2c): coxa with 4 setae (1 simple and 3 sparsely plumose) arranged 1 + 1 + 1 + 1; basis with 11 sparsely plumose setae arranged 1 + 2 + 2 + 3 + 3; endopod 4-segmented with 3 terminal sparsely plumose, 1 terminal sparsely plumose, 2 terminal sparsely plumose and 4 (3 terminal sparsely plumose + 1 subterminal simple) setae, respectively; exopod unsegmented with 1 subterminal plumose and 4 terminal long plumose natatory setae.

Second maxilliped (Fig. 2d): coxa with 1 sparsely plumose seta; basis with 6 setae (2 sparsely plumose and 4 simple) arranged 1 + 1 + 2 + 2; endopod 4-segmented with 3 terminal sparsely plumose, 1 terminal sparsely plumose, 2 terminal sparsely plumose, and 5 (3 terminal sparsely plumose, 1 terminal plumodenticulate and 1 subterminal simple) setae, respectively; exopod unsegmented with 2 subterminal plumose and 4 terminal long plumose natatory setae.

Third maxilliped (Fig. 2e): coxa without setae; basis with 3 (1 plumose and 2 sparsely plumose) setae arranged 1 + 1 + 1; endopod 3-segmented with 3 (2 marginal and 1 subterminal) sparsely plumose, 3 (l terminal simple and 2 subterminal sparsely plumose), 3 terminal long sparsely plumose setae, respectively; exopod unsegmented with 4 marginal plumose and 5 (1 simple and 4 terminal long plumose natatory) setae.

Pereiopods (Fig. 2f): first as a uniramous bud.

Pleon (Figs. 1a, b): with 5 pleonites, fourth pleonite with one pair of posterodorsal simple setae, pleonite 6 fused with the telson; pleopods and uropods absents; anal spine present.

Telson (Figs. 1a, b, f): broad at posterior margin, with 7 + 7 plumose setae (the outer 2 setae plumose only in the inner margin), outermost pair subterminal, inner pair shorter; one row of minute spinules on distal margin between and around bases of the 6 + 6 inner setae.

Discussion

The morphology of the first larval stage of the recently resurrected *O. occidentalis* showed conspicuous dissimilarities when compared with the same larval stage of *O. alphaerostris*, described more than 40 years ago (Sandifer 1974). Unfortunately, the larval morphology scenario for the genus did not receive new descriptions and no additional comparison on larval features among the other 10 recognized species of *Ogyrides* (De Grave & Fransen 2011, Ayón-Parente & Salgado-Barragán 2013, Terossi & Mantelatto 2020) is possible due the lack of descriptions. Additionally, the zoea of *O. delli* described by Packer (1985) is incomplete, with no details and no standard characterization that allows comparison. This background illustrates the importance of new and accurate descriptions of some larvae of Decapoda to fill out the tremendous lack of information.

Despite the similarities (number of appendages, pleonites, and numbers of setae on some appendages) between the first larval stage of *O. occidentalis* presented here and *O. alphaerostris* (Sandifer 1974), some morphological characters were remarkably distinct (see Table I). In *O. alphaerostris*, the rostrum does not reach the extremity of the eyes, while in *O. occidentalis* this structure slightly overreaches the sessile eyes. Total length of larvae was smaller in *O. occidentalis* than in *O. alphaerostris* (1.285 ± 0.01 mm and 2.03, respectively). Regarding CL, Sandifer (1974)

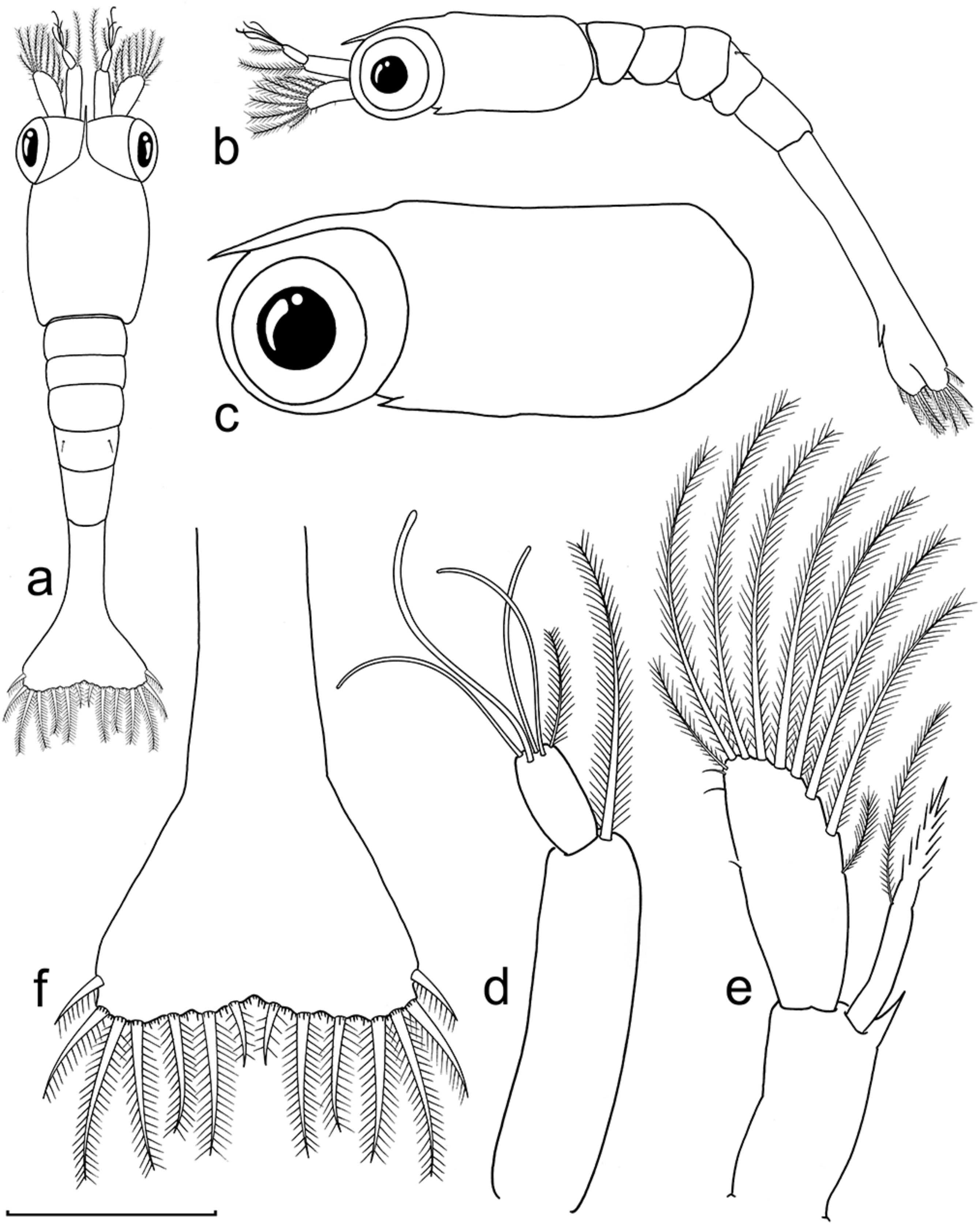

Figure 1. *Ogyrides occidentalis* (Ortmann, 1893), Zoea I. a. Dorsal view; b. Lateral view; c. Magnification of the carapace, lateral view; d. Antennule; e. Antenna; f. Telson. (Scale bar: a, b = 0.5 mm; d, e = 0.125 mm; c, f = 0.25 mm).

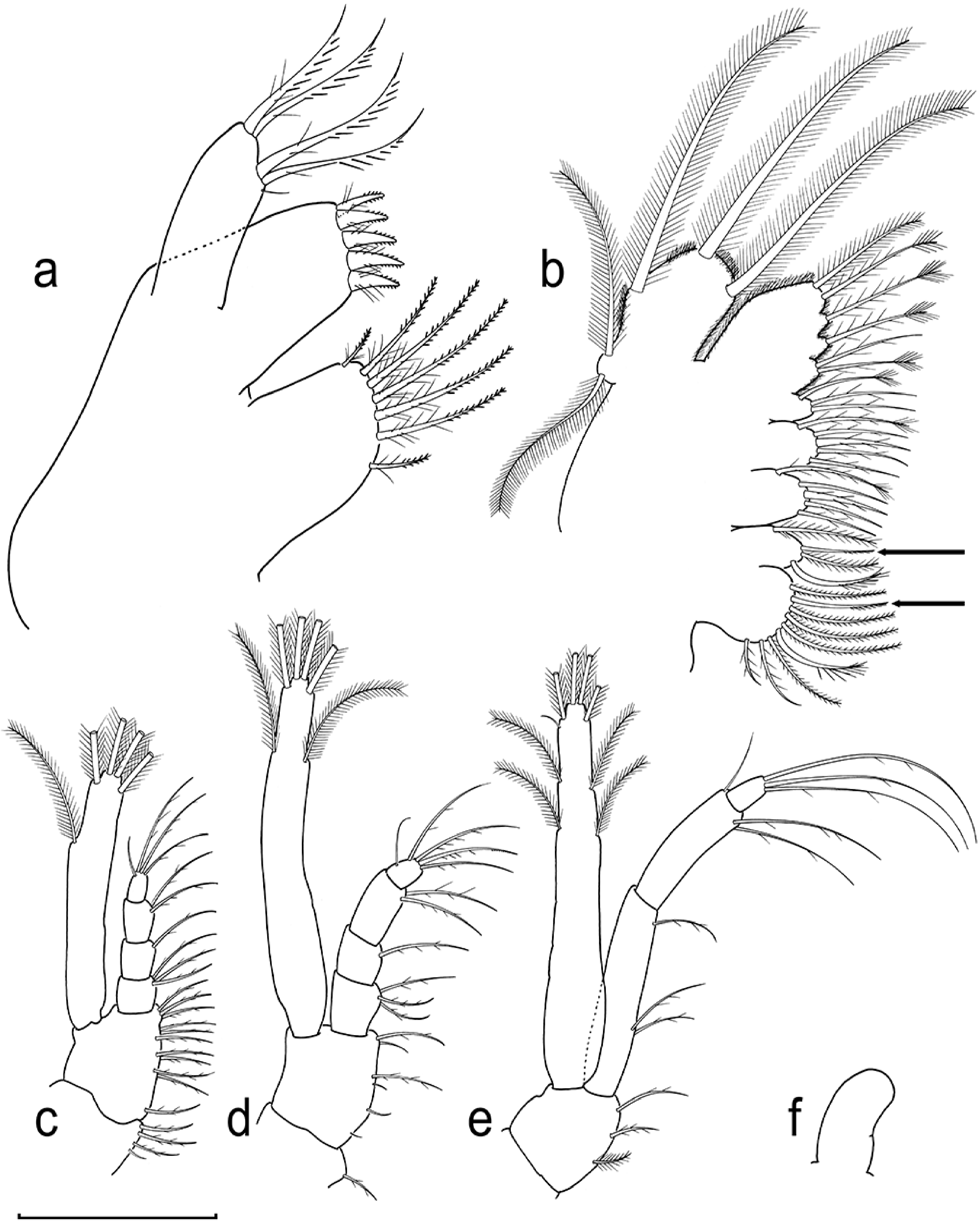

Figure 2. *Ogyrides occidentalis* (Ortmann, 1893), Zoea I. a. Maxillule; b. Maxilla; c. First maxilliped; d. Second maxilliped; e. Third maxilliped; f. First pereiopod. Arrows indicate serrate setae on the coxal endite of maxilla. (Scale bar = a = 0.05 mm; b = 0.1 mm; c-f = 0.15 mm).

did not include any measurement, however, using the scale bars of the original illustrations it is possible to estimate a CL of approximately 0.5 mm, which is also larger than the CL of *O. occidentalis* (0.358 ± 0.001 mm). Additional differences can be noted in the segmentation of some structures: the antenna exopod is 3-segmented and first maxilliped coxa and basis were described as being fused in *O. alphaerostris*, while the antenna exopods are unsegmented, and there is a clear segmentation between coxa and basis of first maxilliped of *O. occidentalis*.

Other differences were observed in the number of setae of some structures, i.e. antenna exopod, maxillule endopods, 2nd maxilliped basis and fourth pleonite (Table 1). We also described all setal types found in the first zoea of *O. occidentalis*, including some structures that were not described for *O. alphaerostris* (coxa of 2nd and 3rd maxillipeds). Except for some plumose setae in the antennule, antenna, maxilla, maxillipeds and telson, Sandifer (1974) had used different terminology for the types of setae.

Setae of decapod crustaceans manifest a variety of structures and perform numerous functions: e.g. cleaning the body surface, providing water flows and chemo - and mechanoreception (Borisov 2016). Accurate descriptions of setal types allow the identification of the level of development of some structures that are decisive to understanding of ecological, taxonomical, and systematic features of the distinct groups of Decapoda. After the present description, it is possible to notice that the first larval stages of *Ogyrides* species exhibit particular features such as a notorious development in mouthparts (maxillule and maxilla). Morphology of the mouthparts provides useful information on feeding habits and prey characteristics (Cox & Johnston, 2003). Furthermore, information about setal types will probably be important after the description of more species of the genus, because in some circumstances, the combination of several morphological characters are necessary for the identification of the zoea I of some caridean species (e.g. Geiselbrecht & Melzer 2009, Mantelatto et al. 2014, Pescinelli et al. 2017).

The differences noted between the two species must be interpreted carefully at this time. In this sense, a future redescription of the zoea I of *O. alphaerostris* would be important to detail the types of setae and to check if some of the observed differences are real or could be the result of misidentification, e.g. segmentation in the antennal scale, absence of segmentation between coxa and basis of the first maxilliped and absence of simple setae in the pleon of *O. alphaerostris*.

The results of this study, especially the differences in some structures (e.g., length of the rostrum and setation of antennal scale) furnished support to recent taxonomical rearrangements for the genus, with resurrection of *O. occidentalis* (Terossi & Mantelatto 2020), suggesting that the zoea I described herein and those described by Sandifer (1974) do not actually belong to the same species. On the other hand, to state whether these dissimilarities refute the synonymization of *O. limicola* and *O. alphaerostris* or reflect natural groups not yet detected by systematic studies based on adult morphology is beyond the scope of the present study, especially if we consider the limited number of species with some described larval stage for the family, i.e., about 15,4% of the current representatives, disregarding *O. delli* (see Introduction for details). Given this context, we can suggest that this family probably needs a taxonomic revision, and a combination of a morphological analysis, including all zoeal stages if possible, with a molecular analysis could be extremely useful in this case.

Besides the importance of larval morphology knowledge to provide useful information in the current taxonomic and phylogenetic context, the present description will also help to identify specimens of *O. occidentalis* in plankton samples, allowing the advance of ecological and biodiversity studies. An increase in the descriptions of species not yet described and redescriptions of some species, as presented here, are thus essential to generate accurate information, what will certainly bring significant gains for future comparative research on biodiversity.

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Author Contributions

João Alberto Farinelli Pantaleão: Contribution to data collection, analysis and interpretation, and manuscript preparation.

Fernando Luis Mantelatto: Substantial contribution in the concept and design of the study, contribution to data collection and critical revision, adding intellectual content.

Rogério Caetano Costa: Substantial contribution in the concept and design of the study, contribution to data collection 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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Article

Seed removal of *Araucaria angustifolia* by native and invasive mammals in protected areas of Atlantic Forest

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Abstract: Araucaria Forest is one of the most threatened tropical forests in the world. Wild pig (*Sus scrofa*) are invasive pig that is expanding through these forests and seed removing that would be available to native fauna. Our aim was to evaluate the rates of seed Araucaria (*Araucaria angustifolia*) removal by both small, medium, and large mammals in areas with and without wild pig. We conducted a seed-removal experiment with three treatments differing in mammals' access to seeds, in areas with and without the occurrence of wild pig. Similar numbers of seeds Araucaria were removed by small, medium and large mammals, even in areas with wild pig. However, we verified that seed removal by small mammals is graduate over time, while large mammals, especially wild pig, remove in one event. So, we recommend long-term studies to investigate competition between wild pig and native biota and the effects of wild pig on seed dispersal and seed survival.

Keywords: *Brazilian Atlantic Forest; Araucaria; Rodents; Wild boar; Feral pigs.*

Remoção de sementes de *Araucaria angustifolia* por mamíferos nativos e invasores em áreas protegidas da Mata Atlântica

Resumo: A floresta de Araucárias é uma das florestas tropicais mais ameaçadas do mundo. O javali (*Sus scrofa*) é um porco invasor que está se expandindo por essas florestas e removendo sementes que estariam disponíveis para a fauna nativa. Nosso objetivo foi avaliar as taxas de remoção das sementes do pinheiro brasileiro (*Araucaria angustifolia*) por pequenos, médios e grandes mamíferos, em áreas com e sem javali. Realizamos um experimento de remoção de sementes com três tratamentos diferentes que limitam o acesso das espécies de mamíferos às sementes do pinheiro brasileiro, em áreas com e sem a ocorrência de javalis. Números semelhantes de sementes de pinheiro brasileiro foram removidos por pequenos, médios e grandes mamíferos, mesmo em áreas com javalis. No entanto, verificamos que a remoção de sementes por pequenos mamíferos é gradativa ao longo do tempo, enquanto que grandes mamíferos, especialmente os javalis, removem as sementes em uma única visita. Portanto, recomendamos estudos de longo prazo para investigar a competição entre javalis e biota nativa e os efeitos dos javalis na dispersão e sobrevivência das sementes do pinheiro brasileiro.

Palavras-chave: *Mata Atlântica; Pinheiro brasileiro; Roedores; Javalis; Porco Asselvajado.*

Introduction

Seed removal by animals is a key process determining the spatial structure of plant populations (Schupp 1988, Wang & Smith 2012) and there is a tendency towards increased seed removal in areas with greater abundance of seed removers and higher plant density (Janzen 1970, 1971). In neotropics, large granivores such the white-lipped peccary *Tayassu pecari* are capable of changing the structure of keystone-tree populations through predation on seeds that change the quantity and distribution of seedlings (Silman et al. 2003). In tropical forests, the presence of such large granivores can maintain higher rodent assemblage diversity by suppressing the populations of dominant rodents via resource competition (Galetti et al. 2015a). This reduces the removal of seeds by small granivores because dominant rodent species are the main removers of keystone-tree seeds (Fleury & Galetti 2006, Galetti et al. 2001, Galetti et al. 2015b).

Araucaria Forest is one of the most threatened tropical forests (Lacerda 2016, Rezende et al. 2018, Ribeiro et al. 2009) and is characterized by Araucaria trees (Araucariaceae: *Araucaria angustifolia*). Due to its restricted distribution, habitat fragmentation, logging, and loss of seed dispersers (Fragoso et al. 2011, Veloso et al. 1992), Araucaria populations are declining (Thomas 2013). The seed cones begin to mature two years after pollination, and development from the primitive carpel to seed takes about four years. Trees begin to set seeds between 12 and 15 years of age (Bittencourt 2007, Thomas 2013) that are dispersed from March to June by both gravity and animals (Mantovani et al. 2004). The species has large seeds (6.5 to 7 g) that are produced in great abundance at a predictable time of relative scarcity of food resources in the Araucaria Forest (Mantovani et al. 2004, Souza et al. 2010). Highly nutritive (average 7g and 14.8 kcal per seed), the Araucaria seeds is considered a key resource for insects, birds, and mammals in the Araucaria Forest (Brocardo et al. 2018, Iob & Vieira 2008, Ribeiro & Vieira 2014).

Beside the historical deforestation, logging and poaching (Brocardo 2017), Araucaria forest have been faced a new enemy, the biological invasion of wild pig (*Sus scrofa*) (Rosa et al. 2017). The wild pig is native to Eurasia and northeast Africa, and is one of the most widespread invasive mammals in the world (Clout & Russell 2007). They are recognized as an important alien ecosystem engineers with high ecological plasticity, changing the soil, vegetation cover and seed bank structure (Barrios-Garcia & Ballari 2012, Cuevas et al. 2012, Ickes et al. 2001, Webber et al. 2010), and competing with native fauna for resources (Hegel et al. 2019, Sanguinetti & Kitzberger 2010). The range of the wild pig is expanding throughout tropical forests (Clout & Russell 2007, Rosa et al. 2017), where they are mainly recognized as seed removers, although they may eventually also act as dispersers (Sanguinetti & Kitzberger 2010, Pedrosa et al. 2019).

The wild pig spread through the Araucaria Forest domain (Rosa et al. 2017) and is consuming seeds of the Araucaria (Deberdt & Scherer 2007, Hegel & Marini 2013, Rosa 2018). However, the effects of this invasion on the removal of Araucaria seeds or the interaction with native removers is still unknown. In addition, the previously studies evaluating seed removal of Araucaria where conducted in areas where large granivores were extinct or have their population dramatically reduced (Iob & Vieira 2008, Ribeiro &Vieira 2014, Vieira et al. 2011). We thus conducted a block experiment that limited the access of Araucaria seeds by mammals to identify seed removers and evaluate the rates of seed removal of both small and large granivores in areas with wild pig (and without white-lipped peccary) and areas without wild pig (and with white-lipped peccary). Beside both wild pig and white-lipped peccary are large granivores, wild pig is recognized as a highly invasive and competitive species (Massei & Genov 2004, Barrios-Garcia & Ballari 2012), so our hypothesis is that, in the area invaded by wild pig, the seed removal will be majoritarian executed by large mammals because of high frequency of occurrence of wild pigs in the area (Mazza et al. 2018).

Material and Methods

Our study was carried out in the Itatiaia National Park (INP) (22°26’14”N, 44°36’3”W), Brazil’s first National Park created in 1937, and in the Private Natural Heritage of Alto Montana Reserve (PRNH) (22º21’08 “N, 44º48’04” W), both located in southeastern Brazil. The INP cover areas of Minas Gerais and Rio de Janeiro states and PRNH is located in Minas Gerais (Figure 1). The protected areas cover 28.084 (INP) and 672 (PRNH) ha of the Brazilian Atlantic Forest domain. Both are in the Serra da Mantiqueira mountain chain, which is considered to be an area of global importance for biodiversity conservation (Myers et al., 2000) and recently considered an irreplaceable protected area (Le Saout et al. 2013). The INP altitude ranges from 600 to 2791 m asl and our study was conducted in its lower portion, between 600 and 1600 m asl, which has a Cwb climate type (altitude subtropical climate), mesothermal with a rainy season in summer (Köppen 1936). The PRNH has altitude ranging from 1500 to 2500 m asl and has a Cfb climate type (temperate oceanic climate), mesothermal, without a dry season (Köppen 1936). The PRNH is dominated by a mixture of seasonal semideciduous montane forest and high-altitude fields characterized by the presence of the Araucaria while the lower part of INP is dominated by montane rain forest with few widely-dispersed individuals of Araucaria (Ururahy et al. 1983, Oliveira-Filho & Fontes 2000).

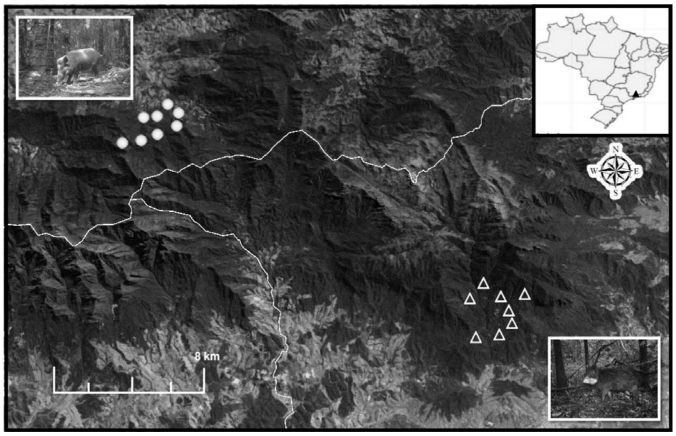

Figure 1. Study area showing the sampled points (blocks) in the area with wild pig and without white-lipped peccaries (circles), and in the area with white-lipped peccaries and without wild pig (triangles). The gray line is the division of the Brazilian states Minas Gerais, São Paulo and Rio de Janeiro.

The region of our study is distribution area of native taiassuids (*Tayassu pecari* - white-lipped peccaries and *Pecari tajacu* - collared peccary) and is suffering with wild pig’ invasion since 2006 (Rosa 2018). In the PRNH occurs 21 native species of medium and large mammals (Mazza et al. 2018), while in INP occurs 22 species of native mammals

(Aximoff et al. 2015). Wild pig occurs in all PRNH territory and is the most frequent mammal species recorded in the area (Mazza et al. 2018), however there is no evidence of its occurrence in the lower portion of INP. The white-lipped peccary is widespread in the lower portion of INP, but there is no recent record of this species in the PRNH, indicating a probable local extinction (De Abreu 2016). In this study, the INP will be referred as the area without wild pig and with white-lipped peccary, and PRNH as the area with wild pig and without white-lipped peccary.

To assess seed removal of Araucaria, we conducted an experiment from April to May 2014 during the seed harvest season, in both areas with and without wild pig. In this period, the experiment was repeated three times at intervals of 15 days. Within each area, we selected eight sampling points, around 1 km far from each other (Figure 1). Each point represented a block with three different treatments to segregate the species with access to seeds (Mileri et al. 2012, Ribeiro & Vieira 2014). We collected the seeds used in the treatment in PRNH area twenty days before the study started. We arranged the treatments two meters apart, with 10 seeds in each using the same sampling design previously tested by Mileri et al. (2012). Each block had one each of the following treatments: (1) Free - seeds arranged side-by-side, forming a circle, on the ground with free access by all animals; (2) Small - seeds placed inside 75 mm wide and 40 cm length PVC pipes which were accessible only by small mammals rodents species. We secure the pipe to the ground with an iron rod to prevent medium and large mammals from rolling the pipe and access the seeds; (3) Large - seeds placed in a bowl 15 cm above the ground, allowing access only by medium and large mammals (see Mileri et al. 2012).

We identify seed removers placing one camera-trap in each point sampled. We used motion-activated digital cameras (Bushnell HD, ©Bushnell Outdoor Products, California, USA) set to take three photos every 30 seconds. The camera-traps were installed in front of experiments and were in continuous operation during the two months of data collection, resulting in 960 camera-traps/nights. Since the camera-traps run automatically over that period, we did not check them to avoid unnecessary disturbance. At the end of sampling, we recovered memory cards and identified species from the image records. We consider as seed removers those species that directly interacted with the seeds or with the pipe and bowl structures, as observed in photographic records. We also count the number of these interactions considering 1-hour interval between the photographs. We also used photographs to count frequency of occurrence of white-lipped peccary and wild pig counting all records of both species considering 1-hour apart for independent observations (Srbek-Araujo & Chiarello 2013).

To test our hypothesis, we considered each block as a replicate and the number of seeds removed as the response variable. To evaluate how seed removal is influenced by area (with and without wild pig), treatment (Free, Small and Large) and frequency of occurrence of large granivores (white-lipped peccary and wild pig), we used Generalized Linear Mixed-effects Models (GLMM) with Poisson distribution for non-normal counting data (Bolker et al. 2008, Zuur et al. 2009). The block (i.e. sampling point) was used as random effect. We constructed models using the "glmer" function available in the "lme4" R software package (R Core Team 2017). We used the Akaike Information Criterion corrected for small samples (AICc) for model selection, to identify the variables that best explain seed removal. The best models were considered as those with ΔAICc lower than 2 (Burnham & Anderson 2002; Burnham et al. 2011).

Results

Of a total of 1440 seeds available in all treatments, 77% (N = 1,111) were removed (see Appendix 1). From seeds removed, 30.2% (N = 336) were in the Large treatment, 34.5% (N = 383) in the Free treatment and 35.3% (N = 392) in the Small treatment. In the white-lipped area 552 seeds are removed, being 35% (N = 193) in the Free treatment, 33% (N =184) in the Small treatment and 32% (N = 175) in the Large treatment. In the wild pig area 559 seed are removed, being 37% (N = 208) in Small treatment, 34% (N = 190) in the Free treatment and 29% (N = 161) in the Large treatment (Figure 2 and 3). The frequency of occurrence of the wild pig was a mean of 17.87 (min = 0; Max = 76), while white-lipped peccary has a frequency of occurrence of a mean of 39.75 (Min = 0; Max = 175).

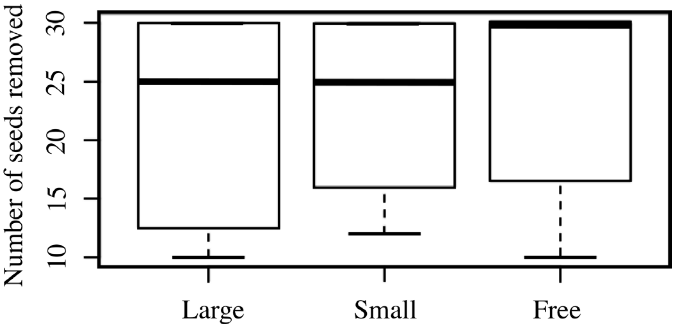

Figure 2. Seed removal in Small (seeds accessible only by small mammals), Large (seeds accessible only by medium and large mammals) or Free (seeds accessible by all mammals) treatments, in PNI area with white-lipped peccary.

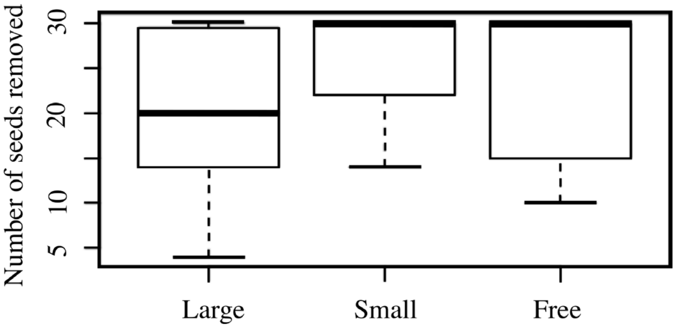

Figure 3. Seed removal in Small (seeds accessible only by small mammals), Large (seeds accessible only by medium and large mammals) or Free (seeds accessible by all mammals) treatments, in PRNH area with wild pig.

From camera-traps, we recorded 115 interactions between species removers and treatments of six taxa: wild pig (N = 9), white-lipped peccaries (N = 3), black-horned capuchin (*Sapajus nigritus*, N = 1), red brocket deer (*Mazama americana*, N = 1), Brazilian squirrel (*Guerlinguetus ingrami*, N = 6), dusky-legged guan (*Penelope obscura*, N =1) and small rodents (N = 94) (see Appendix 2). We pooled all species of small rodents (except *G. ingrami*) into a single category because no reliable identification at species level was possible from the photographs. Although there are apparently more events of seed-removal by rodents, from camera-traps photographs we are able to see that those removals were gradual, with one or two seeds being removed daily, while all seed-removal events made by wild pig and white-lipped peccaries occurred in a single event by one individual. We cannot evaluate the final destination of seeds, but we found many seeds predated by both small and large mammals (see Appendix 3).

Treatment appears as the only variable in the best model influencing seed removal of Araucaria. The frequency of white-lipped peccary also has a positive influence in seed removal of Araucaria as shown by the third and fourth-best model (Table 1).

Table 1. Ranking of the best generalized linear mixed-effects models with poisson distribution to predict the effects of the explanatory variables on seed removal of Araucaria. Variation in AICc (ΔAICc) and Akaike weight (ωi).

Models	AICc	ΔAICc	ωi
Treatment (+)	322.8	0.00	0.21
Null model	323.1	0.29	0.18
White-lipped peccary (+) + Treatment (+)	324.5	1.71	0.09
White-lipped peccary (+)	324.6	1.78	0.09

Discussion

Our results indicate that the amount of seed removed was influenced by experimental treatments and, different of our expectation, the frequency of occurrence of the native large granivore is more important to seed removal than the frequency of occurrence of the invasive large granivore. Even though we do not observe the presence of wild pig as determinant to seed removal of Araucaria, wild pig may be altering forest structure and the resources available for the native fauna (Sanguinetti & Kitzberger 2010).

Large granivores in tropical forests can alter the population structure of trees, changing the quantity and distribution of seedlings through cascading effects of seed-removal rates and the distance of the seed from the parent plant; and indirectly due to competition from other seed removers (Galetti et al. 2015a, Silman et al. 2003). Although Pedrosa et al. (2019), found a positive relationship between Araucaria seeds and passage through the stomach and intestinal tract of wild pig (intact seeds in 56% of the stomachs and in 90% of the analyzed feces), we do not know the effects of the invasive population of wild pig on seedling recruitment and structure of Araucaria populations. So, it may be too early to decree this invasive species “safe” for this ecosystem, especially because wild pig have a historic of seed predation in its native distribution (Gomez et al. 2003). In addition to the consumption and destruction of Araucaria seeds in Araucaria forests, wild pig showed intense herbivory, rooting and soil overturning in areas around Araucaria trees (Hegel & Marini 2013). This can be considered a threat to the integration and maintenance of these forests.

From a native fauna perspective, the overlap of habitat use and diet are part of the competitive interactions between wild pig and native species (Ilse & Hellgren 1995). We could not identify competition between wild pig and native biota for seeds, but our photographic records show that small rodents gradually removed the seeds of Araucaria, returning consecutively to the experiment to feed, while large mammals (native and invasive), on the other hand, visited the experiment site, removing virtually all available seeds in one event. This occurs because species body size, species home range, and species behavior results in differences between resource sharing among rodents and large granivorous with the later needing proportionally more seeds than rodents to feel satiated (Murray 1987). So, when an invasive species like the wild pig is inserted in the trophic relations of a biological community, it can interfere with the foraging dynamics of local populations of native species, resulting in competition for resources, that was not possible to observe with our sampling design.

The history of human occupation in Araucaria forest results in a largely defaunated forest of medium and large mammals (Galetti & Dirzo 2013, Bogoni et al. 2018). On the other hand, small rodents generally are not negatively affected by fragmentation and suffer less from hunting pressure (DeMattia et al. 2006, Pardini 2004), which results in small rodents being commonly observed as main removers of Araucaria seeds (e.g. Iob & Vieira 2008, Solórzano-Filho 2001). Most of the Araucaria seeds removed by rodents were destroyed and less than 5% of seeds were effectively dispersed (Vieira et al. 2011, Solórzano-Filho 2001). However, the massive seed production of Araucaria in a short time (two months) (Mantovani et al. 2004) allows small rodents, especially the scatter hoarding rodents, to play a relevant role in seed dispersal of this tree (DeMattia et al. 2004, Iob & Vieira 2008, Silman et al. 2003). In our wild pig area, the Small treatment account for most of seeds removal, which may be a reflex of the historical absence of large granivores. So, the answer we found may be related to the fact that, in eight years of invasion, wild pigs are still not participating significantly in this dynamic of seed removal, which does not mean that it will not happen. This may be because the wild pig population is not yet large enough for this effect to be noticed, especially considering that the frequency of its occurrence was half that recorded for the white-lipped peccary, or because the wild pig is using other alternative sources of food, since the PRNH region is characterized by the presence of small rural producers who report the common attack of wild pigs on crops and vegetable gardens (Pereira et al. 2019). The area of the white-lipped peccary is surrounded by urban centers and highways. That, and the fact that the white-lipped peccary is more intolerant of changes in the landscape (Keuroghlian & Eaton 2008, Reyna-Hurtado et al. 2012), means that the species does not leave the forest and the forest’s resources are the only food available to the species.

In some landscapes, especially those where the frequency of disturbances has been changed by human activities (Badano & Cavieres 2006), exotic ecosystem engineers, such as wild pig, could eventually replace ecological functions of native ecosystem engineers that are locally extinct (see Novel Ecosystem concept in Hobbs et al. 2006 and Hobbs et al. 2009, Pedrosa et al. 2019). Thus, more studies evaluating the effects of wild pig on the final destination of seeds (i.e. seed dispersal or predation), and on seedlings and adult trees are necessary to understand the role of wild pig in recruitment of Araucaria and other tropical-forest trees. Beside removal of Araucaria seeds were the same in area with or without wild pig, the effects of wild pig may increase in the future for both Araucaria trees and native fauna, especially if no population control is undertaken, since its invasion is recent (< eight years) in our study area. Also, in Araucaria forest, the invasion of wild pig presents an opportunity to study the effects of large granivores on Araucaria populations, in localities where native large granivores are rare or extinct. In any case, we recommend long-term studies to investigate competition between wild pig and native biota and the effects of wild pig on seed dispersal and seed survival in this tropical ecosystem.

Supplementary material

The following online material is available for this article:

Appendix 1 - Seed removal data from the three treatments of the experiment (Free, Small, and Large) in both studied protected areas [Itatiaia National Park (INP) and Private Natural Heritage of Alto Montana Reserve (PRNH)].

Appendix 2 - Seed removers of *Araucaria angustifolia* identified by camera-traps: Figure 1 and Figure 2. Small rodent species; Figure 3. *Sapajus nigritus*; Figure 4. *Sus scrofa*; Figure 5. *Tayassu pecari*.

Appendix 3 - Seeds of *Araucaria angustifolia* predated by A) Small rodents; B) *Tayassu pecari*; and C) *Sus scrofa*.

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Author Contributions

Clarissa Rosa: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Carla Grasiele Zanin Hegel: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Marcelo Passamani: Substantial contribution in the concept and design of the study; Contribution to critical revision, adding intellectual content.

Conflicts of interest

There is no conflict of interest.

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Article

Geographical origin of white honey produced by stingless bees in the Araucaria Forest in Southern Brazil

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Abstract: Honey produced by native stingless bees in the Araucaria Forest region of Rio Grande do Sul, southern Brazil, specifically the municipality of Cambará do Sul, is highly valued for its characteristic white color, floral odor and taste. In this study, we investigated the botanical origin of white honey stored in colonies of five Meliponini species of the genera *Melipona* (n = 3) and *Plebeia* (n = 2). During the production period of white honey, from January to March, flowers were sampled fortnightly along pre-established trails to identify plants used by bees. For all sampled plant species, exsiccates and pollen reference slides were prepared. Honey samples from stingless bees were processed for extraction and preparation of pollen grains for identification of pollen types. In all analyzed honey samples, pollen grains of *Clethra scabra* (Clethraceae) were predominant (between 46-94%). Pollen grains from other botanical families, including Myrtaceae, Fabaceae and Melastomataceae were frequently identified in honey samples of the *Melipona* species, while Cunoniaceae was also found in samples of *Plebeia* species. In this study, we concluded that *Clethra scabra* is predominantly used by Meliponini bees in the production of white honey in the municipality of Cambará do Sul.
Keywords: *bee flora; Clethra scabra; meliponiculture; Meliponini; pollen.*

Origem geográfica do mel branco produzido por abelhas sem ferrão na Floresta de Araucárias no Sul do Brasil

Resumo: Na região da Floresta de Araucária do Rio Grande do Sul, especificamente no município de Cambará do Sul, as abelhas nativas sem ferrão produzem mel característico devido à sua cor branca e gosto apreciado. Neste estudo, investigamos a origem botânica do mel branco armazenado em colônias de cinco espécies de Meliponini dos gêneros *Melipona* (n = 3) e *Plebeia* (n = 2). Durante o período de produção do mel branco, de janeiro a março, as flores foram amostradas quinzenalmente ao longo de trilhas pré-estabelecidas, a fim de identificar as espécies utilizadas pelas abelhas. A partir de amostras de plantas foram preparadas exsicatas e lâminas de referência de pólen. As amostras de mel de abelhas sem ferrão foram processadas para extração e preparo dos grãos de pólen para a determinação dos tipos polínicos presentes. Em todas as amostras de méis branco analisadas os grãos de pólen de *Clethra scabra* (Clethraceae) predominaram (entre 46-94%). Grãos de pólen de outras espécies das famílias botânicas Myrtaceae, Fabaceae e Melastomataceae foram frequentemente identificados nas amostras de méis das espécies de *Melipona,* enquanto Cunoniaceae também o foi nas amostras das espécies de *Plebeia*. Neste estudo, nós concluímos que *Clethra scabra* é predominantemente utilizada pelas abelhas Meliponini na produção de méis branco no município de Cambará do Sul.
Palavras-chave: *Clethra scabra: flora apícola; meliponicultura; Meliponini: pólen*

Introduction

The Araucaria Forest is part of the Atlantic Forest biome, found in South and Southeast Brazil, and in the Argentinian province of Missiones (Backes & Irgang 2002, Oliveira-Filho & Fontes 2000). The total land area covered by this charismatic high altitude forest ecosystem has receded greatly in recent decades, as a consequence of anthropogenic activities, with remaining forest areas representing just 13% of its original coverage (Emer & Fonseca 2011).

Land use change that causes loss and/or fragmentation of natural habitats negatively impacts native bees populations due to loss or change of the floral resources used as food sources and nesting sites (Kremen al. 2004). Stingless bees (Hymenoptera: Apidae: Meliponini) represent about 80% of the floral visitors of the upper stratum of the Atlantic Forest (Ramalho 2004). Therefore, alteration or loss of their populations affects the pollination of native plants (Kevan & Baker 1983, Roubik 1989, Gruchowski-Woitowicz 2020) and the maintenance of regional biodiversity (Brown & Albrecht 2001).

The management of stingless bees can provide rural communities with a sustainable source of income,and promote wider ecosystem restoration (Venturieri et al. 2012). The market for honey produced by stingless bees has grown rapidly in recent years, and is commonly sold at a higher price per kilo than honey produced by *Apis mellifera* Linnaeus, 1758 (Souza et al. 2006). Stingless bee honey occupies a specific niche in the market due to its appeal to consumers interested in healthy living, social justice and sustainable production with native species. However, the production of honey from stingless bees is still incipient and only serves local markets (Alves 2013).

The characteristics of stingless bees' products are closely related to local abiotic (e.g., climate) and biotic variables (e.g., plant community composition, bee species' physiology and life history strategy) (Lira et al. 2014). Knowledge on the botanical origin and the physicochemical properties of honey add value to it (Aboud et al. 2011) and allow the management of the flora and the hives for its production. Pollen analysis can be used to characterize the botanical and geographical origin of honey (Barth 1989), since the composition of the bee pollen differs according to the region or season, indicating patterns and variations of the local flora (Barth 2004). In the northeast of the Rio Grande do Sul State, in the Araucaria Forest ecosystem, *Apis mellifera* produces characteristic honey, called white honey, which has a very particular floral flavor and odor (Cambará do Sul 2020). Stingless bees also produce this "white honey", but in both cases, the botanical origin and potential bee species that produce the white honey, considered a local delicacy, remain poorly understood.

The aim of this study was to identify the floral resources used by stingless bees in the production of white honey in the Araucaria Forest of the Rio Grande do Sul State. The results of this study will benefit regional meliponiculturists, adding value to the honey by providing knowledge of its botanical origin, and guiding hive management in the areas with the flowers detected in this melissopalynological analysis.

Material and Methods

1. *Area of study*

The study was performed in an area of 250 ha located in the municipality of Cambará do Sul (29°02' 51S" 50°08' 40W"), Northeast region of Rio Grande do Sul state, Brazil, with altitudes ranging from 980 to 1047 m (Figure 1). The climate of the region is predominantly Cfb (Köppen classification), temperate humid (Boldrini 2009). Mixed Ombrophilous Forest is characterized by a rich floristic mixture and physiognomy, evident by the presence of *Araucaria angustifolia* (Brazilian pine) of in the upper canopy and with a species-rich forest understory (Veloso et al. 1991, Jarenkow & Budke 2009).

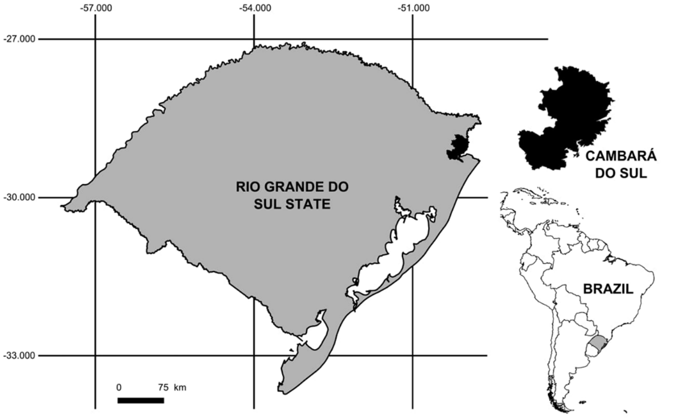

Figure 1. Municipality of Cambará do Sul, RS, Brazil, site of white honey production.

2. *Plant collection and pollen preparation*

In 2008, during the region's peak white honey production period (January to March), flowering plants were collected and the exsiccates prepared to identify the botanical species as potential food sources for bees. Flower buds were packed in paper envelopes to prepare the regional pollen reference collection for comparison with pollen types collected in bee honey samples. Collections were carried out fortnightly along two 2 km trails, both in rural areas and forest edges.

Plants were identified by experts and deposited in the herbarium of the Department of Diagnosis and Agricultural Research (DDPA) of the Secretariat for Agriculture, Livestock and Rural Development of Rio Grande do Sul and the Herbarium of the Pontifical Catholic University of Rio Grande do Sul (PUCRS). The reference slides were prepared by removing the pollen grains from the anthers according to protocol presented by Silva et al. (2014) and subsequently submitted to the acetolysis method (Erdtman, 1960). Three slides were assembled from each plant sample, which was cataloged and deposited in the Museum of Entomology of the Department of Diagnosis and Agricultural Research (DDPA) of the Secretariat for Agriculture, Livestock and Rural Development of Rio Grande do Sul and duplicate of these slides in the Palynological Laboratory of FFCLRP-USP (Faculty of Philosophy, Sciences and Letters at Ribeirão Preto, University of São Paulo). Images of each pollen type were obtained with a camera attached to the optical microscope Leica DM4000B that allowed an increase of up to 2560x.

3. *Honey harvest*

In 2008 and 2009, from January to March, thirty-three samples of honey were obtained from nine (9) Meliponini hives of five species: *Melipona bicolor schencki* Gribodo, 1893 (n = 4), *Melipona torrida* Friese, 1916 (n = 2), *Melipona quadrifasciata quadrifasciata* Lepeletier, 1836 (n = 1), *Plebeia remota* (Holmberg, 1903) (n = 1) and *Plebeia* *emerina* (Friese, 1900) (n = 1). From these species, nineteen (19) samples were obtained from the hives of *Melipona bicolor schencki*, eight (8) from *Melipona torrida*, two (2) from *Melipona quadrifasciata quadrifasciata*, two (2) from *Plebeia remota* and two (2) from *Plebeia emerina*. The honey was collected with the aid of disposable pippetes.

4. *Pollen analyses*

Pollen types present in the honey samples were identified by morphological comparison to the reference collection of the Palynological Laboratory of FFCLRP-USP. Honey samples were prepared firstly according to the methodology described in Rezende et al. (2020), where 10 ml of each honey sample was diluted in 20 ml of distilled water and centrifuged to separate pollen grains. Afterward, the samples were submitted to the acetolysis method and pollen grains were mounted using glycerinated gelatin on sets of three slides sealed with paraffin (Kisser 1935). For the quantitative analysis, 400 pollen grains per sample were counted and were grouped according to the frequency class definition following the adaption by Santos et al. (2012) of the methodology in of Louveaux et al. (1978): predominant pollen (> 45%); secondary pollen (≤ 45% to >15%); important minor pollen (≤ 15% to ≥3%); and minor pollen (< 3%). These analyses were carried in the Palynological Laboratory of FFCLRP-USP.

Results

During the white honey production period, 19 flowering plant species belonging to 12 botanical families were registered. Myrtaceae showed the highest richness (five taxa), followed by Asteraceae (three taxa) and Cunoniaceae (two taxa). However, only pollen grains from six (6) of these families were detected in honey samples. Nonetheless, pollen grains from a further nine (9) botanical families not detected in plant surveys were also found in honey samples (Table 1).

In relation to the samples of white honey, 36 pollen types were detected and distributed among 15 plant families (Table 1). The family Myrtaceae (10) was the richest in pollen types followed by Fabaceae (5), Arecaceae (3), Melastomataceae (3) and Solanaceae (3).

The predominant pollen type (DP) in all honey samples analyzed belonged to the Clethraceae family, with only one pollen type, *Clethra scabra* (Figure 2) with percentages above 45%. The size of the pollen grain (Figure 3) of this plant is classified as very small (<10 μm) and its occurrence in honey indicates a strong nectar collection from its flowers. This honey is colorless white or cloudy white to amber when crystallized. Although in *Melipona quadrifasciata quadrifasciata* honey the proportion of *Clethra scabra* pollen (46.5%) was lower than Fabaceae pollen (47.37%), both families are categorized as predominant pollen (Figure 2).

Myrtaceae was distinguished as important secondary pollen (IIP) in *Melipona torrida* and *Melipona bicolor schencki* honey, and Cunoniaceae as important minor pollen (AP) in the samples of *Plebeia remota* and as important minor pollen (IIP) in the samples of *Plebeia emerina* (Figure 2). Another botanical family with important participation in *Melipona torrida* and *Melipona quadrifasciata quadrifasciata* honey was Melastomataceae (Figure 2).

Although *Melipona bicolor schencki* honey presented 22 pollen types, Clethraceae and Myrtaceae together represent 98.5% of the pollen samples. In *Melipona torrida* honey, the same number of pollen types was found (22) but the contribution of other families was higher, as well as in *Melipona quadrifasciata quadrifasciata* honey (Table 2).

The bee species most faithful to *Clethra scabra* flowers was *Melipona bicolor schencki* during the two years of study (Figure 4). Species of *Plebeia* used a higher percentage of *Clethra scabra* in 2008 and *Melipona torrida* in 2009 (Figure 4). Honey samples from *Melipona quadrifasciata quadrifasciata* were collected only in 2008.

Discussion

Clethra scabra pollen was numerically predominant in all honey samples of the five Meliponini species analyzed, except *M. quadrifasciata*. This species blooms during January and February and, according to Ramalho (2004), the pollen and nectar of its flowers were used by bees as food resources, thus confirming its strong melitophile character. Honey samples from *Melipona bicolor schencki* contained the highest proportion of *C. scabra* pollen. Despite the importance of *Clethra scabra* as a food resource for bees in the summer, other plant families, including Myrtaceae, Fabaceae and Melastomataceae also represented important food resources for the three species of *Melipona*, while Cunoniaceae was mainly used by the two species of *Plebeia* to meet colony nutritional demands. The number of pollen grains of plants with poricidal anthers, such as the Clethraceae and Melastomataceae, could be an effect of intranidal contamination. In the discussion presented by Maia-Silva et al. (2020), the authors mentioned that *Melipona* nests usually present fewer pollen storage pots than honey pots, and that during the mass flowering the bees foraging activity suddenly increases. Then, pollen foragers may not find appropriate storage space, and so deposit their load in any available pots. This behavior seems to be normal to *Melipona* since it has observed several species occurring in different regions (Alves et al., 2012; Nascimento and Nascimento 2012; Maia-Silva et al. 2014, 2016).

Lopes (2012) studied the floristic composition and floral phenology in the same area of study and identified 60 species of flowering plants from January to March and observed that Asteraceae was the richest family by number of species, followed by Myrtaceae, Fabaceae and Melastomataceae. For *Melipona* species, the importance of Myrtaceae, Fabaceae and Melastomataceae seems to be related to the richness of flowering species during this period. However, in the present study, Meliponini showed a clear preference for *Clethra scabra*, that besides providing pollen, also produces a high volume of nectar, the most attractive resource for the Meliponini bees.

One possible factor which could explain the preference of *Melipona* species for flowers of *Clethra scabra* is the presence of poricidal anthers, characteristic of this genus (Rossi 1992). Flowers with these characteristics need agents of pollination capable of vibrating the anthers to remove pollen, and *Melipona* exhibits this behavior (Ramalho et al. 1989). Pollen grains from other species with poricidal anthers as of Melastomataceae were also found in the honey produced by *Melipona* bees (Table 1).

Studies performed in the Atlantic Forest in Rio Grande do Sul, in regions close to the studied area, did not mention visits to flowers of *Clethra scabra* by *Melipona bicolor schencki* (Wilms & Wiechers 1997) and *Melipona torrida* (Hilgert-Moreira et al. 2014). There was also no record of production of the characteristic white honey. Meliponini exhibit high levels of floral constancy, where foraging individuals

Table 1. Flowering plants detected in the white honey production period (January-March 2008, 2009) and pollen types present in stingless bee honey, Cambará do Sul, RS, Brazil.

Flowering plant detected during the period of white honey production		*Pollen Type present in white honey*	
		Amaranthaceae	*Chamissoa* sp.
		Anacardiaceae	Anacardiaceae sp. 1
			Anacardiaceae sp. 2
*Arecaceae	*Butia capitata* (Mart.)Becc	*Arecaceae	Arecaceae sp. 1
			Arecaceae sp. 2
			Arecaceae sp. 3
*Asteraceae	*Gochnatia polymorpha* (Less.) Cabrera subsp. *Floccosa Cabrera*	*Asteraceae	Asteraceae sp. 3
	Vernonia sp.		
	Baccharis anomala DC.		
		Cecropiaceae	*Cecropia* sp.
*Clethraceae	*Clethra scabra* Pers.	*Clethraceae	*C. scabra*
*Cunoniaceae	*Lamanonia ternata* Vell.	*Cunoniaceae	*L. ternata*
	Weinmania paulliniifolia Pohl ex Ser.		*W. paulliniifolia*
Escalloniaceae	*Escallonia bifida* Link & Otto		
		Euphorbiaceae	*Croton* sp.
Loranthaceae	*Tripodanthus acutifolius* (Ruiz & Pav.)		
*Melastomataceae	*Tibouchina* sp.	*Melastomataceae	Melastomataceae sp. 1
			Melastomataceae sp. 2
			Melastomataceae sp. 3
		Fabaceae	*Mimosa* sp. *1*
			Mimosa sp. *2*
			Mimosa sp. *3*
			Mimosa sp. *4*
			Mimosa sp. *5*
*Myrtaceae	*Eucalyptus viminalis* Labill.	*Myrtaceae	*Eucalyptus* sp. *1*
	Eugenia pyriformis Cambess.		*Eucalyptus* sp. *2*
	Myrcia oligantha O. Berg.		*Eucalyptus* sp. 3
	Myrceugenia oxysepala (Burr.) Legr. & Kaus		Myrtaceae sp. 1
	Siphoneugena reitzii D. Legrand		Myrtaceae sp. 3
			Myrtaceae sp. 4
			Myrtaceae sp. 5
			Myrtaceae sp. 6
			Myrtaceae sp. 7
			Myrtaceae sp. 8
		Nyctaginaceae	*Boungaville* sp.
Oleaceae	*Ligustrum lucidum* W. T. Aiton		
		Poaceae	Poaceae
		Portulacaceae	*Talinum* sp.
Rubiaceae	*Borreria capitata* (Ruiz & Pav.) DC.		
		Solanaceae	*Solanum* sp. 1
			Solanum sp. 2
			Solanum sp. 3
Styracaceae	*Styrax leprosus* Hook. & Arn.		
Winteraceae	*Drimys brasiliensis* Miers.		

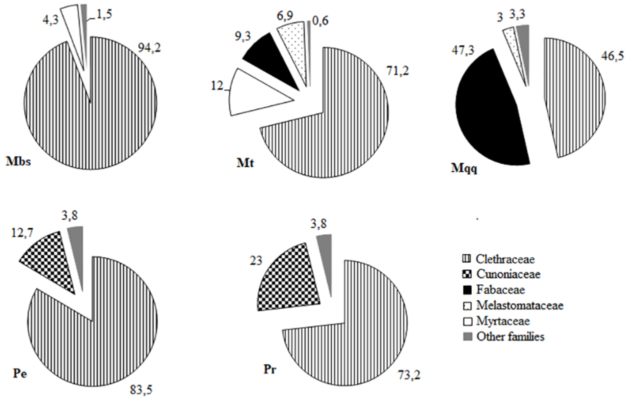

Figure 2. Average percentage of pollen types of the botanical families present in samples of Meliponini honey obtained in Cambará do Sul, RS, Brazil, in 2008/2009. *Melipona bicolor schencki* (Mbs); *Melipona torrida* (Mt); *Melipona quadrifasciata quadrifasciata* (Mqq); *Plebeia emerina* (Pe); *Plebeia remota* (Pr).

Figure 3. *Clethra scabra* pollen grains in polar (left) and equatorial (right) views.

visit only one type of flower during the same foraging trip (Ramalho et al. 1994).

White honey is referred to as a rare product with particular floral flavor and odor that makes it a delicacy in the study region. The high percentage of pollen grains of *Clethra scabra* signifies that this Meliponini honey can be classified as unifloral or monofloral.

This study provides empirical evidence for the processes of geographical indication and Denomination of Origin (DO) to white honey from Cambará do Sul/RS. Geographical indications are collective tools for valuing traditional products linked to certain territories. They have two functions: to add value to the product and to protect the producing region. The DO defines that a particular area has a product whose qualities have exclusive or essential influence because of the characteristics of that place, including natural and human factors (Giesbrecht et al. 2011).

The valuation of white honey of *Clethra scabra* and, consequently, the bee species associated with its production constitute an opportunity for its conservation, since the plant and some bee species that produce this honey, are on the list of endangered species of Rio Grande do Sul (Fundação Zoobotânica 2012).

Conclusions

- *Clethra scabra* was the dominant plant species in all honey samples confirming the indication of this plant to beekeepers for the white honey production;
- The high percentage of *Clethra scabra* pollen grains characterize the honey analyzed as unifloral;
- *Melipona bicolor schencki* was the most specialized stingless bee species in the collection of *Clethra scabra*.

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Author Contributions

Sidia Witter: Substantial contribution to the conception and design of the work; Contribution to data acquisition; Contribution to data analysis and interpretation; Contribution to the writing of the work; Contribution to critical review by adding intellectual content.

Letícia Azambuja Lopes: Substantial contribution to the conception and design of the work; Contribution to data acquisition; Contribution to data analysis and interpretation; Contribution to the writing of the work; Contribution to critical review by adding intellectual content.

Cláudia Inês Silva: Substantial contribution to the conception and design of the work; Contribution to data acquisition; Contribution to data analysis and interpretation; Identification of the botanical origin - pollen morphology description; Contribution to the writing of the work; Contribution to critical review by adding intellectual content.

Bruno Brito Lisboa: Substantial contribution to the conception and design of the work; Contribution to data acquisition; Contribution to data analysis and interpretation; Contribution to the writing of the work; Contribution to critical review by adding intellectual content.

Vera Lucia Imperatriz-Fonseca: Substantial contribution to the conception and design of the work; Contribution to critical review by adding intellectual content.

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Cláudio Augusto Mondin: Substantial contribution to the conception and design of the work; Contribution to data acquisition; Contribution to data analysis and interpretation; Contribution to the writing of the work; Contribution to critical review by adding intellectual content.

Table 2. Relative frequency (%) of pollen types in stingless bees honey, in Cambará do Sul, RS, Brazil, in 2008/2009. Pollen frequency (PF); Species / pollen types (PT); *Melipona bicolor schencki* (Mbs); *Melipona torrida* (Mt); *Melipona quadrifasciata quadrifasciata* (Mqq); *Plebeia emerina* (Pe); *Plebeia remota* (Pr).

	Mbs		**Mt**		**Mq**		**Pe**		**Pr**	
	PF	**PT**	**PF**	**PT**	**PF**	**PT**	**PF**	**PT**	**PF**	**PT**
Amaranthaceae	0	0	0	0	0	0	1	1	0	0
Anacardiaceae	0,2	2	0,04	1	0	0	1	1	0,6	1
Arecaceae	0	0	0,1	2	0,5	1	0,3	1	0,25	1
Asteraceae	0	0	0	0	0	0	0	0	0,1	1
Cecropiaceae	0	0	0,1	1	0,5	1	0	0	0	0
Clethraceae	94,2	1	71,2	1	46,5	1	83,5	1	73,2	1
Cunoniaceae	0,39	2	0,3	2	0,1	1	12,7	1	23	2
Euphorbiaceae	0	0	0	0	0	0	0,1	1	0	0
Fabaceae	0,1	1	9,3	5	47,3	5	0,5	1	0,5	1
Melastomataceae	0,7	3	6,9	2	3	2	0	0	0	0
Myrtaceae	4,3	9	12	6	2	4	0,1	1	0,75	1
Nyctaginaceae	0	0	0	0	0	0	0,5	1	0,75	1
Poaceae	0	0	0	0	0	0	0,2	1	0,1	1
Portulaceae	0	0	0,02	1	0,1	1	1	0	0	0
Solanaceae	0,1	3	0,04	1	0	0	0	0	0,75	0
Undetermined	0,01	1	0	0	0	0	0,1	1	0	0
Total Pollen Types		**22**		**22**		**16**		**11**		**10**

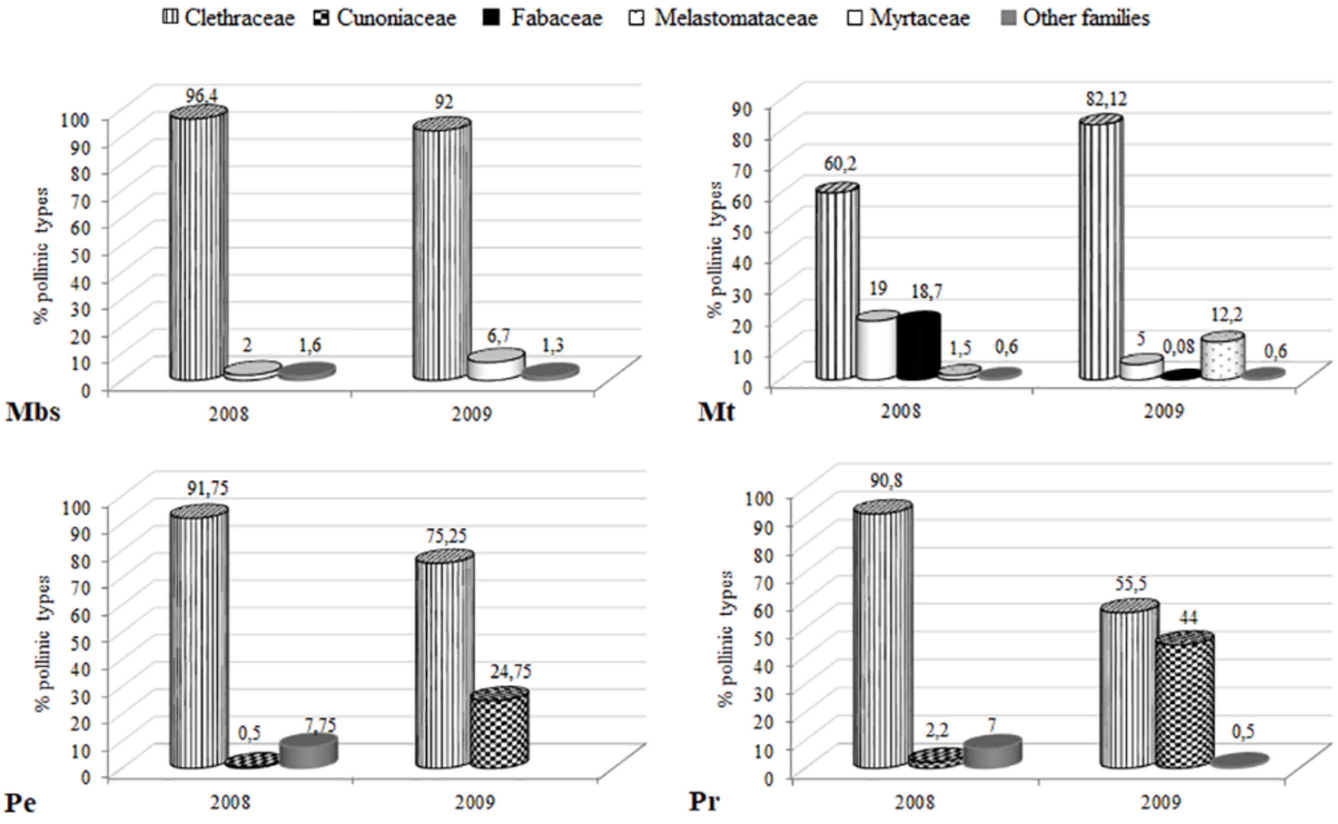

Figure 4. Proportion of botanical families of the pollen grains present in honey of stingless bees *Melipona bicolor schencki* (Mbs); *Melipona torrida* (Mt); *Plebeia emerina* (Pe); *Plebeia remota* (Pr), in Cambará do Sul, RS, Brazil, in 2008 and 2009.

Conflicts of interest

The authors declare no competing interests.

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Article

Foraging preferences of the native stingless bee *Melipona seminigra pernigra* (Apidae: Meliponini) in campo rupestre on canga of Serra dos Carajás, southeastern Amazonia

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Abstract: Honey pollen samples of *Melipona seminigra pernigra* Moure & Kerr 1950 sampled between 2017 and 2019 from experimental apiaries installed in campo rupestre on canga (CRC) vegetation of the Serra dos Carajás aimed to evaluated seasonal floral availability of undisturbed and mining-influenced areas. Around one hundred pollen types were identified mainly belonging to Fabaceae, Myrtaceae and Euphorbiaceae (31, 6 and 5 species, respectively). Mining area presented the highest pollen richness, almost twice those identified in the undisturbed areas. 80% of the pollen types are rare with concentrations ≤ 2,000 pollen grains/10 g, while the remaining were the most abundant, frequent and the primary bee sources. These latter correspond mostly to native plants species such as *Tapirira guianensis* Aubl., *Protium* spp., *Aparisthmium cordatum* (A.Juss.) Baill., *Mimosa acutistipula* var. ferrea Barneby, *Periandra mediterranea* (Vell.) Taub., *Miconia* spp., *Pleroma carajasense* K.Rocha, *Myrcia splendens* (Sw.) DC., *Serjania* spp. and *Solanum crinitum* Lam. All pollen types were identified during both seasons, but higher concentration values are related to the dry period (June-September). The statistical analysis of the pollen data indicated that there was no significant difference between undisturbed and mining-influenced areas, since primary bee sources of this study are widespread used in revegetation of mined areas.

Keywords: *Stingless bees; Honey; Pollen; Amazonia; Iron mining.*

Preferências de forrageamento da abelha nativa sem ferrão *Melipona seminigra pernigra* (Apidae: Meliponini) em campo rupestre na canga da Serra dos Carajás, sudeste da Amazônia

Resumo: O conteúdo polínico de amostras de mel coletadas nos anos de 2017 e 2019 de apiários experimentais de *Melipona seminigra pernigra* Moure & Kerr 1950, instalado dentro de uma vegetação de campo rupestre em um afloramento de canga na Serra dos Carajás, sudeste da Amazônia, foi analisado para entender a variabilidade local dos recursos florais em áreas naturais e perturbadas. Aproximadamente 100% dos tipos polínicos foram identificados e pertencem principalmente às famílias Fabaceae, Myrtaceae e Euphorbiaceae (31, 6 e 5 espécies, respectivamente). Áreas de mineração apresentaram a maior riqueza de pólen, quase o dobro daquelas identificadas em áreas perturbadas. 80% dos tipos de pólen são raros com concentrações ≤ 2.000 grãos de pólen/10g, enquanto que os restantes foram os mais abundantes, frequentes e fontes primárias para as abelhas. Este últimos correspondem principalmente a plantas nativas como *Tapirira guianensis* Aubl., *Protium* spp., *Aparisthmium cordatum* (A.Juss.) Baill., *Mimosa acutistipula* var. *ferrea* Barneby, *Periandra mediterrânea* (Vell.) Taub., *Miconia* spp., *Pleroma carajasense* K.Rocha, *Myrcia splendens* (Sw.) DC., *Serjania* spp. e *Solanum crinitum* Lam. Todos os tipos polínicos foram identificados durante ambas as estações, mas altas concentrações estão relacionadas ao período seco (junho-setembro). A análise estatística indicou que não houve diferença significativa nos dados de pólen de mel entre áreas naturais e áreas anteriormente degradadas, uma vez que as fontes primárias das abelhas deste estudo são amplamente utilizadas na revegetação de áreas mineradas..

Palavras-chave: *Abelhas sem ferrão; Mel; Pólen; Amazônia; Mineração de ferro.*

Introduction

Melissopalynological studies of stingless bees are important to identify the sources of nectar explored by bees and, through this, improve the management of colonies and landscape for a better honey production in both aspects: quality and quantity. Moreover, it can potentially be used to characterize the local flora regarding its diversity and phenology (Absy et al. 1984, Absy & Kerr 1977; Martins et al. 2011). The use of this technique for identifying nectar resources used by social, native stingless bees – Meliponini tribe on Brazil dates from 1960, according to an historical review (Barth 2004; Freitas & Novais 2014; Souza et al. 2018). Some of them have been developed specifically in the Amazon region, in natural and disturbed areas, to identify the origin of pollen in loads carried by forager bees or in the stored honey and beebread (Absy et al. 1980, Marques-Souza 1996, Marques-Souza et al. 1996, Rezende et al. 2019). These are important contributions to understand the floral resources collected by bees, especially considering the high diversity of stingless bees on Brazil and the particularities of each biome. It is also key to practical applications on sustainable development practices for rural communities (meliponiculture and honey production) and/or for the design of restoration strategies (Montoya et al. 2012). Some melissopalynological studies on humid evergreen tropical forest (HETF) areas of this region have shown strong preferences of Meliponini species by *Tapirira guianensis* Aubl. (Anacardiaceae), *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) and *Mimosa pudica* L. (Fabaceae) (Online Resource 1; Absy & Kerr 1977; Oliveira et al. 2009; Rezende et al. 2019). These taxa widespread occur on most of Brazilian phytogeographic domains such as Amazon Rainforest, Caatinga, Central Brazilian Savanna and Atlantic Rainforest (BFG 2015). As a consequence, the distribution of Meliponini species tend to follow the same domains (Camargo & Pedro 2013).

Knowledge regarding bee flora in plant endemism hotspots (Kasecker et al. 2009), such as *campo rupestre* on *canga* at the Serra dos Carajás (Mota et al. 2018), are still incipient (Barth 1987). This particular type of open vegetation consists mostly of herbs and shrubs adapted to the edaphic conditions provided by iron-rich outcrops (Mota et al. 2018) surrounded mostly by evergreen tropical forest. Some Amazonian stingless bees are also poorly known, as is the case with *Melipona seminigra pernigra* Moure & Kerr 1950. This native, abundant species in eastern Amazonia (Moure & Kerr 1950) is considered as one of the most popular species reared by traditional populations for honey production (Nogueira-Neto 1997; Cortopassi-Laurino et al. 2006), as seen also in the Carajás region.

Our aim is to understand the local variability of floral resources utilized by *M. seminigra pernigra* in natural and disturbed areas. For this, we kept colonies of this stingless bee species in experimental meliponaries on *canga*, forest and disturbed areas in the region of Carajás and analyzed their honey samples through melissopalynology. This is an important step for the conservation of bee species and their landscape, providing knowledge about plant species used for honey production and also to help on degraded land restoration strategies in the Carajás region.

1. *Study area*

The Carajás National Forest, a Federal Conservation Unit created in February 1998 comprises an area of approximately 3,930 km^2 (Figure 1a). Represented by discontinuous table-top outcrops surrounded by a matrix of humid evergreen tropical forest (HETF) and seasonally dry forest (SDF). The higher areas (600-800 m a.s.l.) are occupied by lateritic plateaus with *campo rupestre* on *canga* (CRC) vegetation (Mota et al. 2018). This mineral province includes one of the largest iron ore deposits worldwide (Tolbert et al. 1971) and mining operations in the region began in 1985. The group of northern plateaus are known as Serra Norte, where two main areas are being exploited, namely N4 and N5 mines.

The rainfall regime is characterized by a rainy season, regionally known as Amazonian winter from November to May, followed by a dry Amazonian summer between June and October (Lopes et al. 2013). The total annual rainfall of the Carajás region may vary between 1,545 mm and 1,863 mm during the rainy season, with the dry season amounting to values between 159 mm and 321 mm (Silva Júnior et al. 2017). The mean temperature is 27.2°C, with a minimum annual temperature of 26.6°C in January and a maximum annual temperature of 28.1°C in September (Tavares et al. 2018).

Materials and Methods

Four experimental meliponaries were installed in natural (area 1: Trilha da Lagoa da Mata) and disturbed (area 2: Viveiro Florestal; area 3: revegetation of degraded areas – RDA; area 4: N5 mine) areas of Serra Norte (Figure 1a). The area 1 is located in the northeast portion of the N5 mine, Serra Norte. It presents a narrow CRC area of around 33,000 m^2 surrounded by HETF (Figure 1b). The area 2 is a plant nursery area for growing species of the Carajás flora to provide plants for the revegetation of degraded areas (RDA), and it is located ~ 1.2 km southeast of area 1, covering an area of 26,500 m^2 surrounded by HETF and weedy plants; area 3 is close to the N5 mine and located 8.7 km southwest of area 1, and it consists of HETF and SDF towards the southern part of this area (Figure 1c); area 4 is a pit slope in RDA process mainly covered by CRC and weedy plants, and it is located 5.5 km southwest of area 1 and 3.4 km distant of area 3. The main plant species in the four areas are given in Online Resource 2 (Vale-Golder Associates 2011), updated according Viana et al. (2016). *Melipona seminigra* forage range is around 1 km (L. Costa, personal communication), a flight ability similar to that of *Melipona mandacaia* Smith (Kuhn-Neto et al. 2009), so it is unlikely that foraging activity overlapped between areas, with exception to areas 1 and 2.

Due to the expansion dynamics of the N5 mine, the meliponary installed in this area (3) was moved in March 2018 to another location in the same area, while the RDA apiary (4) started in June 2018. Monthly samples were collected from the honey pots of fourteen hives of *Melipona seminigra pernigra* from each apiary. The content, from five to ten honey pots (chosen randomly) was removed during each collection event (Figure 1d, e). 46 samples covering a period of 16 months (September 2017 to January 2019) were harvested.

Honey samples weighing 10 g were diluted using 20 ml of distilled water according to Maurizio & Louveaux et al. (1965). For each sample, a tablet containing 9,666 spores of *Lycopodium clavatum* was added (Stockmarr 1971). These samples were acetolysed following Erdtman (1952), and mounted on microscope slides using glycerin jelly. They were deposited in the pollen library at Instituto Tecnológico Vale (PaliITV, Belém, Brazil). Five hundred pollen grains were counted per sample using a Zeiss Axio Imager M2. Pollen type identification was based on morphological comparisons with PaliITV.

The distribution frequency of the pollen types followed Feller-Demalsy et al. (1987): very frequent (>50%), frequent (20–50%), infrequent (10–20%) and rare (<10%); and groups of pollen concentration according to Maurizio (1975): Group I (<20,000), Group II (20,000–100,000), Group III (100,000–500,000), Group IV (500,000–1,000,000) and Group V (>1,000,000) were adopted to classify pollen types per honey sample. The range of the pollen types per honey sample was calculated using the Shannon–Weaver diversity index (H´; Ludwig and Reynolds 1988). Pielou's evenness index (J'), was calculated to evaluate the degree of heterogeneity (0) or homogeneity (1) of pollen resources (Pielou 1977). Product moment correlation coefficient (PMCC) was applied between pollen richness, H' and J' data.

For statistical analysis, the total pollen concentration (grains/cm^3 unit) of four areas (Trilha da Lagoa da Mata, Viveiro Florestal, N5 Mine and RDA) were counted based on the definition of the seasonal rainfall pattern.

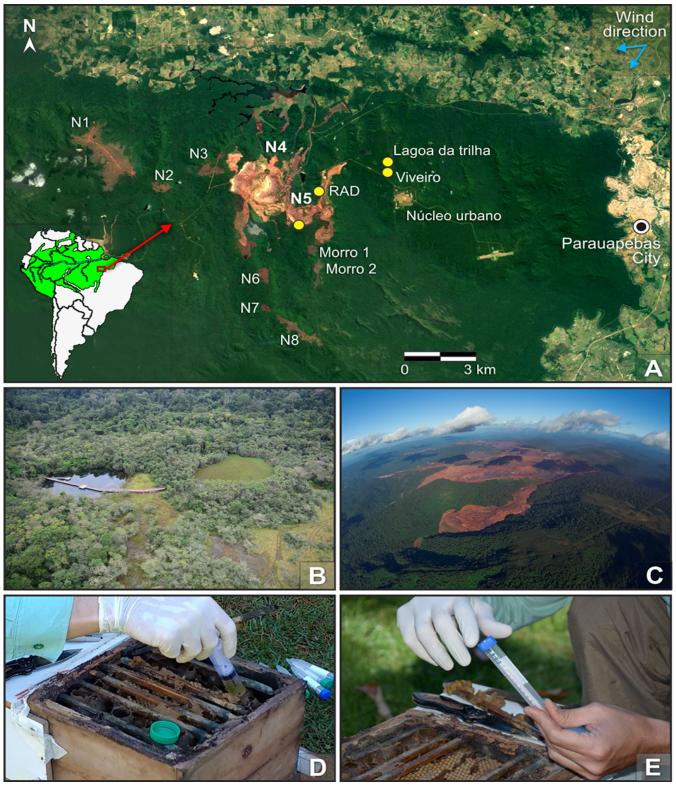

Figure 1. (a) Study site inserted in the northern portion of the Carajás National Forest, southeast Amazonia (N1 to N8 are related mining areas codes). (b) Aerial photography of the Trilha da Lagoa da Mata (small area of canga vegetation surrounded by HETF and SDF. (b) Aerial photography showing the N4 and N5 mining areas surround by canga vegetation (open areas in the right side) and (c) HETF and SDF. (d-e) sampling the honey pots.

The descriptive analyses were performed using common and abundant taxa among sites, and Wilcoxon test for significance difference of each site between periods. Bray-Curtis based non-metric multidimensional scaling (NMDS) has been applied to evaluate species distribution and their relationship between the sites. The NMDS analysis required the vegan R package (version 2.5.3; Oksanen 2016), and visualization were carried out using the ggplot2 package (version 3.4.1; Wickham 2009)

Climatological data covering the collection years were obtained from the meteorological station of Serra Norte de Carajás (OMM: 81860), which covers all studied areas.

Results

1. *Climatological data*

The patterns of wind intensity and direction in the study site from January 2017 to January 2019 show slight variation of wind speed (~ 1 to 2 m/s) with prevailing direction from the E and ENE (Figure 1a). The mean minimum and maximum air temperature for this period was 23.8 ºC and 27.7 ºC, respectively, with the highest values in August to September, while the lowest temperatures occurred in February and July (Figure 2a). Based on the accumulated monthly rainfall (Figure 2a), the dry period extended from June to September, ranging from 67 to 132 mm. The rainy period comprised October to May, with values ranging from 1805 to 2300 mm. The total annual precipitation during 2017 was lower than that for 2018 with values of 1935 and 2367 mm, respectively. The wettest month of the study period was February 2019 (approx. 700 mm) Relative humidity follows the seasonal rainfall pattern, with the lowest values (< 80%) observed during in the dry period. Highest solar irradiance was detected in July with a mean of ~ 220 W/m^2, while the lowest occurred in December, with ~ 140 W/m^2 (Figure 2b).

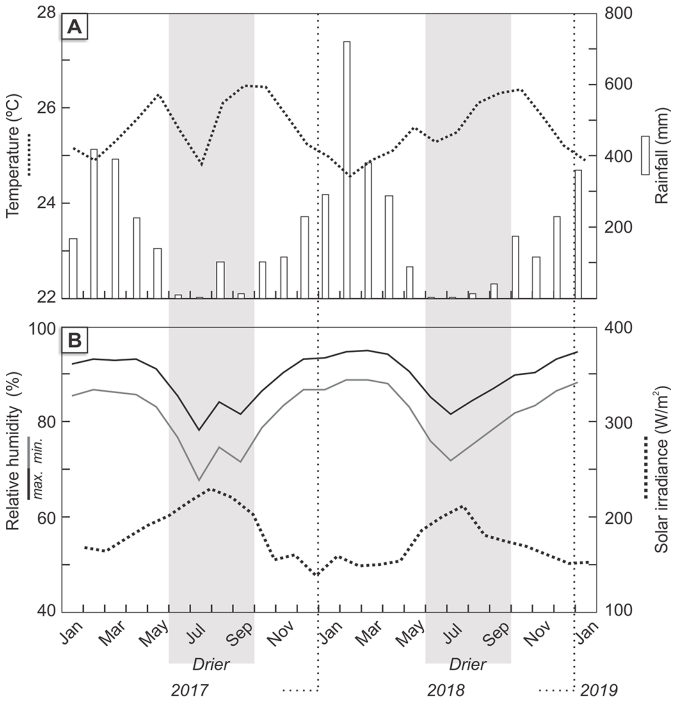

Figure 2. (a) Accumulated monthly rainfall data (mm; right side) and mean air temperature (ºC; left side) at the Carajás Meteorological Station from January 2017 to January 2019. (b) Monthly mean solar irradiance (W/m2; right side) and Maximum and minimum relative humidity (%; left side). Gray bands indicate the drier climate periods.

2. *Pollen assemblage*

A total of 104 pollen types were identified in the 46 honey samples analyzed, belonging to 35 families. Fabaceae, Myrtaceae and Euphorbiaceae were the most representative with 31, 6 and 5 species, respectively (Online Resource 3).

In general, the N5 mine presented the highest pollen richness with 95 pollen types identified. Trilha da Lagoa da Mata and Viveiro Florestal presented around 60 pollen types each, while RDA had 39 types. Maximum pollen concentration per area ranged from 60,895 to 1,521,194 pollen grains/g, which were observed, respectively, in RDA and Viveiro Florestal areas. 80% of the pollen types were found with values < 5,000 grains/10g and < 1%. Thus, the pollen types are generally rare (< 10%; Feller-Demalsy et al. 1987) and belong to group I (≤ 20,000 pollen grains/10 g; Maurizio, 1975) (see Online Resource 3; Online Resource 4).

The remaining 20% of the pollen types are the most abundant and present in all areas along the studied period (Online Resource 3; Online Resource 5, Online Resource 6, Online Resource 7, Online Resource 8). These types were identified as *Tapirira guianensis* (Anacardiaceae), *Protium* (Burseraceae), *Aparisthmium cordatum* and *Alchornea* (Euphorbiaceae), *Mimosa acutistipula* var. *ferrea* and *Periandra mediterranea* (Fabaceae), *Miconia* and *Pleroma carajasense* (Melastomataceae), *Myrcia splendens* (Myrtaceae), *Serjania* (Sapindaceae) and *Solanum crinitum* (Solanaceae) (Figure 3, Figure 4) abundances varied according to the year of study.

Significantly higher pollen concentration was detected on 2018 (Online Resource 5, Online Resource 6, Online Resource 7, Online Resource 8). Thus, for the Trilha da Lagoa da Mata, pollen types of *P. carajasense*, *Miconia*, *Alchornea* and *M. acutistipula* var. *ferrea* abounded in March and April, while *Protium* and *T. guianensis* in August and September (Online Resource 5).

Protium and *T. guianensis* also abounded in June and July in the Viveiro Florestal, where *A. cordatum* was very representative in October to December (Online Resource 6). *Protium* was abundant in June and November in the RDA area (Online Resource 8).

The pollen types *M. splendens* and *M. acutistipula* var. *ferrea* were significantly present from September to December 2018, but not during the same period of the previous year in N5 mine.

According to the mean values of pollen concentration of the most abundant pollen types during the dry and rainy seasons, the pollen of all plant species was identified during both seasons, however in variable abundance. Higher values are generally related to the dry period (June-September). *M. acutistipula* var. *ferrea* is more abundant at the Viveiro Florestal and N5 mine in the dry and wet season, respectively. *P. carajasense* abounded during the wet season in the Trilha da Lagoa da Mata, while it was remarkably important in the dry season in the Viveiro Florestal (Figure 5).

The Shannon-Weaver diversity (H') and Pielou's evenness (J') indexes have strong PMCC (ρ-value ~ 0.7 to 0.9), and moderate to strong correlation with pollen richness (ρ-value ~ 0.5 to 0.9) in each area (Figure 6). Thus, higher H' and J' were related to 2017, while lower values were mainly found during June and July 2018.

3. *Statistical analyses*

Considering the statistical analysis, the overall mean of pollen concentration of all sites shows an overlap of pollen concentration distribution (Figure 7a). RDA samples had the lowest concentration

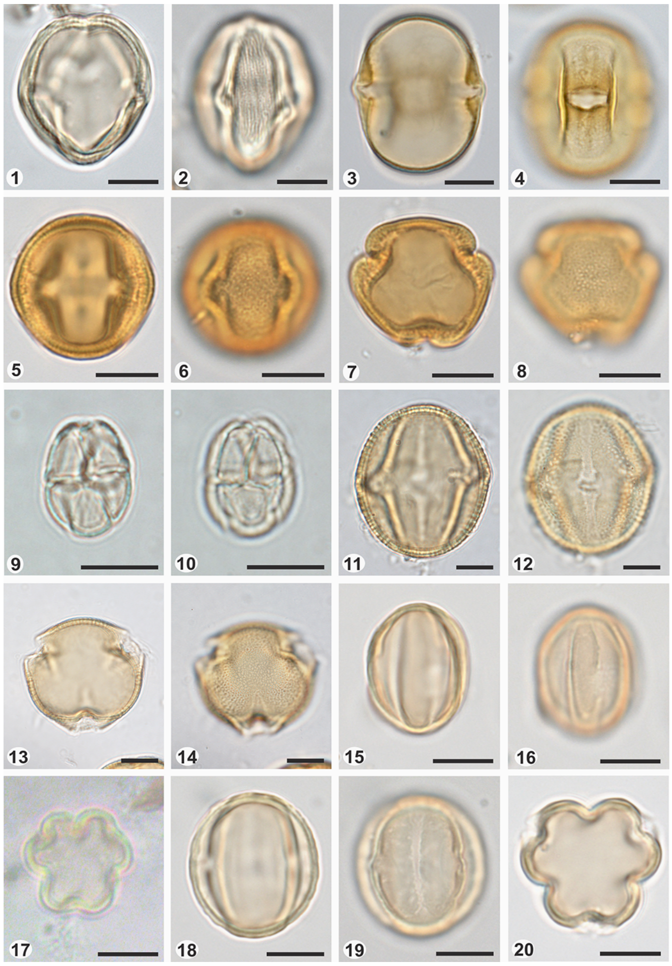

Figure 3. Optical microscopy photomicrographs of the most abundant and frequent pollen types identified in this study. *Tapirira guianensis* (1, 2); *Protium* (3, 4), *Aparisthmium cordatum* (5, 6) and *Alchornea* (7, 8), *Mimosa acutistipula* var. ferrea (9, 10) and *Periandra mediterranea* (11-14), *Miconia* (17, 18) and *Pleroma carajasense* (19, 20). Scale bars: 10 µm.

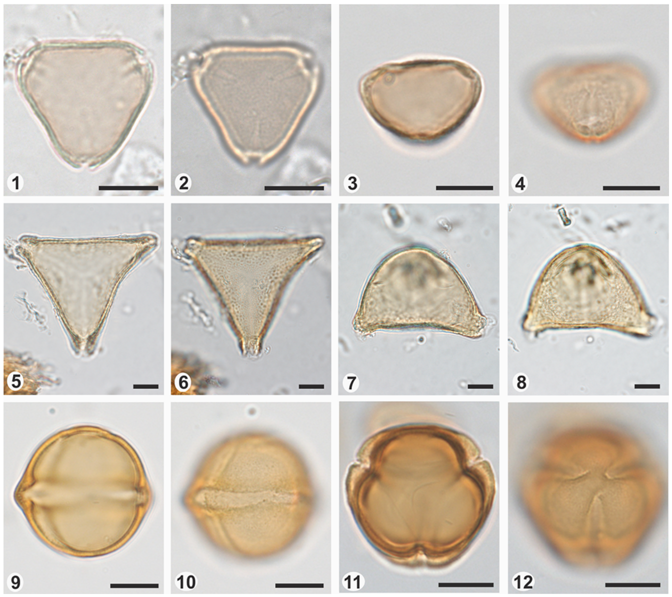

Figure 4. Optical microscopy photomicrographs of the most abundant and frequent pollen types identified in this study. *Myrcia splendens* (1-4), *Serjania* (5-8) and *Solanum crinitum* (9-12). Scale bars: 10 µm.

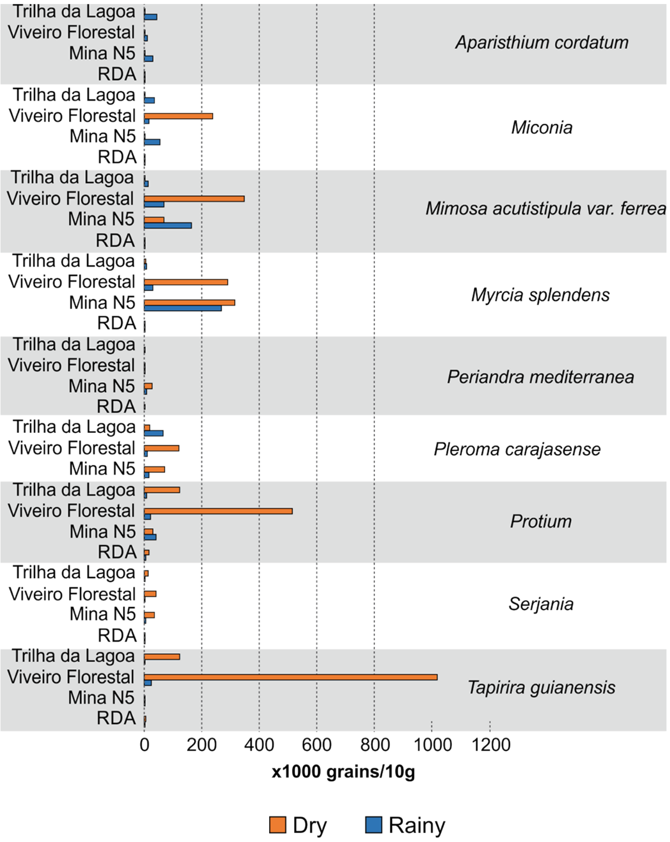

Figure 5. Total pollen concentration of the most abundant, frequent and common plant species of all studied areas considering the different climate periods.

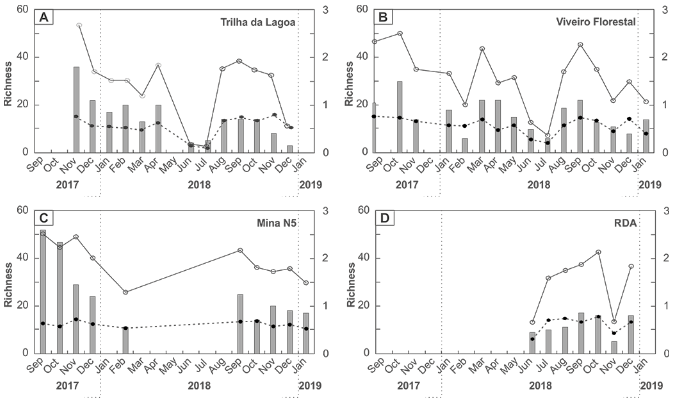

Figure 6. Richness (gray bars), Shannon-Weaver diversity (H'; upper stright line with empty circles) and Pielou's evenness (J'; lower dashed line with black ircles) of the pollen data per sampling area: (a) Lagoa da Trilha, (b) Viveiro Florestal, (c) Mina N5 and (d) RDA areas.

than all other sites for both periods. Diversely, Viveiro Florestal had different abundance for the two periods. Wilcoxon test was applied to evaluate differences between climate periods for each site. The results show no statistically significant difference on sites between periods, except for the Viveiro Florestal (ρ-value = 0.03). In order to evaluate the pollen distribution and similarity among the sites, Non-metric Multidimensional Scaling was the appropriate ordination method because the stress value for two dimensional, which was 0.04. NMDS, pointed to (dis)similarities on sites based on pollen concentration (Figure 7b). RDA samples are more related on both periods due to abundance of the pollen types of *Protium* and *Serjania*. N5 Mine samples are more similar during both periods due to the abundance of *Mimosa acutistipula* var. *ferrea* and *Myrcia splendens*, whereas the Trilha da Lagoa da Mata and Viveiro Florestal (rainy period) have more contribution from *A. cordatum*, *Miconia* and *P. carajasense*. During the dry period, Trilha da Lagoa da Mata and Viveiro Florestal differed from other samples due to their pollen assemblages: the first is mainly composed by *T. guianensis* and *Protium* and the latter has higher influence from several species. According to the Wilcoxon test, Viveiro Florestal demonstrated significant statistical difference for both periods, and therefore the NMDS is coherent with Wilcoxon test. Viveiro Florestal had higher concentration of all pollen assemblages during the dry period, whereas, during the rainy period it has a composition more comparable to the one at the Trilha da Lagoa da Mata and N5 Mine in the same period.

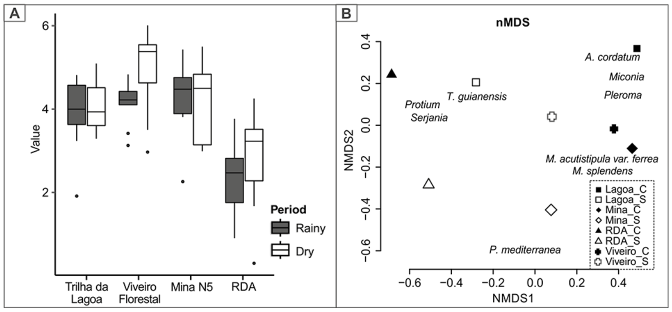

Figure 7. (a) Boxplot of pairwise samples in rainy and dry period. (b) Result of Non-Metric Multidimensional Scaling using honey samples in two distinct periods (dark gray: rainy period; white: dry period). Named pollen taxa used in the analysis are included.

Discussion

Considering the total pollen assemblage identified in honey samples of *M. seminigra pernigra*, around 80% of the pollen types were found to be within the values < 5,000 grains/10g and < 1%. The remaining ~20% are the primary bee sources. The secondary sources (less abundant) are continuously explored, providing a small amount of food that is an alternative resource for the colony (Heinrich 1976). This is especially important when other plants are saturated by other pollinators or flowering diminishes according to seasonal changes. In some occasions, the secondary source moves to a central position in the food supply (Novais & Absy 2013).

Melipona species are a successful generalist group of bees from tropical humid forests that have a close relationship with tree crowns and mass flowering (Ramalho 2004), and consequently play an important role in plant reproduction and natural forest regeneration. In the present experimental study developed at the Carajás National Forest, the honey samples of *M. seminigra pernigra* were constituted mostly by native species pollen.

Considered a native and abundant species, *Mimosa acutistipula* var. *ferrea* Barneby is endemic to north and northeastern Brazil and abundant in the Serra dos Carajás region, occurring on all CRC outcrops studied (Mattos et al. 2018). It features in the Brazilian red list of plants as Data Deficient (DD) (Martinelli & Moraes 2013) and vulnerable (VU) in the List of Threatened Species of Pará State (COEMA-PA 2010). *M. acutistipula* var. *ferrea* forms large single-species stands in both *canga* and altered barren areas (Zappi et al. 2018). The clustered, long, pendulous, sweetly scented inflorescences of *M. acutistipula* var.

ferrea are very rich in pollen (Mattos et al. 2018). This mass flowering is very attractive for *M. seminigra pernigra*. The abundance of the *Mimosa*-type (small) pollen grains in honey samples can be related to the configuration and relatively small pollen size, varying from 10-14 µm (Zappi et al. 2018), which are also released in large amounts according Ferreira and Absy (2017).

P. carajasense is a recently described endemic species from the CRC of Carajás (Rocha et al. 2017), and it has been categorized as a possible Endangered (EN) species according to the IUCN criteria (IUCN 2012). It occurs in large populations both on natural and anthropic areas. This species has attractive short thyrsoid inflorescences with 7–25 campanulate flowers, lilac-pink to purple petals and poricidal anthers (Matos & Santos 2017, Rocha et al. 2017). Vibrating bees (flower or anther buzzing) are favored by poricidal anthers, limiting the access of competing pollinators (De Luca & Vallejo-Marín 2013). *M. seminigra pernigra* have body sizes exceeding the gap between anthers and stigma in *P. carajasense*, and can be considered an efficient pollinator (Solís-Montero & Vallejo-Marín 2017). Considering *Alchornea* spp., nectary stomata is commonly observed on flowers, also in leaves, of *A. discolor* Poepp., *A. acutifolia* Müll. Arg., *A. castaneifolia* (Willd.) A. Juss., *A. glandulosa* Poepp., *A. megalophylla* Müll. Arg., which were extensively described for the study area (R. Secco personal communication; Secco 2004).

Other abundant plant species visited by *M. seminigra pernigra* in the study area, but with wider geographical distribution, are *A. cordatum*, *M. splendens* and *T. guianensis*. These species are commonly observed in forest margins, gallery forest, seasonally dry forest (SDF) and humid evergreen tropical forest (HETF), as well as disturbed areas in the Amazon and elsewhere. *A. cordatum* has racemose inflorescences with female flowers closer to the foliage and distal male flowers, while the two latter are paniculate. All these inflorescences provide large number of flowers per inflorescence. All species are good source of pollen and nectar for bees, as well as resin in the case of *T. guianensis* (Matos & Santos 2017). *P. mediterranea* has also a wide distribution with preference for open ecosystems such as Amazonian savannas, high altitude grasslands, *campo rupestre* on *canga* and other substrates (Mattos et al. 2018). It has racemose inflorescence with few, showy blue to purplish open, zygomorphic pea-shaped flowers with conspicuous nectar guides. These flowers provide both pollen and nectar for bees (Mateus 1998).

Pollen type of *Miconia* is frequently abundant in honey samples of *Melipona* bees in the Amazon region (Absy et al. 1980, Marques-Souza 1996, Oliveira et al. 2009, Ferreira & Absy 2017). The continuous flowering along both dry and wet seasons provides an excellent source of pollen for bees (Renner 1989). *Miconia* has poricidal anthers, supporting selective buzz pollination (Buchmann 1983). However, the identification of *Miconia* to species level using honey samples was not possible due to the stenopalynous pollen found in the genus, and there are at least 20 species of *Miconia* cited for the CRC of Carajás (Rocha et al. 2017). This large genus, one of the biggest in the Brazilian Flora, comprises 288 species in Brazil, 81 of them recorded for Pará state (BFG 2020), and its species present considerable variation in the gap between anthers and stigma. Therefore, it is not possible to be sure whether *M. seminigra pernigra* effectively makes contact with the stigmas.

Pollen types of *Solanum crinitum* were present in lower frequency in honey samples than *Miconia* but were well-represented in the study site. It has also poricidal anthers, but in this case the stamens are fused into a cone and the anthers are almost in contact (Giacomin & Gomes 2018). Thus, bees can vibrate all anthers at once, performing effective pollination (Solís-Montero & Vallejo-Marín 2017). *Melipona* has been commonly observed visiting Solanaceae species in lowland Amazonian forest (Absy et al. 1980), and, according to these authors, its pollen is of great importance for the diet and maintenance of the *Melipona* hives. Extrafloral nectaries are reported for *Solanum* (Anderson & Symon 1985). Predominant occurrence of *Solanum* pollen types with different sizes in the same honey sample of *Melipona* are also reported in the northeastern Brazil (Ferreira & Absy 2015, 2017). However, it is possible that the flowering of *Solanum crinitum* Lam., which is abundant in the study site (Giacomin & Gomes 2018) and certainly very rich in pollen, may has concomitantly occurred with any other nectar provider species. As the pollen extraction from the anthers of *Solanum* is done by vibration (buzz pollination; Buchmann & Cane 1989), the body hairs of *M. seminigra pernigra* may be full of pollen, which are moved to the corbicula. In the hives, the pollen is deposited into pots, but it is tasted by other workers of the colony (Roubik 1989). Likewise, the nectar collected is transferred between workers by trophallaxis before its deposition into the pots. Thus, the contamination of nectar collected from other source with the *Solanum* pollen that is present in the digestive system of the workers may be also a plausible explanation.

Also well represented in honey samples were pollen types of *Protium* and *Serjania*, chiefly at the Trilha da Lagoa da Mata, Viveiro Florestal and N5 mine areas. *Protium* is the commonest component in the pollen assemblage of sampled honey at the RDA area. These taxa present paniculate inflorescences with small, separate sex flowers that provide bees with nectar, resin and pollen (male flowers only), while *Serjania* spp. is a rampant liana with thyrsoid inflorescences with small, sweetly scented flowers that are considered an excellent source of nectar (Matos & Santos 2017). Both genera are commonly observed in transitional areas between CRC and HETF and SDF and provide great availability of resources over a long flowering period (Oliveira et al. 2009).

Following the statistical analysis, there was no significant difference in honey pollen data between the natural and disturbed areas. This is mainly due to the fact that all these abundant and common native taxa are used in revegetation during RDA processes by mining activities (Zappi et al. 2018). However, more studies are necessary to better evaluate the prolonged effects of human interference over plant reproduction.

Regarding the importance of plants for honey production, income generation, and conservation of pollinators, *T. guianensis*, *Protium* spp., *M. splendens*, *M. acutistipula* var. *ferrea*, *Serjania* spp., *P. carajasense* and *A. cordatum* seem to be key species, as well as *Miconia* spp. with some considerations. Indeed, most of *Miconia* species are polliniferous, but it cannot be generalized (T. Vasconcelos, personal communication). Most neotropical Melastomataceae have bee-pollinated flowers with poricidal anthers. However, nectar rewards are known to be produced in about 80 species in eight genera, including *Miconia* (Varassin et al. 2008, Brito et al. 2017). These authors using anatomical methods based on scanning electron microscopy, and serial sections of paraffin-embedded flowers precisely identified nectary stomata on the ovary apex in *Miconia* spp.

These plant species can be used to help recover deforested areas (Zappi et al. 2018) and help improve the resource provisions for the stingless bee populations. For local beekeepers, the incorporation of these plants in the foraging area of beehives can help to improve the amount of honey produced, reflecting positively on income generation.

Conclusions

M. seminigra pernigra uses, preferably, native plants from *cangas* and forests of the Serra do Carajás as its primary sources. Main examples are *Tapirira guianensis* Aubl., *Protium* spp., *Aparisthmium cordatum* (A.Juss.) Baill., *Mimosa acutistipula* var. *ferrea* Barneby, *Periandra mediterrânea* (Vell.) Taub., *Miconia* spp., *Pleroma carajasense* K.Rocha, *Myrcia splendens* (Sw.) DC., *Serjania* spp., and *Solanum crinitum* Lam. However, these primary bee sources (PBS) represent only 20% of the total pollen assemblage, which suggest high dispersion rates of the colony members related to temporal flowering pattern of the PBS. All pollen types were identified during both seasons, but higher concentration values are related to the dry period (June-September). In fact, Shannon-Weaver diversity (H') and Pielou's evenness (J') indexes substantially decrease when PBS are fully available.

The PBS have different floral syndromes. The strategies included condensed spike-shaped inflorescences offering abundant nectar and pollen in *M. acutistipula* var. *ferrea* and *A. cordatum*; buzz pollinated anthers in *P. carajensis*, *Miconia* spp. and *S. crinitum*; and separate sex flowers (*A. cordatum* and *Protium* spp.). The majority of the species had small flowers grouped in inflorescences working as a flowering unit, while only *P. mediterranea* and *P. carajasense* have large, attractive flowers. Of these species, the only strongly zygomorphic flower with nectar guides was *P. mediterranea*.

No significant difference was statically found in honey pollen data between the natural and disturbed areas, which may be related to the widespread use of PBS for mine land rehabilitation, as well as in the remaining vegetation surrounding the mine. However, longer-term data are necessary for better supporting such findings in mining land areas of the study site, including climate change influences.

Supplementary material

The following online material is available for this article:

Online Resource 1 - Melissopalynological records of plant/pollen associated with Meliponini species (Rezende et al. 2019; Rezende et al. 2019; Oliveira et al. 2009; Absy & Kerr 1977; Marques-Souza et al. 2002; Ferreira & Absy 2017.

Online Resource 2 - Main plant species observed in the natural and anthropized areas were the studied apiaries were installed. Humid Evergreen Tropical Forest (HETF), Seasonally Dry Forest (SDF).

Online Resource 3 - Occurrence per area and distribution frequency of the pollen types (Feller-Demalsy et al., 1987): very frequent (>50%), frequent (freq.: 20–50%), infrequent (infreq.: 10–20%) and rare (<10%); and groups of pollen concentration (Maurizio, 1975): Group I (<20,000), Group II (20,000–100,000), Group III (100,000–500,000), Group IV (500,000–1,000,000) and Group V (>1,000,000).

Online Resource 4 - Number of observations per (1) groups of pollen concentration (grains/10g; Maurizio, 1975): Group I (<20,000), Group II (20,000–100,000), Group III (100,000–500,000), Group IV (500,000–1,000,000) and Group V (>1,000,000); and per (2) distribution frequency of the pollen types (percent-%; Feller-Demalsy et al., 1987): very frequent (>50), frequent (20–50), infrequent (10–20) and rare (<10).

Online Resource 5 - Percentage and concentration pollen diagram illustrating the pollen content of the honey samples from Trilha da Lagoa da Mata area. Gray line indicates a gap in the honey sampling.

Online Resource 6 - Percentage and concentration pollen diagram illustrating the pollen content of the honey samples from Viveiro Florestal area. Gray lines indicate gaps in the honey sampling.

Online Resource 7 - Percentage and concentration pollen diagram illustrating the pollen content of the honey samples from N5 mine. Gray line indicates a gap in the honey sampling.

Online Resource 8 - Percentage and concentration pollen diagram illustrating the pollen content of the honey samples from RDA area.

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Conflicts of interest

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

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Article

Breeding biology in a population of Rufous-collared Sparrow (*Zonotrichia capensis*, Statius Müller, 1776) at different elevations in the Tropical Andes

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Abstract: Breeding biology in a population of Rufous-collared Sparrow *(Zonotrichia capensis*, Statius Müller, 1776) at different elevations in the Tropical Andes. Elevation strongly influences the evolution of life history traits associated with bird physiology and reproduction. Since life history traits in birds are modulated by environmental factors that vary with elevation, we expected to find changes in breeding biology traits of *Z. capensis* at different elevations. In this study, we compared the breeding biology (clutch size, egg volume, and reproductive activity) of a non-migratory population of *Z. capensis* in two localities at different elevations (low and high elevations, 1 800 m a.s.l., and 3 800 m a.s.l., respectively) and same latitude in the Colombian Tropical Andes for a one-year period. We found no differences in clutch size between the localities; however, egg volume was higher at high elevation. Furthermore, the reproductive activity differed significantly between localities. We propose that clutch size is likely conserved throughout the altitudinal distribution of *Z. capensis* since the species evolved in Tropical lowlands. On the other hand, the larger egg volume at high elevation could obey local environmental factors that may favor the reproductive success of the population.
Keywords: *Pleistocene; phenology; paramo; life history; reproductive traits.*

Biología reproductiva en una población de Gorrión Copetón (*Zonotrichia capensis*, Statius Müller, 1776) a diferente elevación en los Andes tropicales

Resumen: Biología reproductiva en una población de Gorrión Copetón (*Zonotrichia capensis*, Statius Müller, 1776) a diferente elevación en los Andes tropicales. La elevación influye fuertemente en la evolución de los rasgos de historia de vida asociados con la fisiología y reproducción de las aves. Dado que los rasgos de historia de vida en las aves están modulados por factores ambientales que varían con la elevación, esperábamos encontrar cambios en los rasgos de la biología reproductiva de *Z. capensis* a diferentes elevaciones. En este estudio, comparamos la biología reproductiva (tamaño de puesta, volumen del huevo y actividad reproductiva) de una población no migratoria de *Z. capensis* en dos localidades a diferente elevación (elevación baja 1 800 m snm, y elevación alta 3 800 m snm,) y a la misma latitud en los Andes tropicales colombianos por un período de un año. No encontramos diferencias en el tamaño de puesta entre las localidades; sin embargo, el volumen del huevo fue mayor en elevación alta. Además, la actividad reproductiva difirió entre las localidades. Proponemos que el tamaño de puesta probablemente se conserve en toda la distribución altitudinal de *Z. capensis,* ya que la especie evolucionó en las tierras bajas tropicales. Por otro lado, el mayor volumen del huevo a elevación alta podría obedecer a factores ambientales locales que pueden favorecer el éxito reproductivo de la población.
Palabras clave: *Pleistoceno; fenología; páramo; historia de vida; rasgos reproductivos.*

Introduction

Understanding how life history traits are modeled by natural selection is one of the main questions in evolutionary ecology (Endler 1995). Moreover, environmental factors are relevant to explain the evolution of life history traits in birds (Lack 1968, Badyaev & Ghalambor 2001). Changes in environmental conditions (e.g., temperature, atmospheric pressure, precipitation, and seasonality) that occur in response to variations in elevation and/or latitude have been related to life history evolution (Cody 1996, Boyce 1979, Endler 1986, Badyaev 1997a, Foster & Endler 1999, Quirici et al. 2014). In particular, elevation is considered a factor that affects changes in clutch size, egg volume, and breeding season length in birds (Chabi et al. 2000, Lu 2011, Heming & Marini 2015). However, to our knowledge, there are no population-level studies assessing different elevations at the same latitude in the Tropical region.

The relationship between variation in bird reproductive life history traits, such as clutch or egg size, and elevation remains unclear. For example, previous studies report larger, equal, and/or smaller clutches in breeding populations at high elevation compared to populations at lower elevation (Boyce et al. 2015, Boyle et al. 2016). Differences in life history traits are attributed to variations in climate, predation, and food limitation (Krementz & Handford 1984, Kovshar 1981, Cody 1996, Boyce 1979). Particularly, in Tropical regions, there is insufficient information on how clutch size or egg volume responds to different elevations at the population level (Boyle et al. 2016), or on other reproductive life history traits.

The Rufous-collared Sparrow (*Zonotrichia capensis* Statius Müller, 1776) (Passerine, Emberizidae) is a socially monogamous bird with diverse life histories. This Neotropical passerine has among the largest elevational (from sea level to 4600 m a.s.l.) and latitudinal distributions (from Mexico to Chile) among birds, occupying a wide variety of environments and habitats, such as coastal, paramo, humid forest, and urban areas (Rising et al. 2010, Chapman 1940). Additionally, several studies report differences in life history traits related to reproduction, physiology, ecology, and behavior, among others, throughout its distribution range (Miller & Miller 1968, Moore et al. 2004, Class et al. 2009, Van Dongen et al. 2010). Therefore, this species a good model for investigating differences in reproductive life history traits under contrasting environmental conditions related to elevation. In this study, we identified several differences in the reproductive biology of *Z. capensis* populations at different elevations in the Colombian Tropical Andes. Since life history traits in birds are modulated by environmental factors that vary with elevation, we expected to find changes in the breeding biology traits of *Z. capensis* at different elevations.

Materials and Methods

The Rufous-collared Sparrow has non-migratory populations in Colombia and is found within an elevational range from 1 000 to 3 700 m a.s.l. The sexes are similar in coloration and juveniles differ from adults in several plumage characteristics. At this latitude, individuals breed almost year-round with a locally-defined seasonality; clutch size is two eggs on average and eggs are incubated exclusively by the females (Hilty & Brown 1986, Rising et al. 2011, Miller & Miller 1968).

1. *Study site*

During a one-year period, from 2015 to 2016, we monitored Rufous-collared Sparrow populations in two localities on the western slope of the Colombian Central Andes at different elevations (1 800 and 3 853 m a.s.l.) and similar latitude. In the study area, the species is a year-round resident and shows a continuous distribution. Both localities are located in the Chinchina River basin in the department of Caldas (Figure 1), where precipitation shows a bimodal pattern comprising a minor rainfall season, in relation to the annual average, during June-September and December-March and a major rainfall season occurring during April-May and October-November (Morales-Betancourt et al. 2012). The two sites, namely the low elevation locality (LE) (Alto del Naranjo: 5°00'29.8"N - 75°33'41.2"W; 1 800 m a.s.l.) and high elevation locality (HE) (La Laguna: 4°58'49.3"N - 75°20'06.8"W; 3 853 m a.s.l.), are separated in a straight line by 26 km. Despite their proximity, these sites have different climate conditions (Busi et al. 2020). The LE locality is located in a coffee growing region with an average annual temperature of 20.7ºC (min. 16.8ºC and max. 26.3ºC) and annual rainfall of 2 817 mm. The HE locality is located in a paramo zone with an average annual temperature of 7.1ºC (min. 4.4ºC and max. 10.3ºC) and annual rainfall of 1848 mm (Federación Nacional de Cafeteros (F.N.C) n.d.). The HE locality was covered by a glacier during the Pleistocene until approximately 10 000 years ago (Thouret et al. 1997).

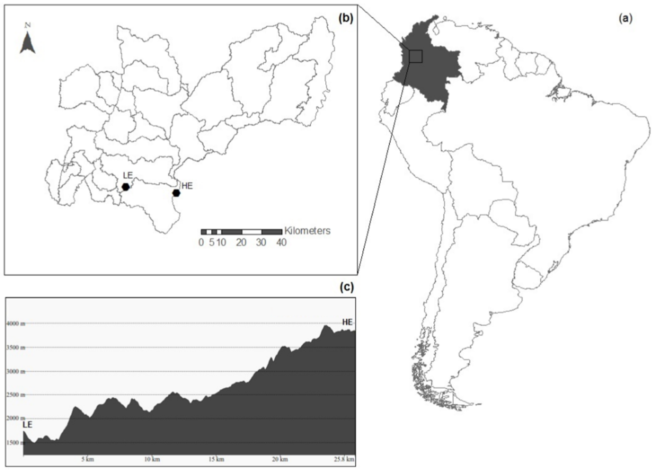

Figure 1. Locations of the study sites along the Chinchina River basin in the department of Caldas, Colombia. The triangle corresponds to LE, low elevation site (1 800 m a.s.l.), and the hexagon corresponds to HE, high elevation site (3 800 m a.s.l.).

2. *Field methods*

We visited each site every other day during one year (from December 2015 to December 2016; December 16 was day 1 of sampling). In each visit, nests were searched within an area of 13 ha to assess clutch size at each locality. The nests were visited every other day to establish the clutch size. Each egg was measured by its length and maximum width using a digital caliper (0.03 mm) to estimate egg volume (Hoyt 1979).

In the same period, we captured Rufous-collared Sparrow individuals using mist nets (12 x 2.5m x 36 mm) to gather morphological evidence of the reproductive stage. The total sampling efforts were 1

120 and 1 680 hours net^{-1} for LE and HE, respectively. We required a total sampling effort of 2 800 hours due to low population densities of approximately two individuals per hectare observed at each locality. The captured birds were marked using bands with a unique color combination for later identification and the individuals were then released at the same capturing place.

We determined the onset of morphological indicators of reproductive stage (MIRS) in order to compare the reproductive activity of individuals from LE and HE localities for one year (Bears et al. 2009). Males were considered to be in reproductive stage when the cloacal protuberance (CP) measured at least 5 mm (Addis et al. 2010). On the other hand, in females, we checked for the presence of a brood patch (BP) since CP size is not a good indicator of MIRS (Miller & Miller 1968).

3. *Data analysis*

To quantify differences in egg volume between the two elevations (high elevation vs. low elevation), we fitted a Linear Mixed Model (LMM) with the Gaussian error distribution using the lme4 R package (Bates et al. 2005). We included egg volume in mm^3 as a response variable, elevation as a factor (LE and HE), clutch size as a predictor (i.e., covariate), and nest identity as a random effect. Due to the reduced number of nests found in the study area, we compared the clutch size between HE and LE with Fisher's Exact Test (2x2 table). Specifically, we contrasted the frequency of nests with a single egg or more than two eggs between localities. We used circular statistics to compare the reproductive activity (MIRS) between both locations. The dates of MIRS were converted to Julian days and then to radians. The reproductive activity data were fitted to density kernels for each locality. Then, we compared the reproductive activity using the overlap coefficient (i.e., high coefficient values indicated more similar activities). The circular statistics analyses were done using the activity (Rowcliffe 2019) and overlap (Ridout & Linkie 2009) R packages. All the procedures and statistical analyses were performed in R 3.6.1 (R Development Core Team 2019).

Results

We observed nests with eggs at LE in three months (April, July, and October, $n = 6$) and at HE in three months (April, May, and June, $n = 6$). Clutch size ranged from one ($n = 2$) to two ($n = 4$) eggs at HE and two ($n = 4$) to three ($n = 2$) eggs at LE, with a mode of two eggs at each elevation. There was no significant difference in clutch size between elevations (Fisher's Exact Test, $P = 0.454$). Rufous-collared sparrow females layed eggs of size 22.2 ± 0.64 mm length and 16.6 ± 0.81 mm width ($n = 10$) at high elevation, and 21.4 ±0.86 mm length and 15.3 ± 0.66 mm width ($n = 15$) at low elevation. The mean egg volume at HE was 3147.7 mm^3 (SD = 346.9) and at low elevation it was 2545.6 mm^3 (SD = 277.6) (Figure 2). We found a statistically significant difference in mean egg volume between elevations, indicating that larger eggs were found at high elevation compared to smaller eggs at low elevation (Table 1). In addition, the LMM also indicated a non-statistically significant relationship between egg volume and clutch size (Table 1).

We captured a total of 46 adult individuals, including 26 and 20 at low and high elevation localities, respectively. At HE, we obtained 19 records of reproductive activity (15 individuals, three recaptures). The highest peaks of reproductive activity occurred in February and September (Figure 3). Moreover, at the LE, we found 24 records of MIRS (22 individuals, two recaptures). Reproductive activity peaks occurred in January, March, July, October, and December (Figure 3). Moreover, the reproductive activity peaks at high elevation occurred before those at low elevation. We found a low reproductive activity overlap between low and high elevations (Figure 3) and the reproductive activity differed significantly between both localities (= 0.35, $P < 0.001$).

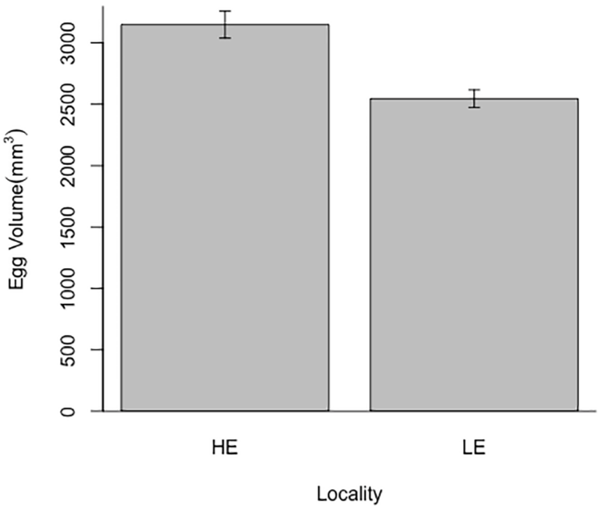

Figure 2. Mean values for egg volume in two localities at different elevations, high elevation (HE) and low elevation (LE). Errors bars represent the standard deviations of the mean.

Table 1. Results of the Linear Mixed Model (LMM) to test differences in egg volume in response to elevation and clutch size. The contrast represents the statistically significant difference between Low Elevation (LE) and High Elevation (HE).

Fixed effects	**ß (SE)**	**t-value**	***p*-value**
Intercept (HE)	3450.51 (295.03)	-11.69	< 0.01***
Contrast (LE-HE)	-453.80 (193.75)	-2.34	0.04*
Clutch size	-171.831 (156.68)	-1.09	0.29
Random effects	σ2		
Nest Identity	33909	–	–
Residuals	61858	–	–

Discussion

We did not find variations in clutch size in relation to elevation. This result disagrees with the findings of Cody (1966) and Winkler (2004). Both authors have suggested changes in clutch size with elevation in the Tropical region, due to the extreme environmental conditions that the individuals experience at high elevation. In particular, changes in clutch size can be associated with low food availability and high energy requirements for reproduction (Badyaev 1997b, Boyle et al. 2016). The Rufous-collared Sparrow populations studied here did not show differences in clutch size. The Rufous-collared Sparrow originated in the Tropical lowlands (Lougheed et al. 2013) so it is likely that clutch size in this species was optimized for lowlands, where a clutch of two is common among near-equatorial locations (Eikenaar et al. 2013, Miller & Miller 1968). Furthermore, this clutch size is conserved by individuals inhabiting a higher elevation. This could be associated with the low

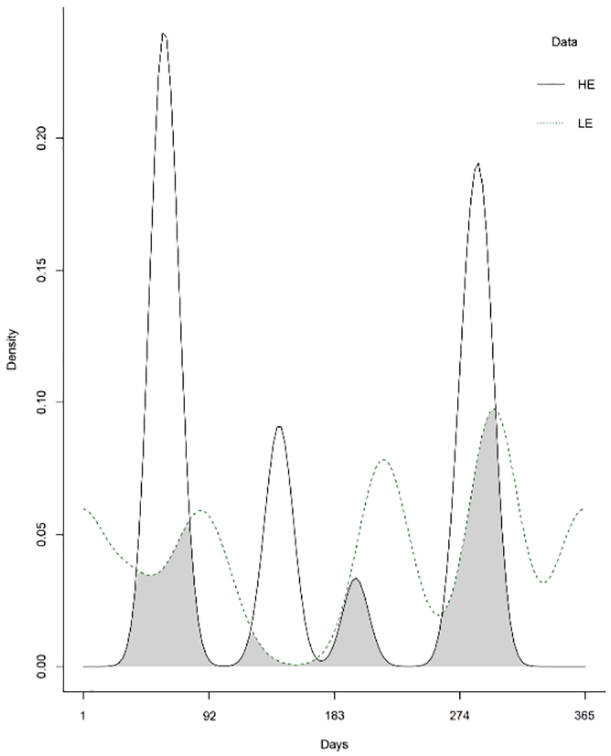

Figure 3. Reproductive activity kernels for high (HE) and low (LE) elevations. Activity kernel curves represent record density throughout 365 days (December 16 was day 1 of sampling).

variability in clutch size observed in our results. In addition, *Z. capensis* likely colonized the locality at HE in this region less than 10 000 years ago, which could be an evolutionarily short time for the appearance of changes in clutch size associated with environmental factors.

The larger egg volume found at high elevation is possibly explained by the embryonic temperature hypothesis, which proposes that egg volume is larger when these are incubated at low environmental temperature and for longer periods due to the energetic requirements of the embryos (Martin 2008). Another possible explanation is that larger eggs lose heat more slowly during incubation recesses because of the lower surface area-to-volume ratio (Rhymer 1988). Larger eggs likely provide an advantage to brood survival in harsh or low-quality environments (Smith & Bruun 1998, Styrsky et al. 1999, Lu 2005). Therefore, larger egg volumes at HE may be related to higher reproductive investment in this trait to possibly produce higher quality offspring (Bears et al. 2009).

The greater amount of evidence of reproductive activity in fewer peaks at HE than LE could be regulated by environmental factors. In fact, local environmental factors, such as rainfall, temperature, and food availability, are supplementary cues that can influence the timing and length of the reproductive season and the development of cloacal protuberance in males (Lack 1966, Wingfield & Kenagy 1991).We found three peaks of reproductive activity at each elevation; two of them occurred at the beginning of the major rainfall season, i.e., March and October, and the other peak occurred during the minor rainfall season, specifically, in July. It is possible that harsher environmental conditions limited the length of the reproductive activity at the high elevation locality as opposed to the low elevation.

Overall, the environment can be an important factor of natural selection, leading to population-level responses to altitudinal gradients in the Tropics. Our results suggest that the Rufous-collared Sparrow that evolved in the lowlands preserves clutch sizes even at different altitudes. As a result, egg volume may have increased at HE to locally favor the reproductive success of the population. However, due to the reduced sample size of our study, these results should be considered with caution.

The negative relationship between clutch size and egg volume found here is similar to that reported by (Heming and Marini 2015). It has been proposed that high energy requirements for reproduction produce a trade-off between clutch and egg size, whereby egg volume decreases as clutch size increases (Stearns 1992; Williams 2012), as it was observed in our study population.

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Author Contributions

Leydy J. Cardona-Salazar (Corresponding Author) performed a substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation and; contribution to manuscript preparation.

Ana Busi performed a substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation and; contribution to manuscript preparation.

Daniela Gómez Castillo performed a contribution to data collection and; contribution to data analysis and interpretation.

Paula A. Ossa-López performed a contribution to data analysis and interpretation; contribution to manuscript preparation.

Fredy A. Rivera-Páez performed contribution to manuscript preparation; contribution to data analysis and interpretation.

Rodrigo A. Vásquez performed a contribution to critical revision, adding intellectual content.

Gabriel J. Castaño-Villa performed a substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation and; contribution to critical revision, adding intellectual content.

Conflicts of Interest

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

Data availability

Dryad, https://datadryad.org/stash/share/dFvmhb6MTtGsbA3E-0OXRnLkkaHqpNQ0CqpreJ82mCc4

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Short Communication

Individual niche trajectories in nesting green turtles on Rocas Atoll, Brazil: an isotopic tool to assess diet shifts over time

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Abstract: In this study, multi-tissue (yolk and carapace) stable isotope analysis was used to assess individual isotopic niche trajectories of nesting green turtles on Rocas Atoll, off northeastern Brazil, and to reveal a diet shift in the temporal dimension. The diet trajectories of individual green turtles were highly directional, with a stronger component towards decreasing values of $\delta^{15}N$ from carapace to yolk. When the green turtles are in their foraging sites (temporal window measured by the yolk samples), they are more herbivores. Conversely, in a broader temporal window, the green turtles demonstrate a carnivore-omnivore strategy, such as represented by heavier $\delta^{15}N$ values in the carapace. This finding confirms a temporal diet shift. This is the first study that applies trophic niche trajectories for sea turtles, adding a new isotopic tool to understand the trophic ecology of these migrant animals.
Keywords: *Chelonia mydas; adult females; stable isotopes; trophic ecology; Atlantic Ocean.*

Trajetórias de nicho individual em tartarugas verdes do Atol das Rocas, Brasil: uma ferramenta isotópica para verificar trocas de dieta ao longo do tempo

Resumo: Neste estudo, a análise de isótopos estáveis em múltiplos tecidos (vitelo e carapaça) foi usada para avaliar as trajetórias individuais de nicho isotópico de tartarugas verdes em nidificação no Atol das Rocas, nordeste do Brasil, e para revelar uma mudança de dieta na dimensão temporal. As trajetórias individuais da dieta de tartarugas verdes foram altamente direcionais, com um componente mais forte na direção de valores decrescentes de $\delta^{15}N$ da carapaça ao vitelo. Quando as tartarugas verdes estão em seus locais de forrageamento (janela temporal medida pelas amostras de vitelo), elas são mais herbívoras. Por outro lado, em uma janela temporal mais ampla, as tartarugas verdes demonstram uma estratégia carnívora-onívora, representada por valores mais elevados de $\delta^{15}N$ na carapaça. Os resultados confirmam uma mudança temporal na dieta. Este é o primeiro estudo que aplica trajetórias de nicho trófico para tartarugas marinhas, adicionando uma nova ferramenta isotópica para entender a ecologia trófica desses animais migrantes.
Palavras-chave: *Chelonia mydas; fêmeas adultas; isótopos estáveis; ecologia trófica; Oceano Atlântico.*

Introduction

The green turtle (*Chelonia mydas* Linnaeus, 1758) is the only sea turtle species known to be herbivore after the individuals' recruitment from oceanic to coastal waters (Bjorndal 1997). However, the contribution of animal matter in its diet can be variable, as demonstrated in the last decade (e.g., Burkholder et al. 2011; Carman et al. 2012; Veléz-Rúbio et al. 2016; Di Beneditto et al. 2017; Fukuoka et al. 2019). Adult sea turtles are true migrants, moving between foraging and breeding sites thousands of kilometers apart. Comparing satellite telemetry data, Shimada et al. (2020) showed that fidelity to specific foraging sites following breeding migrations is common across several sea turtles species. For the green turtle, the authors stated high and long-term fidelity to specific foraging sites, with feeding activity happening during the migration to-and-from the breeding sites.

Stable isotopes are chemical proxies applied as a tool to analyse animals' trophic niches because they allow inferences on food resource use over many temporal scales (Newsome et al. 2007). The stable isotope of carbon ($\delta^{13}C$) represents the food resource origin, with more enriched values (less negative) in coastal than in oceanic waters and in benthic than in pelagic environments. The stable isotope of nitrogen ($\delta^{15}N$) tracks the animals' trophic position and it is more enriched at higher trophic levels, i.e. carnivore and omnivore consumers usually have more enriched $\delta^{15}N$ values in their tissues than herbivore consumers (Fry 2008).

Since each tissue has a specific metabolic rate, its turnover time, i.e. the time within which stable isotopes in tissues are replaced by stable isotopes derived from the food sources, is different (Auerswald et al. 2010). Keratinous tissues, like hair and carapace scutes, are metabolically inert and maintain an isotopic record from the location where they were synthesized, representing the dietary information over a longer period (e.g. several months or years) (Hobson 1999). In metabolically active tissues, such as liver, this information refers to a shorter time, such as a few weeks (Hobson 1999). The vitellogenesis process of sea turtles happens 4 to 6 months before the female's migration to the breeding site, when she is still at the feeding site (Rostal et al. 1998). Thus, the egg yolk represents the dietary information in a narrower temporal window when compared to carapace scutes. By comparing the isotopic profile from different tissues, it is possible to analyse the temporal consistency of individual diet through isotopic niche trajectories (Costa-Pereira et al. 2019). The assumption is that individuals with temporally consistent diets have more similar isotope values across tissues than individuals with temporally variable diets (Martínez del Rio et al. 2009).

Individuals from the same population do not necessarily have temporally consistent foraging strategies, undergoing diet shifts over time, while others may have a more constant diet (Bearhop et al. 2004; Martínez del Rio et al. 2009). Fukuoka et al. (2019) demonstrated by stable isotope analysis and biologging experiments the seasonal diet shift in a juvenile green turtle population from Japanese Pacific waters. The temporal dimension of individual foraging strategies allows for an understanding of habitat use as a whole (Costa-Pereira et al. 2019), which is especially important for true migrant species, such as the sea turtles.

In this study, multi-tissue (yolk and carapace) stable isotopes were used to assess the consistency of trophic niches of nesting green turtles over time. This approach was adapted from Costa-Pereira et al. (2019), whose study with dozens of populations and hundreds of individuals from tropical frogs' species demonstrated the reliability of the trophic niche trajectories to measure the trophic consistency of individuals in a temporal scale. The carapace represents a comprehensive assimilation, integrating diet from several months to years ago, whereas the yolk represents the food assimilation from a few months before oviposition, while the nesting females are still at the feeding sites. If the hypothesis of a consistent dietary pattern over time is supported, yolk and carapace isotope values will be similar, and variation in the individual trophic niche trajectories (lengths and angles) is not expected to differ from a random pattern of changes.

Material and Methods

1. *Sampling*

The sampling of green turtles on Rocas Atoll (03°51'S; 33°49'W) was authorized by the Brazilian Government by the license number 59809. This area is the second largest nesting site for the green turtles in Brazilian waters (Bellini et al. 2013). It is the only atoll in the South Atlantic Ocean, with 7.5 km^2 and located 266 km off the northeastern Brazilian coast. Rocas Atoll is a marine biological reserve; therefore, a pristine area. Bellini et al. (2013) conducted a comprehensive survey to monitor the nesting females in this site from 1990 to 2008. The nesting season occurs from December (beginning, with few nests) to May, with peak from February to April. The average number of nesting females per season is 73, with five nests per individual. The mean remigration period is 3.5 years. The authors estimated that 255 reproductively active females nested on the Rocas Atoll between 1990 to 2008. The authors observed a high site fidelity for the reproductive females in the Rocas Atoll nesting site, confirming its demographic independence in relation to a close reproductive site (Fernando de Noronha Island, 03°51'S; 32°25'W).

In the 2019 nesting season, 22 female individuals were sampled in Rocas Atoll, and sampling began as soon as each female initiated the first egg laying. All females had a healthy appearance, i.e. no visible tumours (Jones et al. 2016). During sampling, each female was measured for curved carapace length, from the nuchal notch to the tip of the longest posterior marginal scute (cm), and microchipped for individual identification (to avoid resampling). Two fresh eggs were sampled during the first laying, rinsed in filtered water, and the yolk separated from other egg fractions. The bulk sample with two yolks (from the same female) was stored in a clean transparent plastic bag and kept frozen (-20°C) until analyses. A carapace fragment (5 cm^2) was sampled from the margin of the anterior scute, close to the nuchal notch. Each fragment was immersed in pure acetone to dissolve any incrustation. Then, it was rinsed in filtered water, dried at room temperature, grounded into a homogeneous powder and stored in a clean plastic vial until analyses. The fragment does not represent the oldest tissue in the carapace, as indicated in Lopéz-Castro et al. (2014), but since all samples were collected in the same way, bias is not expected.

2. *Stable isotopes analysis*

One gram of wet weight of yolk (bulk sample) was freeze-dried for 96 hours and ground into a homogeneous powder. Since yolk has a large amount of lipids (>50% in freeze-dried samples) (Carpentier et al. 2015), the samples were treated using a 2:1 solvent mixture of chloroform and methanol prior to lipid extraction (Bligh and Dyer 1959). The samples were dried at 60°C in an oven for 48 hours to remove the residual solvent. This procedure minimizes bias in $\delta^{13}C$ data interpretation (Post et al. 2007). Since the extraction of lipids can interfere with the $\delta^{15}N$ values (Petitet & Bugoni 2017); the yolk samples were analyzed twice: with and without lipid extraction. For $\delta^{13}C$, the mean values in yolk with and without lipid extraction were -17.9 ± 1.7‰ and -20.0 ± 1.7 ‰, respectively; and for $\delta^{15}N$ they were 7.2 ± 1.3‰ and 6.9 ± 1.3 ‰, respectively.

The ratios of stable isotopes were determined in 0.3-0.4 mg of dry weight of each sample (yolk and carapace) using an organic elemental analyzer (Flash 2000, Thermo Scientific) coupled with a mass spectrometer (Delta V Advantage Isotope Ratio Mass Spectrometer, Thermo Scientific) through the ConFlo-VI interface (Model BR30140, Thermo Scientific) in the Laboratório de Ciências Ambientais at Universidade Estadual do Norte Fluminense Darcy Ribeiro. Reference values were Pee Dee Belemnite (PDB) and atmospheric nitrogen. Samples were analyzed using analytical blanks and urea analytical standards (IVA Analysentechnik-330802174). Analytical control was performed for every 10 samples using a certified isotopic standard (Elemental Microanalysis Protein Standard OAS). The reproducibility was based on triplicates for every 10 samples (±0.2‰, $\delta^{13}C$; ±0.3‰, $\delta^{15}N$).

3. *Individual niche trajectories*

Individual niche trajectories between isotope values of carapace and yolk quantified the temporal consistency of individual diets (Schmidt et al. 2007; Costa-Pereira et al. 2019). The length of trajectories in the bivariate isotopic space ($\delta^{15}N$-$\delta^{13}C$) was calculated by the Euclidean distance between starting points (carapace) and endpoints (yolk) for each individual. The trajectory direction was determined by the (counter clockwise) angle of the line connecting carapace and yolk values in relation to the x-axis ($\delta^{13}C$). The yolk and carapace are dependent measures because part of the dietary information integrated into one tissue is hierarchically integrated to another tissue. Differences between tissues regarding stable isotope values may emerge by temporal variation in environmental baselines and/or differential isotopic route, and not necessarily due to diet variation over time. Since these potential biases should be homogeneous across individuals, they are not expected to bias the results (Schmidt et al. 2007).

The vectors representing the niche trajectory between carapace and yolk for each green turtle individual in the isotopic space represent a dietary shift from the more general life pattern to the period of vitellogenesis. Differences in the angle of change indicate whether and in which direction individuals' isotopic values shift vertically ($\delta^{15}N$ or trophic level shift) and/or horizontally ($\delta^{13}C$ or base carbon sources) over time. The magnitude of the diet shift can be measured by the trajectory length. To compare the observed circular distribution of trajectories with the expected null distribution of random changes, we calculated three statistics: mean trajectory length, standard deviation of trajectory length (as a measure of variability) and Rao's spacing statistic (U). The spacing statistic measures the sum of differences of arc-lengths between adjoining points (ranked by angle) and the regularly spaced arc-lengths expected for the null hypothesis of uniformity ($2\pi/n$) (Pewsey et al. 2013). The U values become larger as directionality increases, with less deviation between individuals' trajectories. The circular statistics were calculated in the R package *circular* (Agostinelli & Lund 2017; R Core Team 2020).

Following the procedure delineated in Costa-Pereira et al. (2019), we used a randomization process to generate stochastic individual niche trajectories in the isotopic space. For each individual, the observed isotopic starting point was kept constant (carapace isotopic values) and the isotopic endpoint was assigned by drawing randomly (without replacement) a pair of yolk isotopic values from the distribution of observed yolk isotope values in the sample of 22 individuals. For each resampling, the endpoint of random trajectories could therefore, assume isotope values observed in the conspecific individuals. The null model tested whether the mean length and standard deviation of observed niche trajectories are compatible with a random expectation, and whether the distribution of trajectory angles support a uniform circular distribution. The null distribution of trajectory statistics was based on 10,000 replicates. Probability values were calculated by the sum of permuted statistics that were equal to or more extreme than the observed, divided by 10,000. The stable isotope values used in this study, as well as the R script to calculate trajectory statistics and the randomization procedure are available as Supplementary material.

Results and Discussion

The multi-tissue isotopes explored the individual trophic consistency of nesting green turtles over the foraging period while individuals stay in the foraging sites (yolk samples), and in a broader temporal scale (carapace samples). Trajectory angles showed directionality towards lighter $\delta^{15}N$ values and heavier $\delta^{13}C$ values (Figure 1). The observed mean and standard deviation of niche trajectory length were smaller than expected by chance ($p < 0.0001$) (Figure 2). The observed sample was more directional than expected under a uniform circular distribution ($U = 176.568$, $p = 0.0083$) (Figure 2). The curved carapace length was very homogeneous across the sample (n = 22 individuals; 112.5 ± 5.0 cm; 107 to 123 cm) and not associated with any isotopic or descriptive variable of the niche trajectory.

The results highlighted that most individuals vary in the consistency of their feeding strategies over time, following a similar pattern. Therefore, a temporal diet shift was noted for the reproductive females that nested at Rocas Atoll during the nesting season of 2019, and this shift was similar within the sample. When the green turtles are in their foraging sites (temporal window measured by the yolk samples), they are more herbivore with stronger association with coastal-benthic waters. Lighter $\delta^{15}N$ values represent lower trophic level, typical in herbivore strategy, whereas heavier $\delta^{13}C$ values are usually associated with coastal-benthic environments (Fry 2008). Conversely, in a broader temporal window, the green turtles demonstrate a carnivore-omnivore strategy, such as represented by heavier $\delta^{15}N$ values in the carapace. Heavier $\delta^{15}N$ values usually represent a greater contribution of animal matter to the consumer diet (Fry 2008), but they could represent variations in isotopic baseline across turtles' habitat. Different sources of nitrogen in turtles foraging sites influence the isotopic profile of turtles from the same trophic level (Ceriani et al. 2012; Pajuelo et al. 2012). However, if the $\delta^{15}N$ values represented the habitat baseline instead of the ingestion of animal matter, we would expect closer $\delta^{15}N$

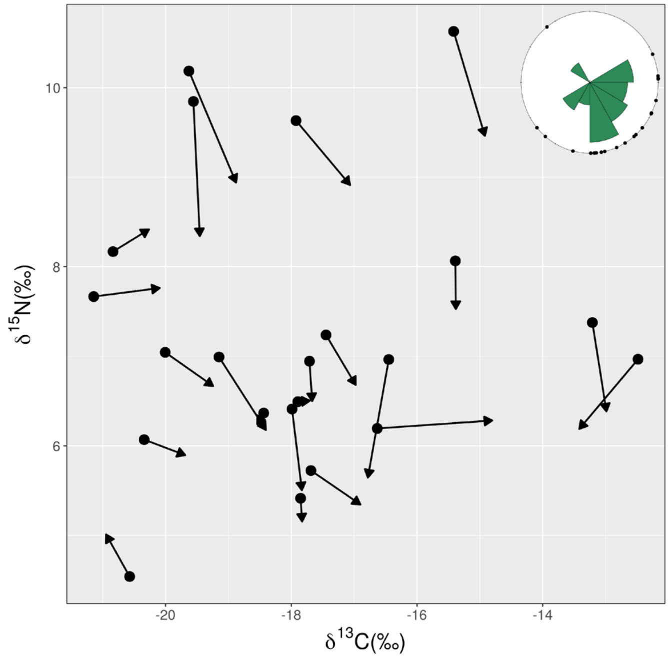

Figure 1. Individual niche trajectories represented by vectors between carapace (black circle) and yolk (black arrow) isotope values, for nesting green turtles on Rocas Atoll, Brazil. The inset represents the observed circular distribution of niche trajectory angles.

values between yolk and carapace in the same turtle, which was noted for only three individuals (Figure 1).

The temporal diet shift demonstrated for the green turtles that nest on Rocas Atoll had a directional pathway, albeit some individual tendencies are noted (Figure 1, Supplementary material). In the four individuals with the highest $\delta^{15}N$ values in the carapace (> 9‰), both oceanic and coastal foraging habitats are represented ($\delta^{13}C$ range), indicating isotopic enriched in different areas. In three individuals, yolk and carapace samples have similar $\delta^{15}N$ values and the temporal diet shift is not evident. Similarities between tissues regarding $\delta^{15}N$ values could represent habitat baseline. Individuals from the same population can vary in their foraging strategies over time, while others may have a more constant diet (Bearhop et al. 2004; Martínez del Rio et al. 2009). The nesting populations of sea turtles represent a mix of individuals from several foraging sites, and satellite telemetry reveals high fidelity to specific sites following breeding migrations (Meylan et al. 2011; Shimada et al. 2020). For the green turtle, Shimada et al. (2020) stated high and long-term fidelity to specific foraging sites, with feeding activity also happening during the migration to-and-from the breeding sites. These features support the individual tendencies described above.

Herbivory is recognized as the predominant feeding habit in adult green turtles (Bjorndal 1997; Burgett et al. 2018), but the adult individuals have enough feeding plasticity to take advantage of other food resources when necessary and available, allowing them to behave temporally like carnivores-omnivores (Burkholder et al. 2011). Agostinho et al. (2020) mentioned the possible foraging activity of nesting green turtles while stay at Rocas Atoll for breeding due to the high diversity of food items locally. Indeed, this area is a foraging site for juvenile green and hawksbill turtles (Bellini et al. 2013). The Rocas Atoll biodiversity includes 143 taxa of macroalgae (Villaça et al., 2010) and a range of zoobenthos and fish species (Moraes et al. 2003; Paiva et al. 2007; Batista et al. 2012; Paiva et al. 2015). The higher $\delta^{15}N$ values for oceanic habitats (more negative $\delta^{13}C$ values) noted in two nesting individuals (Figure 1) could be related to the animal matter ingested around the nesting site, that is an oceanic habitat; or even during the migration to-and-from the nesting site.

Figgener et al. (2019) organized an isotopic database for sea turtles species worldwide, demonstrating the variability of isotopic signatures among them, both inter- and intraspecifically, as well as similarities in the isotopic profile. The green turtle is the second most studied species, with 40% of the available stable isotope studies until November 2018 (Figgener et al. 2019). Meanwhile, data on the isotopic profile of nesting green turtles are still scarce, limited to five studies until the above period (Godley et al. 1998; Hatase et al. 2006; Vander Zanden et al. 2013a; 2013b; Bradshaw et al. 2017). Thus, this study also contributes to the database on $\delta^{13}C$ and $\delta^{15}N$ values in this species (Supplementary material).

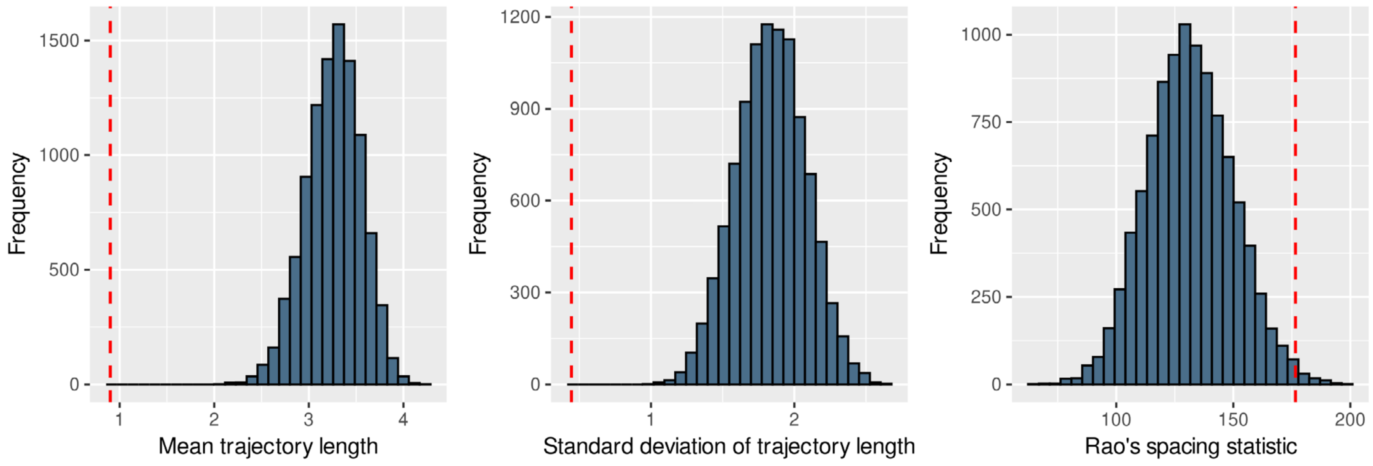

Figure 2. Null distributions of niche trajectory statistics: mean and standard deviation of trajectory length, the Euclidean distance between carapace and yolk stable isotope values from a given green turtle individual, and Rao's spacing statistic, quantifying the degree of directionality in niche trajectories. Red vertical lines indicate observed values.

Despite the small sample size (n = 22), the isotopic niche trajectories had strong statistical support to demonstrate the temporal diet shift for most green turtle individuals that nest on Rocas Atoll, off northeastern Brazil, during 2019 nesting season. To our knowledge, this is the first study that presents this approach for sea turtles, adding a new isotopic tool to understand the trophic ecology of these migrant animals. This approach has potential to be applied in other green turtle populations and/or in other sea turtle species. Since the adult green turtles can feed in the full extent of the habitat (foraging sites, breeding sites and to-and-from), changes in the food availability in these sites and over their migratory routes might compromise the health of the reproductive population. In a global scenario of rapid environmental changes, it deserves concern because sea turtles are long-lived endangered animals, and most species, like the green turtles, have high site fidelity for both feeding and breeding sites.

Supplementary Material

The following online material is available for this article:

Stable isotopes data of nesting green turtles on Rocas Atoll, Brazil (2019 nesting season)

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Author Contributions

Karoline Fernanda Ferreira Agostinho: sampling, methodology.

Leandro Rabello Monteiro: formal analysis, writing - review & editing.

Ana Paula Madeira Di Beneditto: funding acquisition, conceptualization, investigation, writing - original draft, writing - review & editing.

Conflicts of Interest

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

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Coleopterans (Hexapoda, Coleoptera) associated with canopies of *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) in the Brazilian Pantanal

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YAMAZAKI, L., VINDICA, V.F., ROSADO-NETO, G.H., MARQUES, M.I., BATTIROLA, L.D. **Coleopterans (Hexapoda, Coleoptera) associated with canopies of *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) in the Brazilian Pantanal.** Biota Neotropica 21(1): e20200982. https://doi.org/10.1590/1676-0611-BN-2020-0982

Abstract: The study of coleopteran assemblages associated with the canopies of tropical forests can help to expand the knowledge about species diversity and the ecological patterns related to the distribution of this diversity in tropical environments, including wetlands such as the Brazilian Pantanal. In this scenario, the present study examined the effect of temporal variation on the abundance, richness and distribution of the Coleoptera assemblage associated with canopies of *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) throughout the dry and high-water seasonal periods in the Northern Pantanal of Mato Grosso, Brazil. Twelve specimens of *C. fasciculata* were sampled between 2010 and 2011, six per seasonal period, by thermal fogging with insecticide. A total of 1,663 coleopterans were collected, consisting of 1,572 adults and 91 larvae. The adults are distributed into 38 families and 251 species or morphospecies. Chrysomelidae, Curculionidae, Nitidulidae and Tenebrionidae predominated in terms of abundance and richness. Family distribution, abundance, species richness and trophic guilds varied between the dry and high-water periods, demonstrating a relationship with the phenological conditions of *C. fasciculata* in addition to seasonality effect. In conclusion, the seasonality imposed by the hydrological regime and its influence on the phenology of *C. fasciculata* affect the structure of the coleopteran assemblage associated with the canopy of this monodominant vegetation formation in the Pantanal of Mato Grosso.

Keywords: *Beetles; Biodiversity; Seasonality; Wetlands.*

Coleópteros (Hexapoda, Coleoptera) associados às copas de *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) no Pantanal brasileiro

Resumo: O estudo de assembleias de coleópteros associadas às copas de florestas tropicias contribui para a expansão do conhecimento sobre a diversidade de espécies e os padrões ecológicos relacionados à distribuição dessa diversidade em ambientes tropicais, incluindo áreas úmidas como o Pantanal brasileiro. Nesse cenário, o presente estudo avaliou o efeito da variação temporal sobre a abundância, riqueza e distribuição da assembleia de Coleoptera associada às copas de *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) ao longo dos períodos de seca e cheia na região norte do Pantanal de Mato Grosso, Brasil. Doze exemplares de *C. fasciculata* foram amostrados entre 2010 e 2011, seis por período sazonal, utilizando-se termonebulização com inseticida. Um total de 1.663 coleópteros foram coletados, consistindo em 1.572 adultos e 91 larvas. Os adultos se distribuem em 38 famílias e 251 espécies e morfoespécies. Chrysomelidae, Curculionidae, Nitidulidae e Tenebrionidae predominaram em termos de abundância e riqueza. A distribuição das famílias, abundância, riqueza de espécies e agrupamentos em guildas tróficas variaram entre os períodos de seca e cheia, evidenciando uma estreita relação com as condições fenológicas de *C. fasciculata* ocasionadas pelo efeito sazonal dessa região. Conclui-se que a sazonalidade imposta pelo regime hidrológico do Pantanal e sua influência sobre a fenologia de *C. fasciculata* afetam a estrutura da assembleia de coleópteros associados às copas nessa formação monodominante no Pantanal de Mato Grosso.

Palavras-chave: *Áreas inundáveis; Besouros; Biodiversidade; Sazonalidade.*

Introduction

The forest canopy is habitat to a high number of organisms. Different animal assemblages occupy the niches available in those habitats according to their complexity, structure and dynamics as well as their close relationship with the maintenance of forest stability (e.g. Basset et al. 2003a, Erwin 2013, Fotis et al. 2018). In this forest stratum, the availability of a greater variety of food resources is essential for the maintenance of a high number of species such as those of the Order Coleoptera, which participate in various ecological processes in the forest canopy due to its richness and multiple food habitats (Erwin 1983, Hammond et al. 1996, Adis et al. 2010).

Studies on tree canopies have related the interaction of these insects to specific plant hosts, indicating the existence of very close evolutionary relationships between plants and coleopterans involving pollination, herbivory, seed predation, as well as activity as trophic links in food chains (Basset 1992, Ødegaard 2003, 2004, Novotny & Basset 2005). Some coleopterans utilize those habitats in only one of the phases of their life cycle; e.g. Curculionidae and Chrysomelidae species which complete larval development in the soil and migrate to the forest canopy when adult (Adis 1988, Lawrence & Britton 1991), or Cerambycidae species which use the plant hosts when adult and synchronize their larval development on branches fallen to the ground to the flood cycles in flooded areas like the Pantanal (Marques et al. 2014). In addition to the interactions between coleopterans and their plant hosts, these insects are widespread through aquatic and terrestrial ecosystems, constituting the most numerous and diverse Order of the Animal kingdom, where they represent 35% of all known insects (Marinoni 2001, Casari & Ide 2012).

Studies in the Northern Pantanal of Mato Grosso have reported the existence of Coleoptera assemblages with high species richness values associated with different terrestrial habitats (Carneiro et al. 2006, Marques et al. 2016), and plant hosts, including *Attalea phalerata* Mart. (Arecaceae) (Santos et al. 2003, Battirola et al. 2014), *Calophyllum brasiliense* Cambess. (Calophyllaceae) (Marques et al. 2007), and *Vochysia divergens* Pohl. (Vochysiaceae) (Marques et al. 2001, 2006). These studies also demonstrated the temporal variation of those assemblages as a function of the dry and high-water periods, which are characteristic of this Pantanal region. This confirms their complexity and importance for the maintenance of the biodiversity of this floodplain (Junk et al. 2006, 2013, Wantzen et al. 2016, Tomas et al. 2019).

In addition to the specific hydrological conditions in the northern region of the Pantanal, its landscape formed by a mosaic of forest and field units, flooded or permanently dry, makes studies on the diversity associated with these different types of habitats, fundamental to understand the dynamics of this ecosystem (Junk et al. 2006), mainly, assessing groups with high species richness as Coleoptera. In this mosaic of landscapes, monodominant forests stand out, as important elements in the Northern Pantanal region (Nunes da Cunha & Junk 2014), as the formations of *Callisthene fasciculata* (Spr.) Mart. (Vochysiaceae) (Corsini & Guarim-Neto 2000). In these forest formations, considering the specific and in-depth discussions that each taxonomic group needs, specific results to some arthropod groups associated with *C. fasciculata* canopies have already been published as spiders (Yamazaki et al. 2017), and ants (Yamazaki et al. 2016). Despite this, evaluations with other taxonomic groups are still needed to know the real richness of species present in these region.

In view of the importance of conserving wetlands to maintain the great biological diversity of those ecosystems as well as the complexities of coleopteran assemblages in the forest canopy, the present study examines the effect of temporal variation on the abundance, richness and distribution of the Coleoptera assemblage associated with *C. fasciculata* (Spr.) Mart. (Vochysiaceae) canopies as a function of the high-water and dry seasonal periods in the Northern Pantanal of Mato Grosso, Brazil.

Materials and methods

1. *Study area*

Sampling was carried out in a seasonally flooded monodominant forest with predominance of *C. fasciculata,* locally called *"*carvoal*"*, in the northern region of the Pantanal of Mato Grosso, on Porto Cercado road, specifically, at the Alvorada farm (16°26'56" S and 56°24'33" W), in Poconé - MT, Brazil (Figure 1). The local climate is a tropical savannah characterized by dry winters and wet summers, with temperatures ranging between 22 and 32 ºC (Hasenack et al. 2003), and an AW type under the Köppen classification. Annual precipitation varies between 1,000 and 1,500 mm, with periods of rainfall below 10 mm occurring over several months. This region has well-defined seasons, with a rainy period between October and March and a dry period between April and September, resulting in a hydrological cycle with four distinct seasonal periods (high water, receding water, dry period and rising water) (Heckman 1998).

As discussed by Yamazaki et al. (2016, 2017), *C. fasciculata* is a tree that reaches between four and 15 m in height and has a dark, thick and very rough bark (Pott & Pott 1994). The flowering period occurs between September and October, together with the dispersion of seeds generated in the previous year (Custódio et al. 2014). The species is characterized as deciduous, with no leaf fall or partial leaf fall from the rising water to the beginning of the dry period and total leaf fall at the end of the dry period. During the high-water period *C. fasciculata* presents well-developed foliage (Corsini & Guarim-Neto 2000).

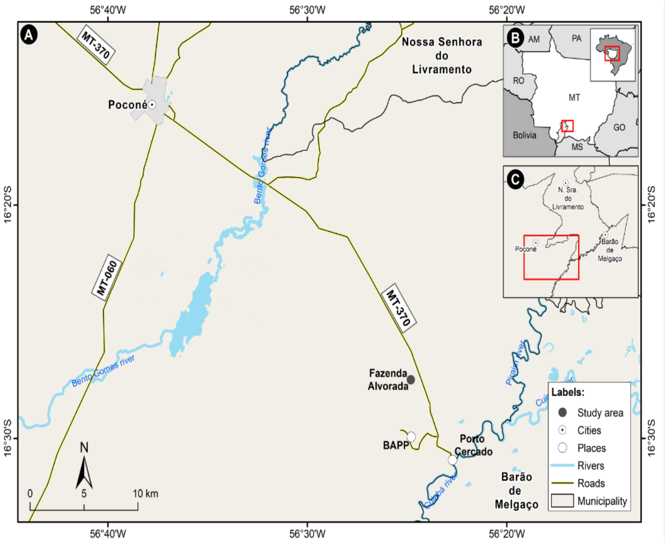

Figure 1. Location of the study area at Alvorada farm (A) in the Northern Pantanal of Mato Grosso, on Porto Cercado road, in Poconé – Mato Grosso, Brazil (B and C).

2. *Methodology*

Canopy fogging was performed in 12 *C. fasciculata* canopies, six during the high water (February/2010) and six in the dry period (September/2011). The trees were selected during the dry and high-water periods, maintaining a minimum distance of 10 m between each specimen. All of them were between 10 and 12 m high, with isolated crown, without interference from branches and foliage of other plants in the same area. The absence of epiphytes, bird nests and colonies of social insects (e.g. ants, bees and termites) was also observed, according to the criteria proposed by Adis et al. (1998), and the methodological procedures of Battirola et al. (2004), and Yamazaki et al. (2016, 2017). The entire diameter at the base of the trees was surrounded by nylon funnels (area of 1 m^2 each) distributed according to the reach and architecture of the canopy, totaling 120 m^2 of sample area (10 m^2 per sampled tree). The base of each collecting funnel contained 92% alcohol in a plastic collecting vial which was suspended 1 m from the ground by ropes tied to neighboring trees (Yamazaki et al. 2016, 2017). During the high-water period, the funnels were suspended 1.5 m from the ground due to raised water levels in the forest (water depth ranged from 0.1 to 0.3 m) (Yamazaki et al. 2016, 2017).

Canopy fogging was carried out for 10 min in each tree, using 0.5% Lambdacialotrin (Icon®) synthetic pyrethroxide diluted in two liters of diesel oil at a concentration of 1% (20 ml), combined with Synergist (DDVP) 0.1% (2 ml). The thermo-fogger used was Swingfog SN50, which produces a strong jet that is directed from the ground to all parts of the canopy. These procedures always occurred at approximately 06h00, when air circulation is less intense, allowing the insecticide cloud to rise slowly through the canopy without dispersing (Adis et al. 1998). Collections were carried out two hours after insecticide application (fogging) on each sampled tree, which the recommended time frame for this action (Adis et al. 1998). Following this procedure, the funnel walls were manually shaken and washed with a spray containing 92% alcohol and the material was packed in collection flasks located at the funnel bottoms (Yamazaki et al. 2016, 2017).

The collected material was transported to the Biological Collection of Southern Amazon (ABAM), at the Federal University of Mato Grosso, in Sinop - MT, Brazil, to be screened and quantified. All adults were identified at the family level according to Bouchard et al. (2011), Triplehorn et al. (2011) and Casari & Ide (2012). After this procedure, they were identified at the lowest possible taxonomic levels (Appendix A) at the Collection Reference of the Laboratory of Ecology and Taxonomy of Arthropods (LETA) at the Institute of Biosciences at the Federal University of Mato Grosso, in Cuiabá - MT, and Padre Jesus Santiago Moure Entomological Collection, in the Department of Zoology at the Federal University of Paraná, in Curitiba - PR, where the control material is deposited. The trophic guilds were determined following Arnett (1963), Erwin (1983), Hammond et al. (1996) and Marinoni et al. (2001).

3. *Data analysis*

The variation in Coleoptera abundance and richness between the dry and high-water periods was evaluated based on the t test. To determine the distribution of Coleoptera clusters between the trophic guilds and between the dry and high-water periods, indirect ordination was accomplished via Non-Metric Multidimensional Scaling (NMDS), using the first ordination axis. Ordination was carried out using quantitative data and the Bray-Curtis similarity measure. Species richness was estimated based on the Bootstrap and Jackknife 1 estimators. The Vegan package (Oksanen et al. 2013) was used for the analyses of NMDS and richness estimates. All analyses were performed using R software version 3.0.1 (R Core Team 2013).

Results

1. *Coleoptera assemblage*

A total of 1,663 coleopterans were sampled from 12 canopies of *C. fasciculata*, consisting of 1,572 adults (94.5%; 13.1 ind./m^2) and 91 larvae (5.5%; 0.8 ind./m^2). The adults were distributed into 38 families and 251 species and morphospecies. The predominant families were Chrysomelidae (447 ind.; 28.4%; 3.7 ind./m^2), Curculionidae (317 ind.; 20.2%; 2.6 ind./m^2), Nitidulidae (188 ind.; 12.0%; 1.6 ind./m^2) and Tenebrionidae (118 ind.; 7.5%; 1.0 ind./m^2), totaling 1,070 individuals (68.7% of the total sampled; 8.9 ind./m^2). Among the 38 families, 14 occurred exclusively in the high-water period (e.g. Nitidulidae, Elateridae and Ptilodactylidae), whereas Hydraenidae, Melyridae, Monotomidae and Ptiliidae where the families which occurred only in the dry period (Table 1).

A total of 251 Coleoptera species and morphospecies were identified. The Bootstrap richness estimator indicated that 306 species should be sampled, whereas Jackknife 1 pointed to the possibility of sampling of 380 species. Therefore, the sampling corresponded to 82 and 66% of the species expected for the assemblage, respectively (Figure 2). The greatest species richness was recorded for Chrysomelidae (40 spp.) and Curculionidae (40 spp.), followed by Staphylinidae (23 spp.), Tenebrionidae (14 spp.) and Elateridae (12 spp.), representing 51.4% of the total richness obtained. Although Nitidulidae was numerically abundant, only five species were recorded, and Staphylinidae exhibited elevated richness, it showed low abundance (53 ind.; 3.4%; 0.4 ind./m^2) (Table 1).

Typophorus florigradus Bechyné, 1961 (Chrysomelidae, Eumolpinae) (272 ind.; 17.3%; 2.3 ind./m^2), *Mystrops* sp. 3 (Nitidulidae, Nitidulinae) (117 ind.; 7.4%; 1.0 ind./m^2), *Pandeleteius* sp. 1 (Curculionidae, Entiminae) (85 ind./5.4%; 0.7 ind./m^2), *Lechriops* sp. 2 (Curculionidae, Conoderinae) (75 ind.; 4.8%; 0.6 ind./m^2) and *Antitypona* sp. 1 (Chrysomelidae, Eumolpinae) (70 ind.; 4.4%; 0.6 ind./m^2) totaled 619 individuals (37.2%; 5.2 ind./m^2), corresponding to the most abundant species in this assemblage (Appendix A).

2. *Temporal variation*

A comparison via indirect ordination of the data of the Coleoptera family (NMDS) between the high-water and dry periods revealed a stress index of 0.12. The result of the t test, which compares the scores of the axis, revealed differences between the distribution of families for the two periods (t test = –10.269; DF = 8.603; $p < 0.001$) (Figure 3) and also that the high-water and dry periods are different regarding abundance (t test = 5.329; DF = 7.167; p = 0.001) and species richness (t test = 4.338; DF = 6.853; p = 0.004) (Figures 4A and B).

With regards the abundance of the Coleoptera assemblage, 1,324 individuals were sampled in the high-water period, consisting of 1,251 adults (94.5%: 20.8 ind./m^2) and 73 larvae (5.5%; 1.2 ind./m^2). The adults were represented by 34 families and 184 species. The most

Table 1. Number of individuals (N), relative abundance (%), density (ind./m^2), richness (S) and trophic guilds of Coleoptera obtained from *Callisthene fasciculata* canopies in the high-water and dry periods in the Northern Pantanal of Mato Grosso. *Saprophage (S), Fungivore (F), Herbivore (H), Predator (P), Decomposer (D), Feeding habit considered secondary ( ).

Families/Subfamilies	HIGH WATER				DRY SEASON				TOTAL				Trophic Guilds*
	N	%	Ind./m^2	S	N	%	Ind./m^2	S	N	%	Ind./m^2	S	
Aderidae	2	0.2	<0.1	2	4	1.2	0.1	3	6	0.4	<0.1	4	S
Anthribidae (Anthribinae)	2	0.2	<0.1	2	2	0.6	<0.1	2	4	0.2	<0.1	4	F
Bothrideridae	20	1.6	0.3	1	4	1.2	0.1	1	24	1.5	0.2	1	H
Brentidae (Apioninae)	8	0.6	0.1	3	12	3.7	0.2	5	20	1.3	0.2	7	H
Buprestidae	2	0.2	<0.1	2	60	18.7	1.0	3	62	3.9	0.5	4	H
Carabidae	58	4.6	1.0	15	13	4.0	0.2	9	71	4.5	0.6	19	P
(Harpalinae)	(48)	(82.8)	(0.8)	(12)	(12)	(92.3)	(0.2)	(8)	(60)	(84.5)	(0.5)	(16)	P
(Paussinae)	(2)	(3.4)	(<0.1)	(1)	-	-	-	-	(2)	(2.8)	(<0.1)	(1)	P
(Trechinae)	(1)	(1.7)	(<0.1)	(1)	-	-	-	-	(1)	(1.4)	(<0.1)	(1)	P
Cerambycidae (Lamiinae)	3	0.2	<0.1	1	-	-	-	-	3	0.2	<0.1	1	H
Chrysomelidae	424	33.9	7.1	30	23	7.2	0.4	18	447	28.4	3.7	40	H
(Eumolpinae)	(353)	(83.2)	(5.9)	(7)	(2)	(8.7)	(<0.1)	(1)	(355)	(79.4)	(3.0)	(7)	H
(Cryptocephalinae)	(12)	(2.8)	(0.2)	(4)	-	-	-	-	(12)	(2.7)	(0.1)	(4)	H
(Galerucinae)	(48)	(11.3)	(0.8)	(12)	(15)	(65.2)	(0.2)	(12)	(63)	(14.1)	(0.5)	(19)	H
(Bruchinae)	(11)	(4.3)	(0.2)	(7)	(6)	(26.1)	(0.1)	(5)	(17)	(3.8)	(0.1)	(10)	H
Ciidae	4	0.3	0.1	2	-	-	-	-	4	0.2	<0.1	2	F
Cleridae	1	0.1	<0.1	1	3	0.9	<0.1	2	4	0.2	<0.1	3	P
Coccinellidae	3	0.2	<0.1	2	10	3.1	0.2	5	13	0.8	0.1	7	P
(Scymninae)	(1)	(33.3)	(<0.1)	(1)	(10)	(100.0)	(0.2)	(5)	(11)	(84.6)	(0.2)	(6)	P
(Sticholotidinae)	(2)	(66.7)	(<0.1)	(1)	-	-	-	-	(2)	(15.4)	(<0.1)	(1)	P
Corylophidae	20	1.6	0.3	5	4	1.2	0.1	2	24	1.5	0.2	5	P
Cucujidae	16	1.3	0.3	6	3	0.9	<0.1	3	19	1.2	0.2	8	P (F)
Curculionidae	220	17.6	3.7	29	97	30.2	1.6	18	317	20.2	2.6	40	H
(Baridinae)	(1)	(0.4)	(<0.1)	(1)	(1)	(1.0)	(<0.1)	(1)	(2)	(0.6)	(<0.1)	(2)	H
(Ceutorhynchinae)	(1)	(0.4)	(<0.1)	(1)	-	-	-	-	(1)	(0.3)	(<0.1)	(1)	H
(Conoderinae)	(76)	(34.5)	(1.3)	(2)	(6)	(6.2)	(0.1)	(4)	(82)	(25.9)	(0.7)	(6)	H
(Cryptorhynchinae)	(6)	(2.7)	(0.1)	(4)	(43)	(44.3)	(0.7)	(2)	(49)	(15.5)	(0.4)	(6)	H
(Curculioninae)	(28)	(12.7)	(0.5)	(9)	(16)	(16.5)	(0.3)	(4)	(44)	(13.9)	(0.4)	(11)	H
(Cyclominae)	(1)	(0.4)	(<0.1)	(1)	-	-	-	-	(1)	(0.3)	(<0.1)	(1)	H
(Entiminae)	(91)	(41.4)	(1.5)	(2)	(12)	(12.4)	(0.2)	(1)	(103)	(32.5)	(0.9)	(2)	H
(Erirhininae)	(2)	(0.9)	(<0.1)	(2)	(2)	(2.1)	(<0.1)	(1)	(4)	(1.3)	(<0.1)	(3)	H
(Mesoptiliinae)	(7)	(3.2)	(0.1)	(3)	(2)	(2.1)	(<0.1)	(1)	(9)	(2.8)	(0.1)	(3)	H
(Molytinae)	(1)	(0.4)	(<0.1)	(1)	(1)	(1.0)	(<0.1)	(1)	(2)	(0.6)	(<0.1)	(2)	H
(Platypodinae)	(1)	(0.4)	(<0.1)	(1)	(2)	(2.1)	(<0.1)	(1)	(3)	(0.9)	(<0.1)	(1)	F
(Scolytinae)	(5)	(2.3)	(0.1)	(2)	(12)	(12.4)	(<0.1)	(2)	(17)	(5.4)	(0.1)	(2)	F
Elateridae	67	5.4	1.1	12	-	-	-	-	67	4.3	0.6	12	H
(Agrypninae)	(62)	(92.5)	(1.0)	(7)	-	-	-	-	(62)	(92.5)	(0.5)	(7)	H
Histeridae	2	0.2	<0.1	2	-	-	-	-	2	0.1	<0.1	2	P
Hydraenidae	-	-	-	-	1	0.3	<0.1	1	1	0.1	<0.1	1	P
Lampyridae	9	0.7	0.1	4	-	-		-	9	0.6	0.1	4	P
Latridiidae	3	0.2	<0.1	2	15	4.7	0.2	5	18	1.1	0.1	5	F
Limnichidae	1	0.1	<0.1	1	-	-	-	-	1	0.1	<0.1	1	S
Melolonthidae	4	0.3	0.1	2	-	-	-	-	4	0.2	<0.1	2	H
(Melolonthinae)	(1)	(25)	(<0.1)	(1)	-	-	-	-	(1)	(25)	(<0.1)	(1)	H
(Rutelinae)	(3)	(75)	(<0.1)	(1)	-	-	-	-	(3)	(75)	(<0.1)	(1)	H
Melyridae	-	-	-	-	2	0.6	<0.1	2	2	0.1	<0.1	2	P
Monotomidae	-	-	-	-	1	0.3	<0.1	1	1	0.1	<0.1	1	F
Mordellidae	2	0.2	<0.1	2	-	-	-	-	2	0.1	<0.1	2	P
Nitidulidae (Nitidulinae)	188	15	3.1	5	-	-	-	-	188	12	1.6	5	S
Phalacridae	2	0.2	<0.1	2	3	0.9	<0.1	3	5	0.3	<0.1	3	H
Phengodidae	1	0.1	<0.1	1	-	-	-	-	1	0.1	<0.1	1	P
Ptiliidae	-	-	-	-	1	0.3	<0.1	1	1	0.1	<0.1	1	D
Ptilodactylidae	21	1.7	0.3	1	-	-	-	-	21	1.3	0.2	1	H
Pitnidae	20	1.6	<0.1	8	19	5.9	0.3	4	39	2.5	0.3	8	H

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(Dorcatominae)	(9)	(45.0)	(0.1)	(2)	(10)	(52.6)	(0.2)	(2)	(19)	(48.7)	(0.2)	(2)	H
(Mesocoelopodinae)	(11)	(55.0)	(0.2)	(6)	(9)	(47.4)	(0.1)	(2)	(20)	(51.3)	(0.3)	(6)	H
Scarabaeidae (Scarabaeinae)	2	0.2	<0.1	1	-	-	-	-	2	0.1	<0.1	1	D
Scirtidae	1	0.1	<0.1	1	2	0.6	<0.1	1	3	0.2	<0.1	2	H
Scydmaenidae	11	0.9	0.2	3	1	0.3	<0.1	1	12	0.8	0.1	4	P
Silvanidae	1	0,1	<0.1	1	-	-	-	-	1	0.1	<0.1	1	F
Staphylinidae	27	2.2	0.4	15	16	5.0	0.3	12	53	3.4	0.4	23	P
(Aleocharinae)	(12)	(44.4)	(0.2)	(7)	(7)	(43.7)	(0.1)	(6)	(19)	(35.8)	(0.2)	(12)	P
(Oxytelinae)	(2)	(7.4)	(<0.1)	(2)	(3)	(18.7)	(<0.1)	(1)	(5)	(9.4)	(<0.1)	(2)	P
(Paederinae)	(8)	(29.6)	(0.1)	(3)	(3)	(18.7)	(<0.1)	(3)	(11)	(20.7)	(0.1)	(5)	P
(Pselaphinae)	(2)	(7.4)	(<0.1)	(2)	(2)	(12.5)	(<0.1)	(1)	(4)	(7.5)	(<0.1)	(3)	F
(Scaphidiinae)	(3)	(11.1)	(<0.1)	(1)	(1)	(6.2)	(<0.1)	(1)	(4)	(7.5)	(<0.1)	(1)	F
Tenebrionidae	96	7.7	1.6	12	22	6.8	0.4	6	118	7.5	1.0	14	S
(Alleculinae)	(62)	(69.7)	(1.0)	(5)	(10)	(45.4)	(0.2)	(2)	(72)	(64.9)	(0.6)	(5)	H
(Diaperinae)	(18)	(20.2)	(0.3)	(3)	(12)	(54.5)	(0.2)	(4)	(30)	(27.0)	(0.2)	(5)	S
(Lagriinae)	(7)	(0.6)	(0.1)	(1)	-	-	-	-	(7)	(0.4)	(0.1)	(1)	S
(Pimieliinae)	(5)	(5.6)	(0.1)	(2)	-	-	-	-	(5)	(4.5)	(<0.1)	(2)	S
Throscidae	5	0.4	0.1	3	2	0.6	<0.1	1	7	0.4	0.1	4	H
Zopheridae	5	0.4	0.1	5	1	0.3	<0.1	1	6	0.4	<0.1	6	F
(Colydiinae)	(1)	(20.0)	(<0.1)	(1)	(1)	(100.0)	(<0.1)	(1)	(2)	(33.3)	(<0.1)	(2)	P (F)
(Monominae)	(3)	(60.0)	(<0.1)	(3)	-	-	-	-	(3)	(50.0)	(<0.1)	(3)	H
(Zopherinae)	(1)	(2.00)	(<0.1)	(1)	-	-	-	-	(1)	(16.7)	(<0.1)	(1)	F
Total adults	1,251	94.5	20.8	184	321	94.7	5.3	110	1,572	94.5	13.1	251	-
Total immatures (larvae)	73	5.5	1.2	-	18	5.3	0.3	-	91	5.5	0.8	-	-
TOTAL	1,324	100.0	22.1	184	339	100.0	5.6	110	1,663	100.0	13.9	251	-

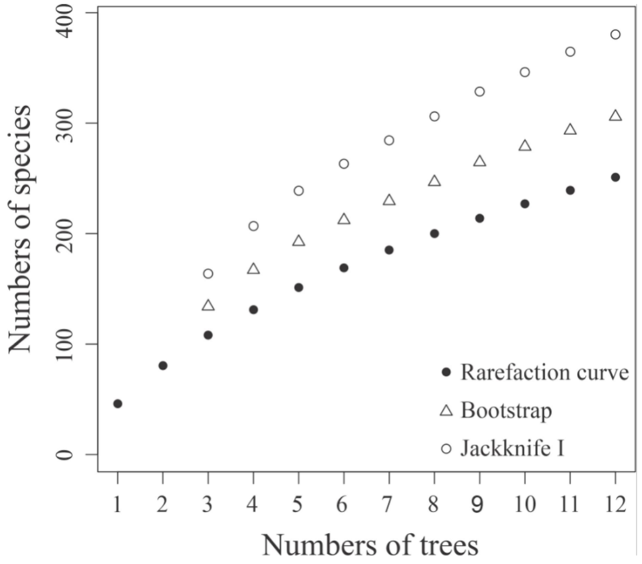

Figure 2. Observed (rarefaction curve) and estimated (Bootstrap and Jackknife 1) species richness of the Coleoptera community associated with *Callisthene fasciculata* canopies during the high-water (trees 1 to 6) and dry (trees 7 to 12) periods in the Northern Pantanal of Mato Grosso.

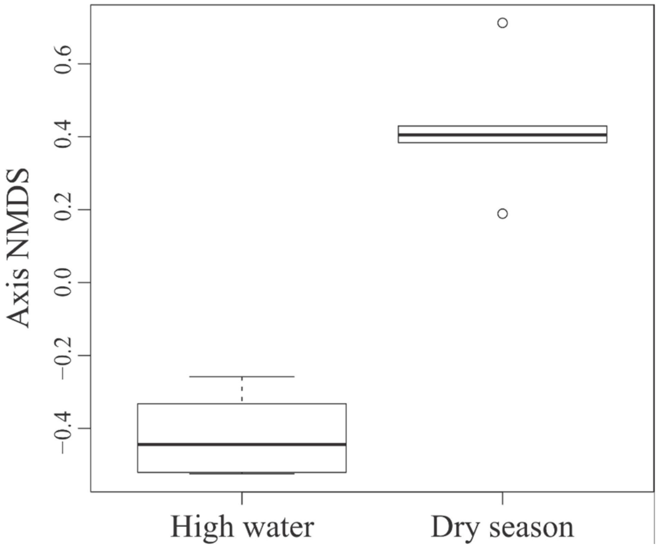

Figure 3. Comparison of scores of the NMDS axis generated from the distribution of 38 Coleoptera families on *Callisthene fasciculata* between the high-water and dry periods in the Northern Pantanal of Mato Grosso.

abundant families were Chrysomelidae (424 ind.; 33.9%; 7.1 ind./m^2), Curculionidae (220 ind.; 17.6%; 3.7 ind./m^2) and Nitidulidae (188 ind.; 15.0%; 3.1 ind./m^2), followed by Tenebrionidae (96 ind.; 7.7%; 1.6 ind./m^2) and Carabidae (58 ind.; 4.6%; 1.0 ind./m^2) (Table 1). During the dry period, only 339 coleopterans were collected (2.4%; 5.6 ind./m^2), 321 of which were adult (94.7%; 5.3 ind./m^2) and 18 larvae (5.3%; 0.3 ind./m^2). The adults are distributed into 25 families and 110 species. Curculionidae (97 ind.; 30.2%; 1.6 ind./m^2) and Buprestidae (60 ind.; 18.7%; 1 ind./m^2) were the most abundant. Carabidae, Chrysomelidae, Curculionidae and Tenebrionidae had their abundance reduced during the dry period, whereas that of Buprestidae rose in that period.

Among the adult coleopterans, 141 species (56.2%) occurred exclusively in the high-water period, and 68 species (27.1%) were sampled only in the dry period. The other 42 species were common to both seasonal periods (Appendix A). In the high-water period, Chrysomelidae (30 spp.), Curculionidae (29 spp.), Carabidae (15 spp.), Staphylinidae (15 spp.), Elateridae (12 spp.) and Tenebrionidae (12 spp.) represented 62.1% of the total richness (Table 1). *Typophorus florigradus* (Chrysomelidae, Eumolpinae) was the dominant species (270 ind.; 21.6%; 4.5 ind./m^2),

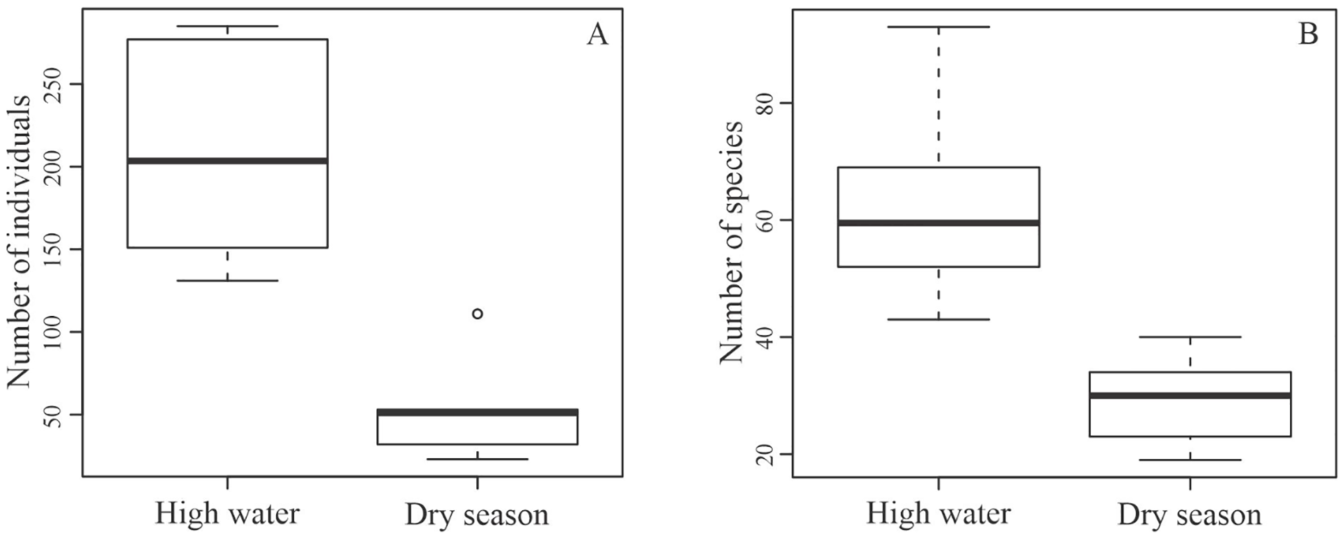

Figure 4. Comparison between abundance (A) and richness (B) of the Coleoptera community on *Callisthene fasciculata* canopies between the high-water and dry periods in the Northern Pantanal of Mato Grosso.

followed by *Mystrops* sp. 3 (Nitidulidae, Nitidulinae) (117 ind./9.4%; 1.9 ind./m^2), *Lechriops* sp. 2 (Curculionidae, Conoderinae) (75 ind.; 6.0%; 1.2 ind./m^2), *Pandeleteius* sp. 1 (Curculionidae, Entiminae) (73 ind.; 5.8%; 1.2 ind./m^2) and *Antitypona* sp. 1 (Chrysomelidae, Eumolpinae) (70 ind.; 5.6%, 1.2 ind./m^2) (Appendix A). In the dry period, Chrysomelidae and Curculionidae were the most diversified, with 18 species each, followed by Staphylinidae (12 spp.) and Carabidae (9 spp.) (Table 1). Buprestidae sp. 1 (58 ind.; 18.1%; 1.0 ind./m^2) and Cryptorhynchini sp. 1 (42 ind.; 13.1%; 0.7 ind./m^2) were the most abundant species in this period.

3. *Trophic guilds*

The coleopterans associated with the *C. fasciculata* canopies were distributed into five clusters of trophic guilds. Considering the abundance of individuals, herbivores (1,050 ind.; 66.8%; 8.7 ind./m^2) predominated, followed by saprophages (241 ind.; 15.3%; 2.0 ind./m^2), predators (221 ind.; 14.1%; 1.8 ind./m^2), fungivores (57 ind.; 3.6% and 0.5 ind./m^2) and decomposers (3 ind.; 0.2%; < 0.1 ind./m^2). The main herbivores were Chrysomelidae and Curculionidae. The most abundant saprophages corresponded to Nitidulidae and Tenebrionidae. Carabidae and Staphylinidae comprised the most numerous predators, whereas Platypodinae and Scolytinae (Curculionidae) and Latridiidae occurred at the highest frequency among the fungivores. The decomposers were represented by Scarabaeidae and Ptiliidae (Table 1).

In terms of species richness, the herbivores predominated, with 131 species (52.2%), followed by predators (78 spp.; 31.1%). Despite their reduced abundance, fungivores appeared with similar richness (21 spp.; 8.4%) to that of saprophages (19 spp.; 7.6%), while only two species were decomposers (0.8%). The indirect ordination of behavioral guild data (NMDS) between the high-water and dry periods resulted in a stress index of 0.04. The result of the t test, which compares the scores of the axis, showed the difference between the distribution of the clusters into guilds for the two periods (t test = –5.571; DF = 9.717; p > 0.001) (Figure 5).

The herbivores were more abundant both in the high-water (836 ind.; 66.8%; 13.9 ind./m^2) and in the dry period (214 ind.; 66.7%; 3.6 ind./m^2). In the high-water period, the herbivores were followed by the saprophages (225 ind.; 18.0%; 3.7 ind./m^2) and predators (166 ind.; 13.3%; 2.8 ind./m^2), whereas the fungivores and decomposers had the lowest abundance (24 ind.; 1.9%; 0.4 ind./m^2). During the dry period, the herbivores were followed by the predator beetles (55 ind.; 17.1%; 0.9 ind./m^2) and fungivores (35 ind.; 4.4%; 0.6 ind./m^2), while the saprophages and decomposers were the trophic clusters of lowest representativeness (17 ind.; 5.3%; 0.3 ind./m^2).

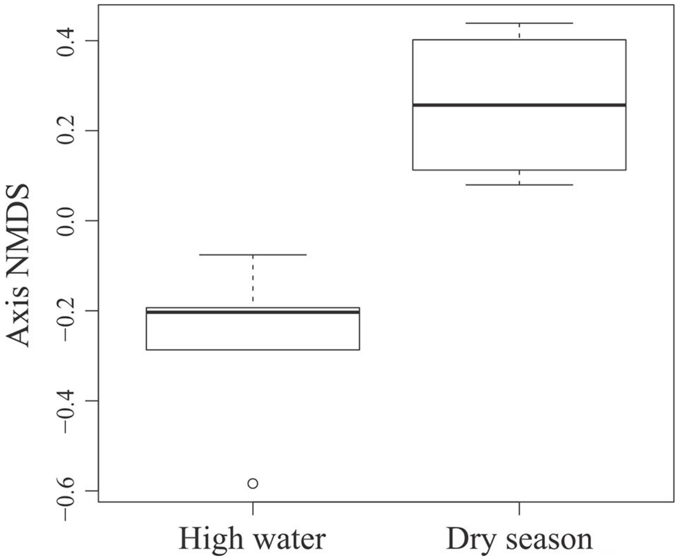

Figure 5. Comparison of the NMDS axis scores generated from the distribution of the five clusters into trophic guilds of Coleoptera on *Callisthene fasciculata* canopies between the high-water and dry periods in the Northern Pantanal of Mato Grosso.

Discussion

The Coleoptera assemblage associated with canopies of *C. fasciculata* has its temporal dynamics influenced in terms of abundance, richness and distribution according to the seasonality of the Pantanal biome. Distinct assemblages occur between the dry and high-water periods, reflecting the specificities of this floodplain as well as association with the phenological characteristics of this host tree. In *C. fasciculata*, the adult coleopterans were distributed into 38 families and 251 species and morphospecies.

The results obtained in our study are similar to those found by Marques et al. (2006) analyzing Coleoptera assemblages in canopy of *V. divergens*, another tree species that forms monodominant forests in the Pantanal and belongs to the Vochysiaceae family. Marques et al. (2006) obtained 256 species/morphospecies in 32 families of Coleoptera. Different results were obtained by Santos et al. (2003) and Battirola et al. (2014) on *A. phalerata* Mart. (Arecaceae) canopies in that same region of the Pantanal, showing that palm trees canopies are habitat to Coleoptera assemblages richer in species, when compared to the species of Vochysiaceae. Santos et al. (2003) obtained 326 species/morphospecies in 48 families during the dry season, while Battirola et al. (2014) found 467 species/morphospecies distributed in 43 Coleoptera families during the high-water period. Palm trees differ from other tree types, because of their specific canopy structure and architecture, which is likely due to organic matter accumulation in the remaining leaf sheaths adhered to the plant stem, which provides specific sites of reproduction, feeding and shelter for the associated species, like insects, as well as due to their more stable phenology (Santos et al. 2003, Battirola et al. 2007, Marques et al. 2009).

Canopies can be considered an important habitat model (Nadkarni 1994, Adams et al. 2017). Variations in host plant phenology, including the periods of fructification, flowering and leaf turnover may influence the availability of resources and niches available to the fauna (e.g. Basset et al. 2003b, Castaño-Meneses 2014). Evergreen plants such as palm trees maintain the resource and niche levels more stable throughout the year compared to plants with extreme phenological variations such as *C. fasciculata.* This species has specific periods of leaf turnover, flowering and fructification, and during the high-water period in the Pantanal, its canopy is characterized by full and mature foliage, whereas in the dry period there is a partial leaf fall and the canopy structure is markedly changed (Corsini & Guarim-Neto 2000). Therefore, it is possible that those variations in the phenology of *C. fasciculata* influenced the total Coleoptera richness, elevating its number during the high-water and reducing it during the dry period in this Pantanal region.

An example of association between host plant phenology and coleopterans can be observed in bruchins (Coleoptera: Chrysomelidae), which synchronize their life cycles to the phenology of their host plants, where flowering provides food to adults and fructification provides substrate to oviposition and larval development (Ribeiro-Costa & Almeida 2009). In studies conducted in the Pantanal of Mato Grosso, bruchins are always sampled as components of Coleoptera assemblages, but with low abundance among the Chrysomelidae (Marques et al. 2001, 2006, 2007, Santos et al. 2003, Battirola et al. 2014). Another species that can be highlighted in this study is *T. florigradus* (Chrysomelidae, Eumolpinae). Yamazaki et al. (2020) stated that the record of *T. florigradus* in association with *C. fasciculata* raises an important discussion about studies on the association between Chrysomelidae and its host plants. Such studies are essential for understanding phylogenetic or ecological theories related to the evolution of host selection and chemical interactions between insects and plants with emphasis on chemical prospecting. Oftentimes, these studies are focused on the host plant rather than on the insects or practices of use, conservation and protection of biodiversity (Flowers & Janzen 1997).

In *C. fasciculata* canopies, Chrysomelidae, Curculionidae and Staphylinidae are the families with greatest species richness. The dominance of these coleopterans is commonly observed in studies investigating the forest canopy of different regions (Erwin 1983, Floren & Linsenmair 1998, Wagner 2000, Santos et al. 2003, Ødegaard 2003, Marques et al. 2006, 2007, 2009, Adis et al. 2010, Battirola et al. 2014). According to Basset (2001), these groups correspond to the invertebrates with greatest richness in the canopy of tropical forests, which is explained by their eating habits, form of exploitation of these forests, and variety of habitats found.

In Uganda, Africa, Wagner (2000) found that Staphylinidae, Curculionidae and Chrysomelidae were the taxa with greatest species richness, whereas Latridiidae, Chrysomelidae and Staphylinidae were the most abundant. Curculionidae and Chrysomelidae were the families with greatest abundance and species richness in studies conducted in Panamá (Ødegaard 2003), similarly to results obtained in the Amazon (Erwin 1983) and in Malasya (Floren & Linsenmair 1998). Farrel & Erwin (1988) emphasized that the tree canopy architecture is a key factor determining the diversity of Staphylinidae and other groups of predator beetles, exerting a greater influence than the very floristic composition of the habitat.

Regarding the variation between the dry and high-water periods in *C. fasciculata* canopies, a higher number of species occurring only once, greater abundance and greater total richness were common to the high-water period. Marques et al. (2006) observed greater abundance and richness of Coleoptera during the high-water period, but a higher number of single-occurrence species during the dry period in *V. divergens* canopies. The authors associated this result with the flowering of the host plant that occurs during this period. Accordingly, the seasonal variation typical of the Pantanal ecosystem might have influenced the structure and composition of the Coleoptera assemblage on *C. fasciculata*, considering that the structure of canopy arthropod assemblages may vary throughout seasonal periods due to variations imposed by the hydrological regime in that region (Castilho et al. 2005, Marques et al. 2011, Battirola et al. 2014, 2016, 2017a, b, Yamazaki et al. 2016, 2017).

The assemblage organization based on clusters of trophic guilds demonstrated the predominance of herbivorous, saprophages and predator beetles, which corroborates the results obtained by Marques et al. (2006, 2007) and Battirola et al. (2014). Fungivores were also found among the most numerous trophic guilds in the studies of Marques et al. (2006) and Battirola et al. (2014) in the same region, contrasting with the present results. The low representativeness of decomposer beetles also conflicts with data obtained in the same region (Santos et al. 2003, Marques et al. 2007, 2009, 2011).

The distribution of clusters into trophic guilds allows us to evaluate the exploitation of resources by those insects. *Callisthene fasciculata* occurs in monodominant formations; i.e., forests where over 50% of the individuals correspond to the same plant species (Connell & Lowman 1989). This vegetation structure may affect the distribution of resources to the fauna, considering the lower number of tree species in its composition. Because of this factor, resource distribution can be more homogenized and less diversified, facilitating the access to and maintenance of species adapted to those conditions over others less adapted and thus dependent on a greater variety of resources (e.g. Southwood 1961, Root 1973).

Overall, it can be concluded that the temporal dynamics of the Coleoptera assemblage associated with *C. fasciculata* canopies is maintained by the hydrological seasonality of the Pantanal of Mato Grosso, which was evinced by variations in abundance, richness and distribution of the assemblage. Seasonality acts as a primary ecofactor in this biome (Junk et al. 1989, 2006, 2013, Wantzen et al. 2016), regulating the spatial-temporal dynamics of the animal and plant assemblages, exerting direct effects on the vegetation structure and its phenology, which, indirectly, interfere with the dynamics of the assemblages associated with specific plant hosts such as *C. fasciculata*. Based on those results, we emphasize the need for maintaining and conserving the hydrological conditions of the Pantanal and its vegetation formations to preserve the stability of ecosystems and, consequently, their rich biodiversity associated with this Neotropical floodplain.

Supplementary Material

The following online material is available for this article:

Appendix A - Species and morphospecies, number of individuals (N), relative abundance (%) and density (Ind./m^2) of adult Coleoptera obtained from *Callisthene fasciculata* canopies in the high-water and dry periods in the northern Pantanal of Mato Grosso, Brazil.

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Author Contributions

Lúcia Yamazaki: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Vanessa França Vindica: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Germano Henrique Rosado-Neto: Substantial contribution in the concept and design of the study; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Marinêz Isaac 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; Contribution to critical revision, adding intellectual content.

Leandro Dênis Battirola: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of interest

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

Ethics

The authors confirm that the manuscript has been submitted solely to this journal and is not published, in press, or submitted elsewhere; Confirm that all the research meets the ethical guidelines, including adherence to the legal requirements of the study country. Confirm that you have prepared a complete text minus the title page, acknowledgments, and any running headers with author names, to allow blinded review.

Data availability

The data is not yet available on digital platforms, however all the material is deposited in Entomological collections as reported in the manuscript.

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Inventory

Saprophytic flies in impacted areas of the Belo Monte Dam, Pará, Brazil (Diptera: Mesembrinellidae, Neriidae, Ropalomeridae, and Sarcophagidae): community composition, abundance, and species richness

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Abstract: The present study aims to document the community composition, abundance, and species richness of saprophytic fly species (Mesembrinellidae, Neriidae, Ropalomeridae, and Sarcophagidae) of the Volta Grande region of the Xingu River, a poorly sampled area impacted by the Belo Monte hydroelectric dam. Five collecting trips were carried out between 2014 and 2016, when traps baited with fermenting bananas were used. A total of 154 specimens, three genera, and six species were collected of Mesembrinellidae; 196 specimens, three genera, and seven species of Neriidae; 272 specimens, three genera, and six species of Ropalomeridae; and 624 specimens, 22 species and 10 genera of Sarcophagidae. Species accumulation curves for all families except Sarcophagidae demonstrated a strong tendency towards stabilization, showing that sampling efforts were sufficient to record most of the targeted species. *Laneela perisi* (Mariluis, 1987) (Mesembrinellidae) is a new record for the state of Pará. Among Ropalomeridae, *Apophorhynchus amazonensis* Prado, 1966, is a new record for Pará. Among Sarcophagidae, *Helicobia aurescens* (Townsend, 1927) is newly recorded from the Brazilian Amazon, and *Ravinia effrenata* (Walker, 1861) and *Titanogrypa larvicida* (Lopes, 1935) are new records for Pará.
Keywords: *anthropic impact, insect, invertebrate, tropical forest.*

Moscas saprofíticas na área sob influência da barragem de Belo Monte, Pará, Brasil (Diptera: Mesembrinellidae, Neriidae, Ropalomeridae e Sarcophagidae): composição, abundância e riqueza de espécies

Resumo: O presente estudo teve como objetivo documentar a composição, abundância e riqueza das espécies de moscas saprófitas (Mesembrinellidae, Neriidae, Ropalomeridae e Sarcophagidae) da Volta Grande do rio Xingu, uma área pouco amostrada e impactada pela Hidrelétrica Belo Monte. Foram realizadas cinco expedições de coleta entre 2014 a 2016, quando foram utilizadas armadilhas contendo banana em fermentação como isca. Foram coletados 154 espécimes, três gêneros e seis espécies de Mesembrinellidae; 196 espécimes, três gêneros e sete espécies de Neriidae; 272 espécimes, três gêneros e seis espécies de Ropalomeridae; e 624 espécimes, 22 espécies e 10 gêneros de Sarcophagidae. As curvas de acumulo de espécies para todas as famílias, exceto Sarcophagidae, demonstraram uma forte tendência à estabilização, mostrando que o esforço amostral foi suficiente para registrar a maioria das espécies-alvo. *Laneela perisi* (Mariluis, 1987) (Mesembrinellidae) é um novo registro para o estado do Pará. Entre os Ropalomeridae, *Apophorhynchus amazonensis* Prado, 1966, é um novo registro para o Pará. Em relação aos Sarcophagidae, *Helicobia aurescens* (Townsend, 1927) é um novo registro para a Amazônia Brasileira e *Ravinia effrenata* (Walker, 1861) e *Titanogrypa larvicida* (Lopes, 1935) são novos registros para o Pará.
Palavras-chave: *impacto antrópico, inseto, invertebrado, floresta tropical.*

Introduction

Biological inventories are essential for recording and describing the local biodiversity of some taxa from a given area, at a determined time and space where this information is lacking (Silveira et al. 2010), representing the first step towards the creation of an effective biodiversity conservation strategy and rational use of the environments (Margules & Austin 1991, Kim 1993, Santos 2003). In some cases, these inventories represent the only information available on the biota of an altered area or the historical record of previous occurrences of a given taxon (Tonetti et al. 2017). They are also the source of new species and new records (Brown et al. 2018). In addition, standardized inventories are the baseline for successful monitoring programs, which are a useful tool to evaluate rates of change of natural populations (Morrison et al. 2008, Silveira et al. 2010). As the data produced by inventories are essential to the establishment of sound conservation plans (Santos 2003), they should preferably be performed in places where the biota is, to some degree, extirpated or vulnerable to extinctions (Kim 1993) or where high species richness, endemism, or biogeographic and phylogenetic significance can be found (Erwin 1991, Vane-Wright et al. 1991, Kim 1993).

The Amazon basin of Brazil corresponds to about 6 million km^2, where approximately 10% of known animal and plant species occur, making it one of the most biodiverse tropical forests on the planet (Silva & Garda 2011). This diversity, however, is threatened due to anthropic impacts from activities such as deforestation (Vieira et al. 2008), cattle ranching (Barona et al. 2010), and hydroelectric dam construction (Alho et al. 2011). However, considering the geographic extension of this region and the rates of environmental changes, there are proportionally few studies on its biota and these are usually concentrated near research centers and access routes (*e.g.* roads, large rivers) (Oliveira et al. 2016). One of the most impacting projects in the history of the Amazon is the Belo Monte hydroelectric dam, located on the Xingu River, in northern Brazil (Fearnside 2006, Tófoli et al. 2017). Since its construction, this hydroelectric dam has caused many drastic environmental changes in terrestrial and aquatic habitats, but the full impacts will take time to manifest (Tófoli et al. 2017). This is of special concern because the biota of Xingu region is still little-known, and many taxa of this region are endemic (Lujanand & Conway 2015, Pérez 2015, Tófoli et al. 2017).

Inventories of terrestrial fauna are usually carried out on taxonomic groups that are both diverse and abundant taxa, easy to collect, and relatively easy to identify, such as vertebrates, butterflies, especially frugivores, and ants (Freitas et al. 2003, Santos 2003). However, other groups of insects also exhibit these characteristics, such as some dipteran families. Diptera is one of the most diverse orders of insects, with about 160,000 described species worldwide (Pape et al. 2011), and one of the most ecologically diverse (Marshall 2012). Some dipteran families are saprophytic, feeding mainly on rotting fruits, such as Mesembrinellidae, Neriidae, and Ropalomeridae (Guimarães 1977, Buck 2010, Ibanez-Bernal 2010, Gomes et al. 2013). Even Sarcophagidae, which most species feed on dead animals, are commonly found visiting rotting fruits (Lopes 1975, Pape & Dahlem 2010). The species of these families are suitable organisms for comprehensive biodiversity inventories because they are abundant in baited traps and are relatively easy to identify, mainly due to the publication of taxonomic revisions with keys to genera and species, such as for the Mesembrinellidae (Guimarães 1977, Bonnato 2001, Kosmann et al. 2013), Neriidae (Sepúlveda et al. 2013a, b, 2014), and Ropalomeridae (Marques & Ale-Rocha 2005, Marques-Costa & Ale-Rocha 2005, Alves & Ale-Rocha 2006, Kirst & Ale-Rocha 2012).

Therefore, the main goal of this study is to provide the first comprehensive description of the composition, species richness, and geographical distribution of the fauna of saprophytic flies obtained with a standardized protocol in forested environments in the Volta Grande of the Xingu River, an area impacted by the Belo Monte hydroelectric dam. These data will be useful in long-term monitoring studies, helping to understand changes in the community of four families of Diptera caused by the construction of a major hydroelectric dam.

Material and Methods

1. *Area of study*

The study was carried out in the Direct Influence Area (AID) of the Belo Monte hydroelectric dam, located between lower and middle Xingu River, covering five municipalities in the state of Pará: Altamira, Anapú, Brasil Novo, Senador José Porfírio, and Vitória do Xingu (Figure 1) (Salomão et al. 2007). The region is located in the Amazon biome, with original vegetation classified as dense ombrophilous forest, as well as floodplain forests on river banks (IBGE, 2012). Regional climate is classified as type Am, according to the Køppen system, with a mean annual temperature of 26ºC, annual precipitation of 2.289 mm, and relative humidity between 78% and 88% (Sousa-Junior et al. 2006). Rainfall presents intense seasonal variations, with wet season between December and May and dry season during the other months of the year (Santos et al. 2015).

2. *Collection, taxonomy, and preparation of samples*

Insect samples from the "Terrestrial Ecosystem Monitoring Project" of the Belo Monte hydroelectric dam were utilized in this study. The sample design is based on eight "RAPELD" modules (Magnusson et al. 2005). Each module is composed of two parallel transects 5 km long, separated by 1 km (Figure 1). From each transects six side trails each 250 m long, were established. The side trails accompany relief contour lines, so they are sinuous rather than straight. Geographic coordinates of the eight modules are as follows: 03°40'37"S, 52°30'39"W (Module 1), 03°19'31"S, 52°15'42"W (Module 2), 03°19'43"S, 52°03'42"W (Module 3), 03°30'12"S, 52°40'43"W (Module 4), 03°29'37"S, 52°53'23"W (Module 5), 03°19'38"S, 52°52'19"W (Module 6), 03°15'12"S, 52°47'35"W (Module 7), 03°04'40"S, 52°46'13"W (Module 8).

Five collection campaigns were carried out between 2014 and 2016. The first was in July 2014; the second in January and February 2015; the third in July and August 2015; the fourth in February and March 2016, and the fifth in July 2016.

Samples were collected using fruit-fly traps (Medeiros & Klazko 1999) made from 2-liter plastic bottles on which insects could enter through four openings of 2.5 by 0.5 cm. In each trail four traps were placed, the first at 10 m from the main trail, the second at 90 m, the third at 150 m, and the fourth at 250 m. Fermenting bananas used as bait were crushed and mixed with 1% dry bakers' yeast (*Saccharomyces cerevisiae*) and fermented for 36 hours. Traps were left open in the field

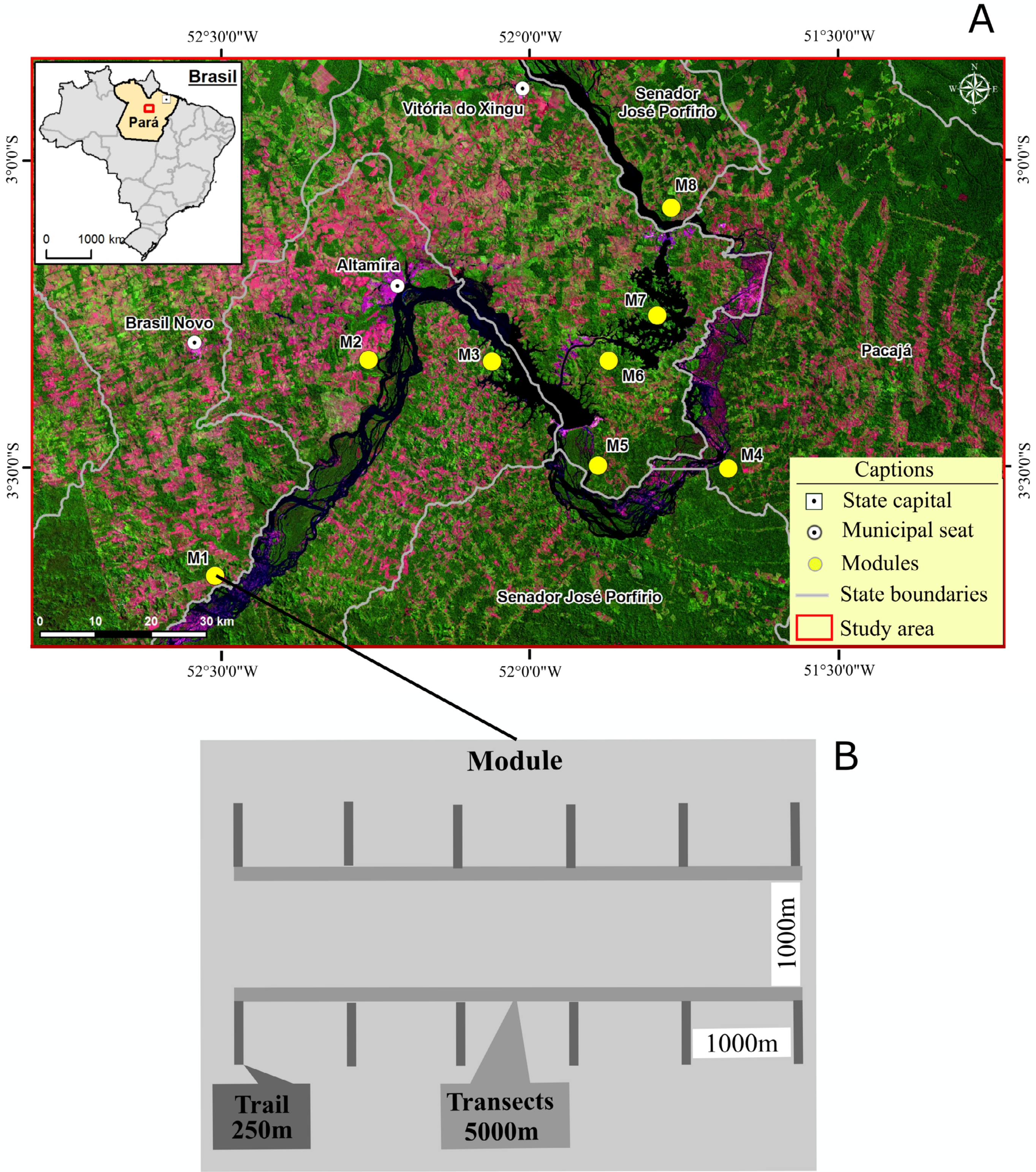

Figure 1. A. Location of the study area, on the middle Xingu River, in the Belo Monte area, Pará, Brazil. **B.** Schematic illustration of the sampling protocol.

for 48 hours, and every 24 hours insects were removed and the bait exchanged for freshly fermented banana mash.

Initially, collected insects were frozen before being passed to 90% ethyl alcohol after preliminary sorting. At the entomology laboratory in the Museu Paraense Emílio Goeldi (MPEG) insects were further sorted and all specimens of Sarcophagidae, Neriidae, Mesembrinellidae, and Ropalomeridae were pinned and labeled, except for the Sarcophagidae, where only male specimens were mounted and counted. The flies (pinned and in alcohol) are deposited in the entomological collection of MPEG.

Fly specimens were identified to species level with identification keys and by comparison with identified specimens in the MPEG collection. For the identification of Mesembrinellidae, keys were used from Guimarães (1977), Bonatto (2001), and Kosmann et al. (2013); for Neriidae the keys were by Carvalho-Filho & Esposito (2008) and Sepúlveda et al. (2006a, b); for Ropalomeridae the keys were by Prado (1966), Marques & Ale-Rocha (2005), Marques-Costa & Ale-Rocha (2013), Ale-Rocha & Alves (2006), and Kirst & Ale-Rocha (2012); and for Sarcophagidae the keys and other taxonomic tools were by Lopes (1939, 1946, 1985, 1987), Lopes & Tibana (1987), Tibana & Xerez (1985), Carvalho & Mello-Patiu (2008), Carvalho-Filho & Esposito (2012), and Buenaventura & Pape (2013). Only male specimens of Sarcophagidae were identified at specific level, since identification is based mainly on features of male genitalia.

3. *Ecological analyses*

Side trails were considered to be sample units, and samples consisted of all captures in the trail over all collection periods. In this way, there were 16 transects with 96 samples. In order to evaluate if the sample effort was sufficient to estimate species richness in the area, Chao1, Chao2, Jackknife1 (first order Jackknife), Jackknife2 (second order Jackknife), ICE, ACE, and Bootstrap were calculated with EstimateS 9.0 (Colwell 2013). Values obtained for different estimators were not discrepant, so only Jackknife1 was used, which is a strong estimator based on abundances and takes into account rare species, i.e., singletons and doubletons (species represented by one or two individuals, respectively); uniques and duplicates (number of species occurring in a single sample and two samples, respectively) (Colwell & Coddington 1994).

Results

In total, 1246 specimens were identified of those collected in traps baited with rotting bananas in Belo Monte, as follows: Mesembrinellidae (154 specimens), Neriidae (196), Ropalomeridae (272), and Sarcophagidae (624). Among these, one Sarcophagidae (*Helicobia aurescens* (Townsend, 1927)) is newly recorded from the Brazilian Amazon, and five species are new records for the state of Pará: *Laneela perisi* (Mariluis, 1987) (Mesembrinellidae), *Apophorhynchus amazonensis* Prado, 1966 (Ropalomeridae), *Ravinia effrenata* (Walker, 1861), and *Titanogrypa larvicida* (Lopes, 1935) (Sarcophagidae).

1. *Mesembrinellidae*

Six species and three genera of Mesembrinellidae were collected (Table 1). The largest genus was *Mesembrinella* with three species, and *Laneella* was the least species-rich with only one species (Table 1). The three most abundant species were *Mesembrinella bicolor* (Fabricius, 1805) (74% of all Mesembrinellidae specimens collected), *Eumesembrinella quadrilineata* (Fabricius, 1805) (19%), and *Laneella perisi* (Mariluis, 1987) (12%). The least abundant species was *Mesembrinella belardiana* (Aldrich, 1922), with only two specimens (Table 1).

Analysis of species richness estimated seven Mesembrinellidae species, compared with an observed richness of six species (Figure 2A). In order to verify the efficiency of the sampling, the behavior of the rare species in the samples was observed. There were one unique, one singleton, one doubleton, and one duplicate for Mesembrinellidae. The species accumulation curve showed a strong tendency towards stabilization, indicating that the collection effort was sufficient to inventory the Mesembrinellidae (Figure 2A). The uniques and doubletons curves of Mesembrinellidae also showed a tendency to stabilize (Figure 2B).

2. *Neriidae*

Three genera and seven species of Neriidae were collected (Table 1). The most species-rich genera were *Glyphidops*, with four species, and *Nerius*, with two species (Table 1). The most abundant species were *Glyphidops filosus* (Fabricius, 1805), with 77% of Neriidae specimens, and *Nerius pilifer* Fabricius, 1805, with 27% (Table 1). Abundances of the remaining species totaled less than 10%. The least abundant species were *Glyphidops flavifrons* (Bigot, 1886) and *Glyphidops vittatus* (Cresson, 1912), each with only one specimen (Table 1).

As for the behavior of rare species in the sample, two uniques, two singletons, no doubletons and no duplicates were collected. The species accumulation curve showed a strong tendency towards stabilization (Figure 3A), but this was not observed for the curves of uniques, singletons, doubletons, and duplicates (Figure 3B). Despite the lack of stabilization of the rare species curves, an estimate of nine species was obtained, only two more than the number collected (Figure 3A), indicating that the sampling effort was sufficient to record most of the species of the locality.

3. *Ropalomeridae*

Six species and three genera of Ropalomeridae were collected (Table 1). The largest genus was *Ropalomera* with four species, but the abundance of each species was equal to or less than two individuals (Table 1). Only one species of *Willistoniella*, *W. pleuropunctata* (Wiedemann, 1824) was collected, and this was the most abundant species (97.4% of collected specimens).

Analysis of the patterns of species incidence and abundance for Ropalomeridae estimated eight species, as opposed to an observed richness of six species (Figure 4A). As for the behavior of the rare species, two uniques, two singletons, one doubleton, and one duplicate were collected. The species accumulation curve showed a strong tendency towards stabilization (Figure 4A), indicating that sampling effort was sufficient to document the local fauna. Curves for uniques and singletons were stabilized, whereas doubletons and duplicate curves appear to have not reached stability for Ropalomeridae (Figure 4B).

Table 1. Community composition and species abundances of saprophytic flies in the study area, on the middle Xingu River, in the Belo Monte direct impact area, PA, Brazil (families Mesembrinellidae, Neriidae, Ropalomeridae and Sarcophagidae).

Familia	Species	Adundance	Relative abundance (%)
Mesembrinellidae		154	
	Eumesembrinella quadrilineata (Fabricius, 1805)	19	12,30%
	Eumesembrinella randa (Walker, 1849)	3	1,90%
	Laneella perisi (Mariluis, 1987)	12	7,80%
	Mesembrinella belardiana (Aldrich, 1922)	2	1,30%
	Mesembrinella bicolor (Fabricius, 1805)	114	74%
	Mesembrinella batesi (Aldrich, 1922)	4	2,60%
Neriidae		196	
	Cerantichir peruana (Hennig, 1937)	4	2%
	Glyphidops etele Aczél, 1961	5	2,60%
	Glyphidops filosus (Fabricius, 1805)	151	77%
	Glyphidops flavifrons (Bigot, 1886)	1	0,30%
	Glyphidops vittatus (Cresson, 1912)	1	0,30%
	Nerius pilifer Fabricius, 1805	27	13,80%
	Nerius plurivittatus Bigot, 1886	7	3,60%
Ropalomeridae		272	
	Apophorhynchus amazonensis Prado, 1966	2	0,70%
	Rhopalomera clavipes (Fabricius, 1805)	1	0,40%
	Rhopalomera stictica (Wiedemann, 1828)	2	0,70%
	Rhopalomera tessellata Prado, 1966	1	0,40%
	Rhopalomera tibialis Walker, 1852	1	0,40%
	Willistoniella pleuropunctata (Wiedemann, 1824)	265	97%
Sarcophagidae		624	
	Argoravinia rufiventris (Wiedemann, 1830)	2	0,30%
	Dexosarcophaga (*Farrimyia*) *carvalhoi* (Lopes, 1980)	1	0,20%
	Duckemyia latifrons Kano & Lopes, 1969	1	0,20%
	Helicobia aurencens (Townsend, 1927)	1	0,20%
	Helicobia borgmeieri Lopes, 1939	1	0,20%
	Helicobia morionella (Aldrich, 1930)	2	0,30%
	Helicobia pilifera Lopes, 1939	21	3,40%
	Lepidodexia (*Notochaeta*) *bogotana* (Enderlein, 1928)	4	0,60%
	Oxysarcodexia amorosa (Schiner, 1868)	2	0,30%
	Oxysarcodexia avuncula (Lopes, 1933)	4	0,60%
	Oxysarcodexia bakeri (Aldrich, 1916)	16	2,60%
	Oxysarcodexia fringidea (Curran & Walley, 1934)	3	0,50%
	Oxysarcodexia intona (Curran & Walley, 1934)	1	0,20%
	Oxysarcodexia thornax (Wiedemann, 1830)	95	15,20%
	Peckia (*Euboettcheria*) *collusor* (Curran & Walley, 1934)	1	0,20%
	Peckia (*Peckia*) *chrysostoma* (Wiedemann, 1830)	23	3,70%
	Peckia (*Sarcodexia*) *lambens* (Wiedemann, 1830)	15	2,40%
	Ravinia belforti (Prado & Fonseca, 1932)	18	2,90%
	Ravinia effrenata (Walker, 1861)	142	22,80%
	Retrocitomyia retrocita (Hall, 1933)	2	0,30%
	Titanogrypa (*Cucullomyia*) *larvicida* (Lopes, 1935)	21	3,40%
	Tricharaea (*Sarcophagula*) *occidua* (Fabricius, 1794)	248	39,70%

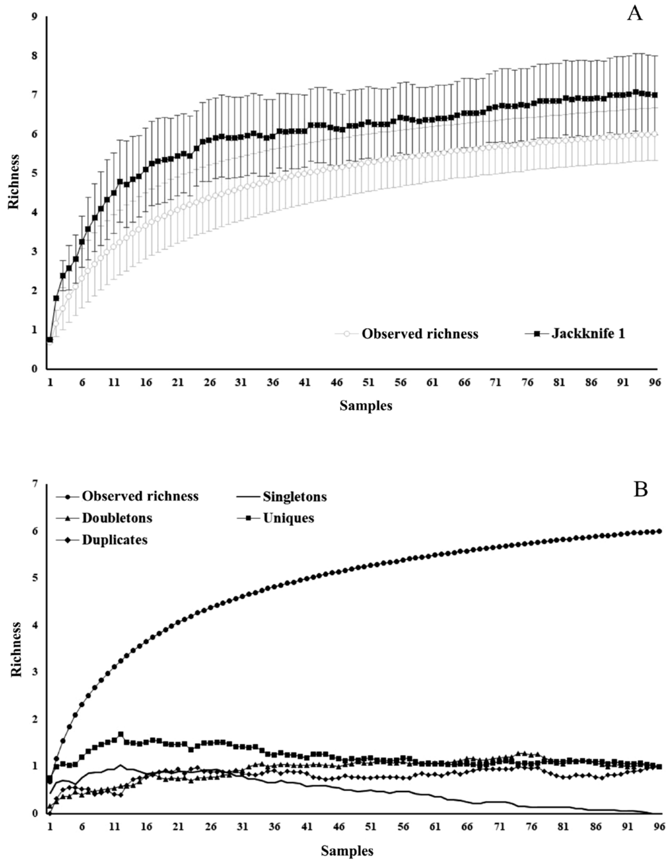

Figure 2. Mesembrinellidae. A. Species accumulation curve (observed richness [Mao Tau] and estimated species richness [Jackknife1]). B. Rare species richness estimation.

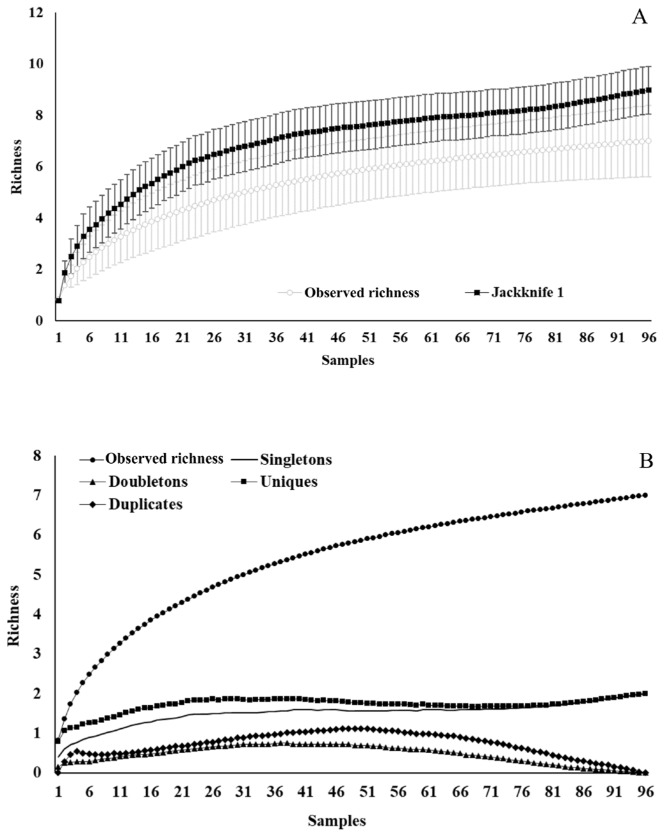

Figure 3. Neriidae. A. Species accumulation curve (observed richness [Mao Tau] and estimated species richness [Jackknife1]). B. Rare species richness estimation.

4. *Sarcophagidae*

Sarcophagidae was the most abundant family with 624 specimens in at least 11 genera and 22 species (Table 1). The most species-rich genera were *Oxysarcodexia* (six species) and *Helicobia* (four species). The genera with only one species each were *Argoravinia*, *Dexosarcophaga*, *Duckemyia*, *Lepidodexia*, *Titanogrypa*, and *Tricharaea*. Despite this, *Tricharaea* (*Sarcophagula*) *occidua* (Fabricius, 1794) was the most abundant species, with 39.7% of collected specimens (Table 1). The second most abundant species was *Ravinia effrenata* (Walker, 1861), with 22.8% of collected specimens (Table 1).

Analysis of species incidence and abundance patterns for Sarcophagidae estimated 28 species, as opposed to an observed richness of 22 species (Figure 5A). As for the behavior of the rare species, four uniques, five singletons, five doubletons, and six duplicates were collected in the samples. The species accumulation curve did not reach the asymptote (Figure 5B), indicating that sampling effort was not sufficient to fully document the local fauna. Curves of uniques and singletons stabilized, whereas doubletons and duplicate curves appear not to have reached stability for Sarcophagidae (Figure 5A).

Discussion

Species accumulation curves for all families except Sarcophagidae demonstrated a strong tendency towards stabilization, showing that sampling efforts were sufficient to record most of the targeted species. This result indicates that traps baited with rotting fruit are effective in inventories of Mesembrinellidae, Ropalomeridae, and Neriidae. Non-stabilization of the species accumulation curve of Sarcophagidae has also been observed in studies performed in Brazil utilizing traps baited with carrion (Souza et al. 2011, 2015), and it may be related to the great number of species in the tropical environments (Pape 1996).

The six fly species newly reported from state of Pará reinforce the need for more research on the biodiversity in the Xingu region and other poorly sampled areas of the state. Pará is the second largest state in Brazil and one with the greatest levels of deforestation and forest degradation of the Brazilian Amazon (Fonseca et al. 2015). The loss or drastic degradation of natural vegetation in this state could cause the local or overall extinction of some species of invertebrates, even before they are properly studied taxonomically or ecologically and even before they are formally reported for the region.

In all the ecological studies published on Mesembrinellidae carried out in the Amazon, only the genera *Eumesembrinella* and *Mesembrinella* have been recorded (Esposito & Linhares 2002, Amat 2010, Esposito et al. 2010, Gomes et al. 2013, Amat et al. 2016). In the present study, a species of the genus *Laneella*, *L. perisi* (Figure 6A) was also collected, as the third most abundant species of its family (Table 1). This difference may be related to the kind of bait utilized, since in the other studies performed in the Amazon, rotting beef lung was utilized

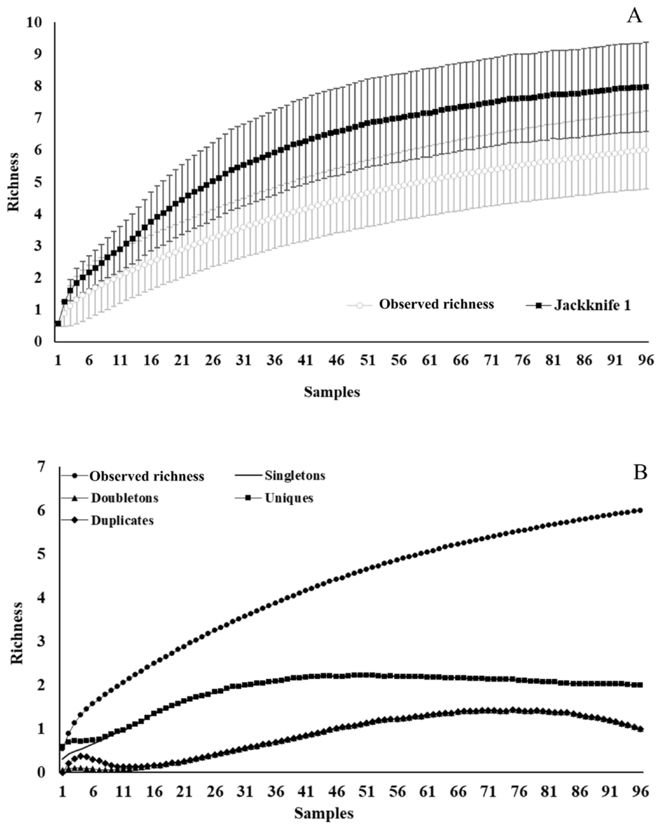

Figure 4. Ropalomeridae. (A) Species accumulation curve (observed richness [Mao Tau] and estimated species richness [Jackknife1]); (B) Rare species estimation.

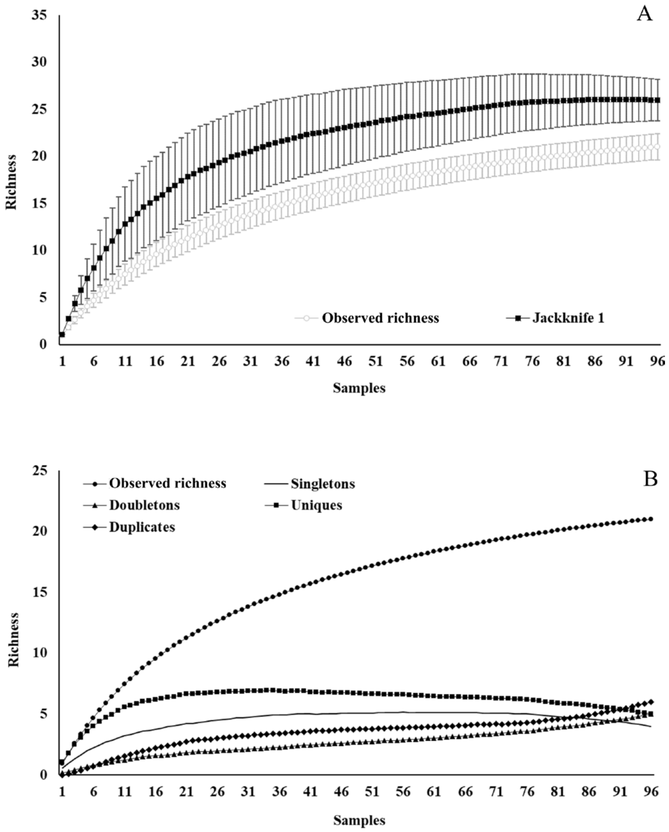

Figure 5. Sarcophagidae. (A) Species accumulation curve (observed richness [Mao Tau] and estimated species richness [Jackknife1]); (B) Rare species richness estimation.

as bait. However, Gomes et al. (2013) also used banana-baited traps in the Caxiuanã National Forest, but they did not collect *L. perisi*.

In ecological studies undertaken in southeastern Brazil, the three reported genera were also found in this study, but the *Laneella* species collected there was *L. nigripes* (Guimarães, 1977) (Ferraz et al. 2010, Barbosa et al. 2014). *Laneella perisi* has been recorded for Colombia and Ecuador (Wolff et al. 2012) and in Brazil for the state of Rondônia (Bonatto 2001), but it is nevertheless widely distributed throughout the Brazilian Amazon (Carvalho-Filho et al., in preparation). Therefore, this is a new record for the state of Pará. Most likely, *L. perisi* is associated with the Amazonian biome and *L. nigripes* with the Brazilian Atlantic Forest. In the study by Ferraz et al. (2010), undertaken in the Tinguá Reserve, state of Rio de Janeiro, the most abundant species of Mesembrinellidae was *L. nigripes*, while studies in the Amazon indicate the most abundant species as *Eumesembrinella randa* (Esposito & Linhares 2002, Amat 2010, Esposito et al. 2010, Souza et al. 2015, Amat et al. 2016). In the present study, however, the most abundant species was *Mesembrinella bicolor*, while only three specimens of *E. randa* were collected (Table 1).

The genus *Mesembrinella* has 13 described species (Guimarães 1977), but only the three collected in this study have been reported from carrion baits in other Amazonian localities, where *M. bellardiana* is generally the most abundant species, followed by the less abundant *M. bicolor* (Esposito & Linhares 2002; Amat 2010, Esposito et al. 2010, Gomes et al. 2013, Amat et al. 2016). These three species are widely distributed in forested habitats in Brazil (Guimarães 1977) where, as observed in ecological studies, they are the most abundant species making them an important component of the fauna of Mesembrinellidae.

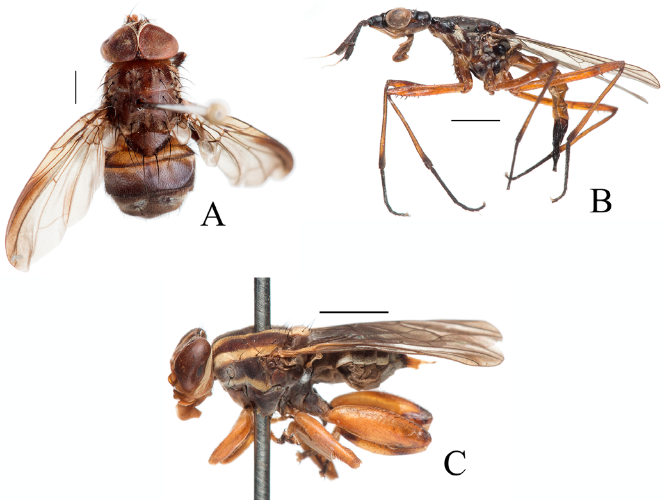

Figure 6. A. *Laneella perisi*, dorsal view. B. *Cerantichir peruana*, lateral view. C. *Apophorhynchus amazonensis*, lateral view. Scale bars = 2 mm. Photos by C.A.C. Favacho.

The genus *Eumesembrinella* contains four described species (Guimarães 1977, Bonatto 2001), but only two were collected in this study, as they were in other ecological studies in the Amazon (Esposito & Linhares 2002, Amat 2010, Esposito et al. 2010, Gomes et al. 2013, Amat et al. 2016). In these studies, *E. randa* is usually the most abundant species, a pattern observed in Belo Monte as well (Table 1). This species is very common in primary and secondary forests, but this has not been observed for *E. quadrilineata* (Carvalho-Filho et al., in preparation), suggesting that *E. randa* is a habitat generalist and possibly a competitor in regard to *E. quadrilineata*.

Despite recent advances in the taxonomy of Neotropical Neriidae (Sepúlveda et al. 2013a, b, 2014), no study on the community composition, abundance, and species richness of this family has been published until now. Studies on the taxonomy and systematic of Neriidae were based on specimens deposited in natural history museum that were obtained in sporadic and non-systematized collections.

Most neriid species collected in this study belong to the genus *Glyphidops* Enderlein, 1922, which is the most species-rich in the Neotropical region, with 14 described species (Sepúlveda et al. 2014). The most abundant species was *G. filosus*, with 77% of the collected specimens (Table 1). The least abundant species were *G. flavifrons* and *G. vittatus*, each with one specimen (Table 1). The species *G. filosus* presents a wide geographical distribution, ranging from Central America (Honduras) to southern Brazil (Sepúlveda et al. 2014) and is one of the most numerous species in collections of Neriidae (Carvalho-Filho & Esposito 2008, Sepúlveda et al. 2014). This could be the most abundant species of *Glyphidops* and all of Neriidae throughout their distribution. However, more ecological studies on this family are needed to see if this pattern holds for other localities in the Neotropical region.

Nerius (Fabricius, 1805) has 10 described species (Aczél 1961, Steyskal 1968) with two in the Brazilian Amazon (Carvalho-Filho & Esposito 2008) that were collected in this study, of which *N. pilifer* was the second most abundant species of the family (Table 1). *Glyphidops flosus* and *N. pilifer* are both widely distributed species that have been collected in primary and secondary forests, plantations, and even urban gardens (Carvalho-Filho et al., in preparation).

Cerantichir peruana (Figure 6B) have been reported from Brazil (Acre and Pará) by Carvalho-Filho & Esposito (2008) and later also registered for Rondônia and Mato Grosso (Sepúlveda et al. 2013a). This species is rarely collected in Brazil, and few specimens are deposited in Brazilian collections (eight in MPEG and three in the Jesus Santiago Moure Entomological Collection of the Federal University of Paraná) (Carvalho-Filho & Esposito 2008, Sepúlveda et al. 2013a).

Most of the collected specimens of Ropalomeridae (97.4%) belong to *W. pleuropunctata*. The genus *Willistoniella* Mik, 1895 has four described species (Marques & Ale-Rocha, 2005), but only one, *W. pleuropunctata*, was collected in this study. This species has a wide geographical distribution, ranging from Mexico to Argentina (Marques & Ale-Rocha, 2005). In a study on the population variation of Ropalomeridae in Manaus, two genera, *Ropalomera* Wiedemann, 1824 and *Willistoniella*, were collected, and 92% of the specimens belonged to the genus *Willistoniella* (Marques et al. 2004), suggesting that species of this genus, especially *W. pleuropunctata*, may be the most abundant Ropalomeridae in several parts of the Amazon, a fact that may be related to the generalist habit of the species (Marques et al. 2004). In the study by Marques et al. (2004), all four species of *Willistoniella* were reported, but the abundance of each was not mentioned.

The genus *Apophorhynchus* Williston, 1895 holds three species, none of which have been reported for the state of Pará (Marques & Ale-Rocha 2005). Thus, *A. amazonensis* Prado, 1966 (Figure 6C), newly collected in this study, is a new occurrence for Pará, while in Brazil the species had been recorded only for Amazonas and Roraima (Marques-Costa & Ale-Rocha, 2005, Ale-Rocha & Alves 2006). This species seems to have a wide distribution in the central Brazilian Amazon, but as there are relatively few collections in western Pará, where the Belo Monte is located, this species has not been previously recorded for the state.

Ropalomera is the most species-rich genus of the Ropalomeridae, with about 16 described species (Kirst & Ale-Rocha 2012). In this study four species were collected, but all with abundances equal to or less than two specimens.

Previous studies on Sarcophagidae community composition, abundance, and species richness undertaken in Amazonia have been based on specimens collected with carrion-baited traps mainly containing bovine lung (Esposito & Linhares 2002, Amat et al. 2011, Souza et al. 2011, 2015), since many species have larvae that feed on decaying animal tissue (Pape & Dahlem 2010). Nevertheless, fermenting fruit is also attractive for these flies, as Lopes (1975) observed, since the number of genera and species collected with fruit was substantial when compared to carrion. With bovine lung bait, Esposito & Linhares (2002) collected five genera and seven species in the Caxiuanã region of Pará, and Souza et al. (2011) collected 10 genera and 23 species in the Urucu region of Amazonas, whereas fruit bait in Belo Monte attracted 11 genera and 22 species.

Most species attracted to fermenting fruit were also collected in studies using carrion (Esposito & Linhares 2002, Amat et al. 2011, Souza et al. 2011, 2015). However, *Lepidodexia bogotana* (Enderlein, 1928) had not been reported in any previous study. Most species of the genus *Lepidodexia*, whose biology is known, are vertebrate and invertebrate parasites (Pape & Dahlem 2010), and therefore are not generally collected with baited traps.

Although the community composition included many species shared with studies that used carrion, species abundances were different between types of bait. The most abundant species in the present study was *Tricharaea occidua*, which was also the most abundant in Souza et al. (2015). However, in studies by Esposito & Linhares (2002) and Souza et al. (2011) the most abundant species were *Peckia chrysostoma* (Wiedemann, 1830) and *Peckia lambens* (Wiedemann, 1830), respectively, when rotting beef lung was utilized as bait. These two species were collected in this study, but with relative abundances below 4% (Table 1). Souza et al. (2011) collected only two specimens of *T. occidua*, while Souza et al. (2015) captured 8.452 specimens, a difference that may be related to the type of sampled environment as well.

Ravinia effrenata is a new occurrence for the state of Pará, since in Brazil it was recorded only for Roraima, Maranhão, Minas Gerais, and Pernambuco (Lopes & Leite 1990, Souza et al. 2015, Mello-Patiu et al. 2014, Barbosa 2019). This was the second most abundant species in this study, and no specimens were reported by Esposito & Linhares (2002) or by Souza et al. (2011). This fact may be related to the kind of bait utilized, since in all other ecological studies performed in the

Brazilian Amazon rotting bovine lung or fish were utilized (Esposito & Linhares 2002, Amat et al. 2011, Souza et al. 2011).

The species *Titanogrypa larvicida* (Lopes, 1935) is also a new occurrence for the state of Pará, since this species had been registered only for Distrito Federal, Minas Gerais, Paraíba, Pernambuco, Rio Grande do Norte, Rio de Janeiro, and São Paulo (Pape 1996, Carvalho & Linhares 2001, Barros et al. 2008, Moretti et al. 2008, Barbosa et al. 2009, Rosa et al. 2011, Alves et al. 2014, Barbosa 2019). This species has been reported in forensic studies using porcine and rat carcasses as baits (Barros et al. 2008, Moretti et al. 2008, Barbosa et al. 2009, Rosa et al. 2011), but fermenting fruit is also attractive as observed here.

The genus *Oxysarcodexia* Townsend, 1917 is one of the most characteristic taxa in ecological and forensic studies that use animal carcasses (Barros et al. 2008, Carvalho & Linhares 2001, Moretti et al. 2008, Barbosa et al. 2009, Rosa et al. 201, Souza et al. 2011, 2015). This sarcophagid genus is one of the most abundant and widespread in the Neotropical region, but the biology of many species is still unknown (Pape & Dahlem 2010). Larvae of this genus have been reared in vertebrate feces and in carrion (Pape & Dahlem 2010). Despite this, they were also numerous in traps containing fermenting fruit bait in Belo Monte, since six species were collected, more than in the studies of Esposito & Linhares (2002) and Souza et al. (2011), who used bovine lung as bait. Lopes (1975) obtained 11 species in butterfly traps baited with bananas, indicating that fermenting fruit is a good bait for species of this genus.

The most abundant *Oxysarcodexia* species in the present study was *O. thornax* (Wiedemann, 1830), which was the third most abundant sarcophagid species. In Souza et al. (2011), *O. thornax* was also the most abundant species, while in Souza et al. (2015) it was the second most abundant species of the genus. This species is widely distributed in the Americas and has been introduced in other mainland (Pape 1996) and is very common in all kinds of environments, including cities (Carvalho-Filho et al, in preparation). This fact suggests that it possesses wide ecological plasticity, allowing this species to explore many environments, where it is one of the most abundant flesh flies, and to consume various kinds of food, including rotting animal tissues, feces, and fermenting fruits.

The second most species-rich genus in the present study was *Helicobia*, with four species. In Souza et al. (2011) and Barros et al. (2008) only one species of this genus was collected and in low abundance. However, in Souza et al. (2015) seven species were obtained, indicating that animal carcasses and decomposing fruit are attractive for this genus. *Helicobia aurescens* (Townsend, 1927) is a new record for the Brazilian Amazon, since in Brazil it was recorded for Distrito Federal, Mato Grosso do Sul, Minas Gerais, Paraná, Pernambuco, Rio de Janeiro, and São Paulo (Pape 1996, Barros et al. 2008, Mello-Patiu et al. 2014, Barbosa 2019, Toma et al. 2020).

The genus *Peckia*, which is one of the most species-rich in the Neotropical region (Buenaventura & Pape 2013), was the third genus with the most species (three species) collected in the present study (Table 1). In studies of Sarcophagidae from the Amazon and the Brazilian northeast, all of which used bovine lung as bait, this genus was the one with most species. Souza et al. (2015) obtained 11 species and Souza et al. (2015) 12 species. In addition, in other studies (Esposito & Linhares 2002, Amat *et al*. 2011, Souza et al. 2011, 2015), some *Peckia* species, such as *P. lambens* and *P. chrysostoma*, were relatively abundant, which was not observed in the present study. The greater abundance of *Peckia* species with bovine lung bait may be related to the larvae being predominantly necrophagous and requiring protein to complete their development (Pape & Dahlem 2010).

The results shown in this study represent the first data on a saprophytic fly community in a poorly sampled and threated region of the Brazilian Amazon. Since the sampling was performed following a standardized protocol, the data shown here can be compared to other studies that follow the same protocol in other Amazonian regions. In addition, the new data can be utilized in faunal monitoring of the region under impact of Belo Monte hydroelectric dam.

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Author Contributions

Camila Lorena Damasceno Queiroz: Contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Caroline Costa De-Souza: Contribution to manuscript preparation; data analysis and interpretation.

Hermes Fonseca de Medeiros: Contribution in the concept of the study; data collection; contribution to critical revision, adding intellectual content.

William Leslie Overal: Contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Arleu Barbosa Viana-Junior: Data analysis and interpretation; contribution to critical revision, adding intellectual content.

Fernando da Silva Carvalho-Filho: Contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Conflicts of Interest

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

Ethics

Specimens were collected under IBAMA / SISBio permit number 251/2013.

Data availability

Data were compiled from the published literature, and appropriately cited in the text. Specimens deposited in the entomology collection of the Museu Paraense Emílio Goeldi will be registered in Specify and published on-line.

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Inventory

Vascular plant checklist in an area of extreme biological importance: filling gaps in the Caparaó National Park-ES, Brazil

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Abstract: Regional floristic lists are essential for defining biodiversity conservation strategies and are key to assist in filling knowledge gaps. They aim to provide a data source for applying tools to reduce extinction rates and to conserve ecosystems. Herein we present the results of an inventory of vascular plants in a rainforest in the Caparaó National Park (CNP) and approach their implications for conservation and management of this protected area and the surrounding communities. We conducted botanical expeditions between the years 2012 and 2017 in a montane and upper-montane forest of the CNP. We found 361 species distributed in 78 families and 181 genera. The study area is home to new species for science that have recently been described in other publications outside that location, and 4 new records for Espírito Santo State; also 43 species listed in different extinction threat categories (VU, EN and CR) and another 190 categorized with lesser concerns (LC and NT). The families with the highest species richness were: Melastomataceae (41 spp.), Lauraceae and Myrtaceae (30), Orchidaceae (26), Rubiaceae (24), and Asteraceae (20). Our results contribute to greater knowledge of the CNP flora, of the montane environments in Brazil and the vegetation of Espírito Santo state, in addition to demonstrate the importance of this protected area to the conservation Atlantic Forest biodiversity.

Keywords: *Neotropical region, Atlantic Forest, nature conservation, endangered species, regional flora.*

Checklist de plantas vasculares em uma área de extrema importância biológica: preenchendo lacunas no Parque Nacional do Caparaó-ES, Brasil

Resumo: As listas florísticas regionais são essenciais para definir estratégias de conservação da biodiversidade e importantes instrumentos para preencher lacunas de conhecimento. O objetivo foi fornecer uma base de dados a partir de uma listagem de espécies como ferramenta para conservação e manejo do ecossistema. Apresentamos aqui os resultados de um inventário de plantas vasculares em uma Floresta Ombrófila Densa no Parque Nacional Caparaó e abordamos suas implicações para a conservação e manejo desta área protegida e das comunidades do entorno. Realizamos expedições botânicas entre os anos de 2012 e 2017 na vertente capixaba dessa floresta ombrófila no parque. Foram encontradas 361 espécies distribuídas em 78 famílias e 181 gêneros. A área de estudo abriga novas espécies para ciência que foram recentemente descritas em outras publicações fora dessa localidade, e 4 novos registros para o Espírito Santo; também 43 espécies listadas em diferentes categorias de ameaça de extinção (VU, EN, CR) e outras 190 categorizadas com menores preocupações (LC e NT). As famílias com maior riqueza de espécies foram: Melastomataceae (41 spp.), Lauraceae e Myrtaceae (30), Orchidaceae (26), Rubiaceae (24) e Asteraceae (20). Nossos resultados contribuem para um maior conhecimento da flora do Parque, dos ambientes montanos no Brasil e da vegetação do Espírito Santo, além de demonstrar a importância dessa área protegida para a conservação da biodiversidade da Mata Atlântica.

Palavras-chave: *Região neotropical, Floresta Atlântica, conservação da natureza, espécies ameaçadas.*

Introduction

Regional floristic listings consist of important tools for designing conservation plans and actions for different ecosystems, and forms the essential basis for biodiversity management, developing of regional flora projects, supporting knowledge expansion. Despite the need for cataloguing biological diversity globally, especially in biodiversity hotspots (Myers et al. 2000, Mittermeier et al. 2004), the number of large expeditions like those carried out in the past by great naturalists, for instance, von Martius, Saint Hilaire and more recently A.C. Brade, were considerably reduced (Christenhusz & Byng 2016) and there are no more. However, regional floristic surveys have been growing in the tropical region, enabling estimation of plant species richness in these tropical regions (Slik et al. 2015).

Brazil is the country that has the greatest plant richness in the world, with an estimated 34,459 species of vascular plants (BFG 2018). However, the state of knowledge on flora is still incipient in many regions of the country, as occurs in the Atlantic Forest, including protected areas (Lima et al. 2015, Zappi et al. 2016, Oliveira et al. 2017), which have the conservation of plant species among their goals. Despite this, many species of vascular plants are listed as "data deficient" (Sousa-Baena et al. 2014), compromising actions for their conservation.

The lack of floristic studies in forest remnants in Brazil, especially those in which the access is difficult higher, such as mountain areas, leads to a lack of data in estimating the richness, as well as the occurrence of species (Giulietti et al. 2009). Thus, the restricted knowledge on the distribution of species due to the low number of floristic inventories in some regions creates gaps and makes it harder to make decisions of public politics, since these studies work as tools for identifying potential places for the conservation, or even for establishing new strategies in areas already under protection regimes (IUCN 2017). Floristic inventories, in addition to generating information about the species composition of a certain area, also enable the feed a database that will serve as the basis for other taxonomic and ecologic studies, as well as studies on the restoration of degraded areas (Souza et al. 2009).

Given these justifications and for being recognized as an important Atlantic Forest remnant, as well as being in a priority area for conservation and having extremely high biological importance (Ministério do Meio Ambiente - MMA 2007), the efforts to know the flora in the Caparaó National Park (CNP) has started with the pioneering study by Brade (1942) has been expanding over the last decade (Mazine & Souza 2008; Forster & Souza 2013, Couto et al. 2016, Machado et al. 2016, Zorzanelli et al 2016, Araujo et al. 2018, Campos et al. 2018), beyond fascicles published by the herbarium "Guido Pabst" (GFJP). Recently, the list of plants from CNP was made available on the digital platform "Catálogo de Plantas das Unidades de Conservação do Brasil" (https://catalogo-ucs-brasil.jbrj.gov.br/), where there are 1,789 species of 714 genera and 198 botanical families, of these, 1,292 are angiosperms, 37 lycophytes, 262 ferns and 198 are avascular plants (Carrijo et al. 2020).

Therefore, herein we present the results of a floristic inventory of vascular plants in a rainforest in the CNP and approach its implications for the handling and conservation of this protected area. Moreover, we collaborate to fill the knowledge gaps for mountain environments in Brazil. Our intent is to show that floristic listings of plant species are indispensable tools for biodiversity conservation and that they should be encouraged.

Materials and methods

1. *Study area*

The Caparaó National Park (CNP) is a protected area located between Espírito Santo and Minas Gerais states (Figure 1), within the Serra da Mantiqueira mountain range (20º18' - 20º37'S and 41º42' - 41º52'W). The park has 31,853.12 ha area, with 79% of being within the state of Espírito Santo (ICMBio 2015). It is located in the Atlantic Forest domain and protects different vegetation formations, such as montane forests (Dense Ombrophilous Forest and Semi-deciduous Seasonal Forest), high-altitude grasslands, and inselbergs (IBGE 2012, ICMBIO 2015, Couto et al. 2016, Campos et al. 2018). The Dense Ombrophilous Forest are exclusive to the Espírito Santo state side of the Park.

The CNP surrounding areas consist mostly of agricultural and cattle ranching, with emphasis on coffee and cattle farming, as well as a recent growth in areas destined to silviculture. Activities with potential negative impacts for the CNP are performed in surrounding areas, i.e. the use of pesticides, irregular deforestation for expanding agricultural areas (ICMBIO 2015). We also highlight anthropogenic pressures that may cause ecological unbalance in the park, such as unauthorized hunting, illegal extraction of palm hearts (*Euterpe edulis* Mart.), and the occurrence of forest fires (personal observation).

Floristic expeditions were concentrated in the Santa Marta valley (central point in the sampling area: 20º29'27,7''S 41º45'15,6W), located in the municipality of Ibitirama - ES. The valley has an elevation range from 870 and 2480 m (Figure 1). Vegetation types reported to the area are Montane and Upper Montane Dense Ombrophilous Forests, according to the classification by IBGE (2012). The weather in the Ibitirama municipality is defined as Cwb according to the Köppen classification, adapted to Brazil by Alvares et al. (2013), being characterized as humid subtropical with dry winters and mild summers, and the region has annual precipitation of 1,284 mm and average annual temperatures of 16.8 ºC.

The vegetation in the beginning of the valley at lower elevation has different forest succession stages due to its wood extraction past, also presenting exotic/invading species (i.e. *Eriobotrya japonica* (Thunb.) Lindl.), representing one of the exotic species that exist inside the protected area (Field observation; not collected). The signs of anthropogenic activity are reduced throughout the valley, given that the vegetation is extremely preserved in the highest areas and with an observed great presence of epiphytes (personal observation).

1.2 Data collection

We performed botanic expeditions between 2012 and 2017 to collect specimens comprising all life forms in order to compose the floristic listing to the Santa Marta valley (CNP). We collected fertile plant samples along trails using the walk-over survey method (Filgueiras et al. 1994), as well as samples in plots, covering an elevation gradient of around 700 m (900-1,600 m). We identified the species through pertinent literature as dichotomous keys of regional floras (*e.g.*, Wanderley et al. 2005, 2012, Melhem et al. 2007, Martins et al. 2009) and compared our materials to images available in virtual herbaria such as the Herbário Virtual - Reflora (http://reflora.jbrj.gov.br/reflora/herbarioVirtual/) and the Jabot (http://jabot.jbrj.gov.br/v2/consulta.php). Duplicates were sent to group experts at BHCB, CEPEC, HUFSJ, RB, SPSF, and UPCB

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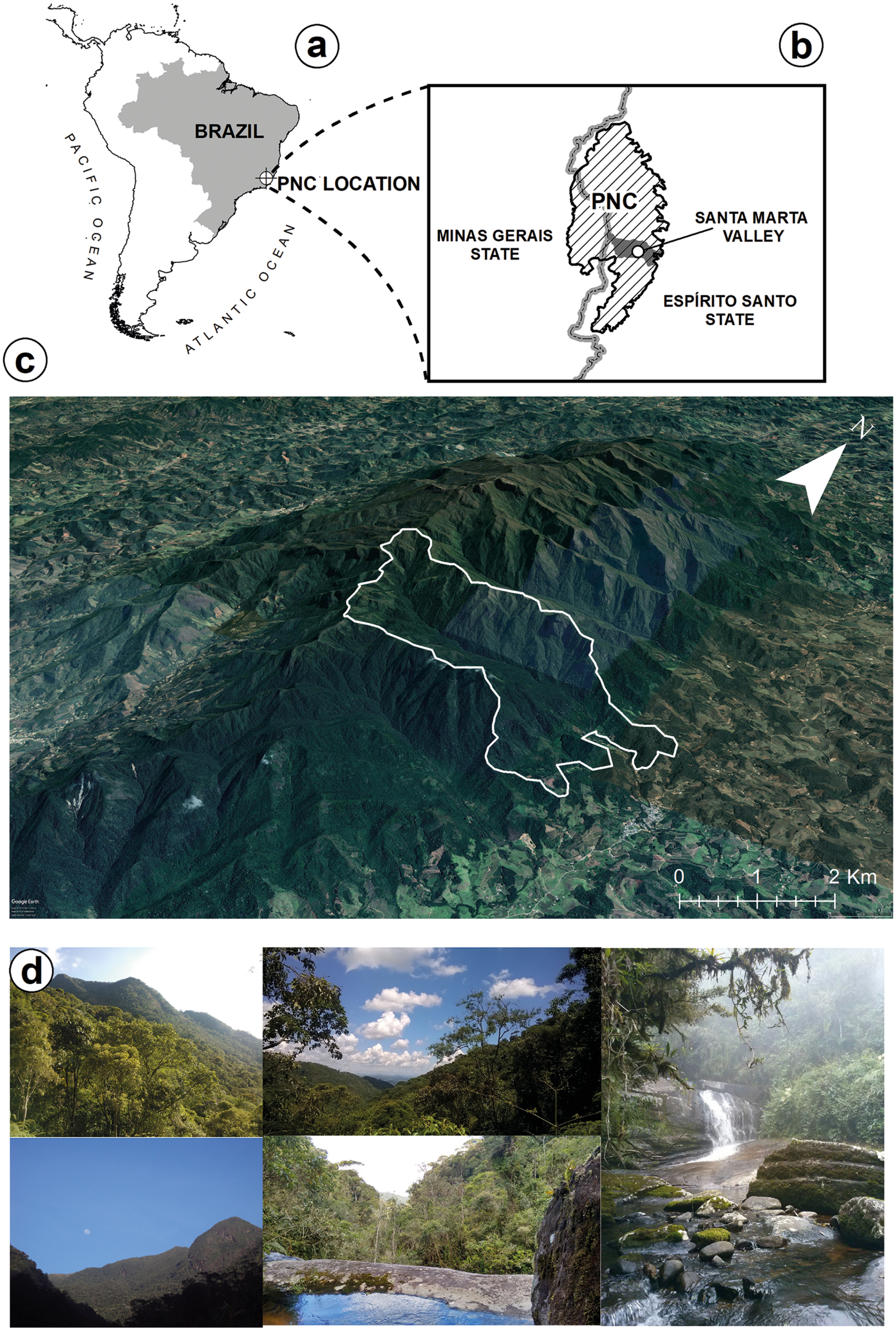

Figure 1. Location map (a; b), relief representation (c), and pictures (d) of the Santa Marta valley, Caparaó National Park (PNC), Brazil. Photo: Araujo, E.A.

herbaria – acronyms according to Thiers (2019) – for confirmation of the species. We have incorporated the specimens in the collection of the VIES and CAP Herbaria. In addition, sterile materials from plot sampling were included in the listing. These were reviewed by experts in their respective families and deposited in a didactic collection of CAP Herbaria. The exotic / invasive species were disregarded in this study. We try to keep a distance from the collection points of any area of human interference and therefore we prioritize including only native species.

Botanical families were classified according to the system proposed by the Angiosperm Phylogeny Group (APG IV 2016) for angiosperms, The Pteridophyte Phylogeny Group (2016) for ferns and lycophytes, and Christenhusz et al. (2011) for gymnosperms. We confirmed name orthography, authorship and synonyms through consulting "Flora do Brasil" 2020 online (<http://floradobrasil.jbrj.gov.br/>), complementing it with data from the online platform of the Missouri Botanical Garden (http://www.tropicos.org) and The Plant List (http://www.theplantlist.org/).

Potentially threatened species were verified in the following lists: a) Red List of Espirito Santo state (Fraga et al. 2019); b) Red List of Flora of Brazil/CNC Flora (Martinelli & Moraes 2013; CNCFlora 2018); and c) The IUCN Red List of Threatened Species (IUCN 2017). New records of species from Espírito Santo state were compiled after the confirmation of group experts, as well as its verification in the database of the "Flora do Brasil" 2020 online (<http://floradobrasil.jbrj.gov.br/>) and Carrijo et al. (2020).

Results

We catalogued 361 species of vascular plants (Figure 2, Table 1) in the Santa Marta river valley, belonging to 78 botanical families (70 angiosperms and eight ferns and lycophytes), and 181 genera. The families with highest species richness were Melastomataceae (41 spp.) Lauraceae and Myrtaceae (30), Orchidaceae (26), Rubiaceae (24), Asteraceae (20), Piperaceae (15), Solanaceae (15) and Bromeliaceae (12), which altogether sum 59% of all compiled species. The most well represented genera regarding number of species were: *Miconia* (23 spp.), *Ocotea* (18), *Myrcia* (12), *Psychotria* (9), and *Eugenia, Mikania*, *Peperomia* and *Leandra* (8).

We found 3 new records for the State of Espírito Santo: *Alsophila salvinii* Hook. (Cyatheaceae); *Pleroma foveolatum* (Naudin) Triana; (Melastomataceae); *Pilea hilariana* Wedd. (Urticaceae), and three possible new species that are under investigation *(Psychotria* sp., *Sloanea* sp. and *Solanum* sp.*)*, which are recent taxonomic discoveries and are being described.

Our results have also pointed out the existence of 43 species (12%) listed as threatened of extinction on The IUCN Red List of Threatened Species, in the Red Book of Brazilian Flora, and on the list of endangered flora species in the State of Espírito Santo (Table 1). The families that have the highest number of species categorized as threatened of extinction were: Myrtaceae (7 spp.), Lauraceae (6), Melastomataceae and Orchidaceae (5), Begoniaceae and Monimiaceae (4).

Based on our field observations during the inventory process, it was possible to notice a gradual change in the plant community throughout the valley (data on the ecology of plant communities are being published). To exemplify this differentiation, we observed some species occurring restrictedly at certain elevation, such as *Alsophila setosa* Kaulf., *Cupania ludowigii* Somner & Ferrucci, *Euterpe edulis* Mart. and *Sorocea bonplandii* (Baill.). W. C. Burger et al. that were only observed between approximately 1,100 m and 1,400 m of altitude, while species such as *Baccharis oblongifolia* (Ruiz & Pav.) Pers., *Miconia longicuspis* Cogn., *M. molesta* Cogn., and *Weinmannia pinnata* L. only occurred in elevations above 1,400 m. Some species were observed throughout the whole sampled altitude range, e.g., *Alchornea triplinervia* (Spreng.) Müll.Arg., *Cyathea atrocastanea* Labiack P.E. et Matos F.B., *Dendropanax cuneatus* (DC.) Decne. & Planch., *Myrcia splendens* (Sw.) DC., and *Myrsine gardneriana* A.DC. Species such as *Bathysa australis* (A.St-Hil.) K.Schum. and *Leandra melastomoides* Raddi mainly occurred in low elevations (1,000 m), especially in areas close to water streams. *Maxillaria caparaoensis* Brade is an endemic species to the CNP, with few sheets deposited in herbaria. *Cyathea atrocastanea* Labiack P.E. et Matos F.B. and *Vochysia santaluciae* M.C. Vianna & Fontella are endemic species of Espírito Santo, originally described for the Estação Biológica de Santa Lúcia in the Santa Teresa region.

Discussion

The new records for the state of Espírito Santo revealed in our study reflects the history of research efforts in certain places regarding the flora of the state (Carrijo et al. 2020, Dutra et al. 2015, Araújo et al. 2018), especially for montane environments. Our data show that the Santa Marta Valley houses 20.2% (361 species) of the vascular plants from CNP (Carrijo et al. 2020), in addition our list includes 88 species of local flora not yet documented, representing an increase of approximately 1%. These knowledge gaps make it harder to map the species and biodiversity distribution correctly, and makes delimitation of endemic areas imprecise, being one of the main obstacles to obtain actual understanding and to establish proper plans for biodiversity conservation (Hopkins 2007, Oliveira et al. 2016). Our findings are relevant for contributing to filling this knowledge gap about the flora in the state, in addition to helping to reduce the current lack of knowledge on the biodiversity in certain locations within Brazilian protected areas (Oliveira et al. 2017).

We have also registered the third known occurrence of *Freziera atlantica* Zorzanelli & Amorim (Pentaphylacaceae). This specie was described in 2016 with samples collected in the Papuã Mountains-BA and in the Valentim Mountains-ES (Zorzanelli et al. 2016), given that these mountains belong to the surrounding areas of the Caparaó Mountains. We have also included a species for the recently described science, *Myrcia altomontana* Sobral & Zorzanelli (Myrtaceae) (Sobral et al. 2017) in our list.

Plant communities of montane ecosystems usually present higher rates of endemism than ecosystems of lower elevations (Gentry 1995), which makes the diversity in these places more vulnerable to climate change due to the specialization degree developed by the species colonizing these environments (Eller et al. 2015, 2016). Expanding knowledge on montane environments has been increasingly important as a support to avoid species loss (Bertoncello et al. 2011).

This list was performed in an area defined by the Brazilian government as priority for the conservation and with extremely high biological importance (MMA 2007). It is one of the first listings for vascular plants in forests above 1000 m in Espírito Santo state. Our

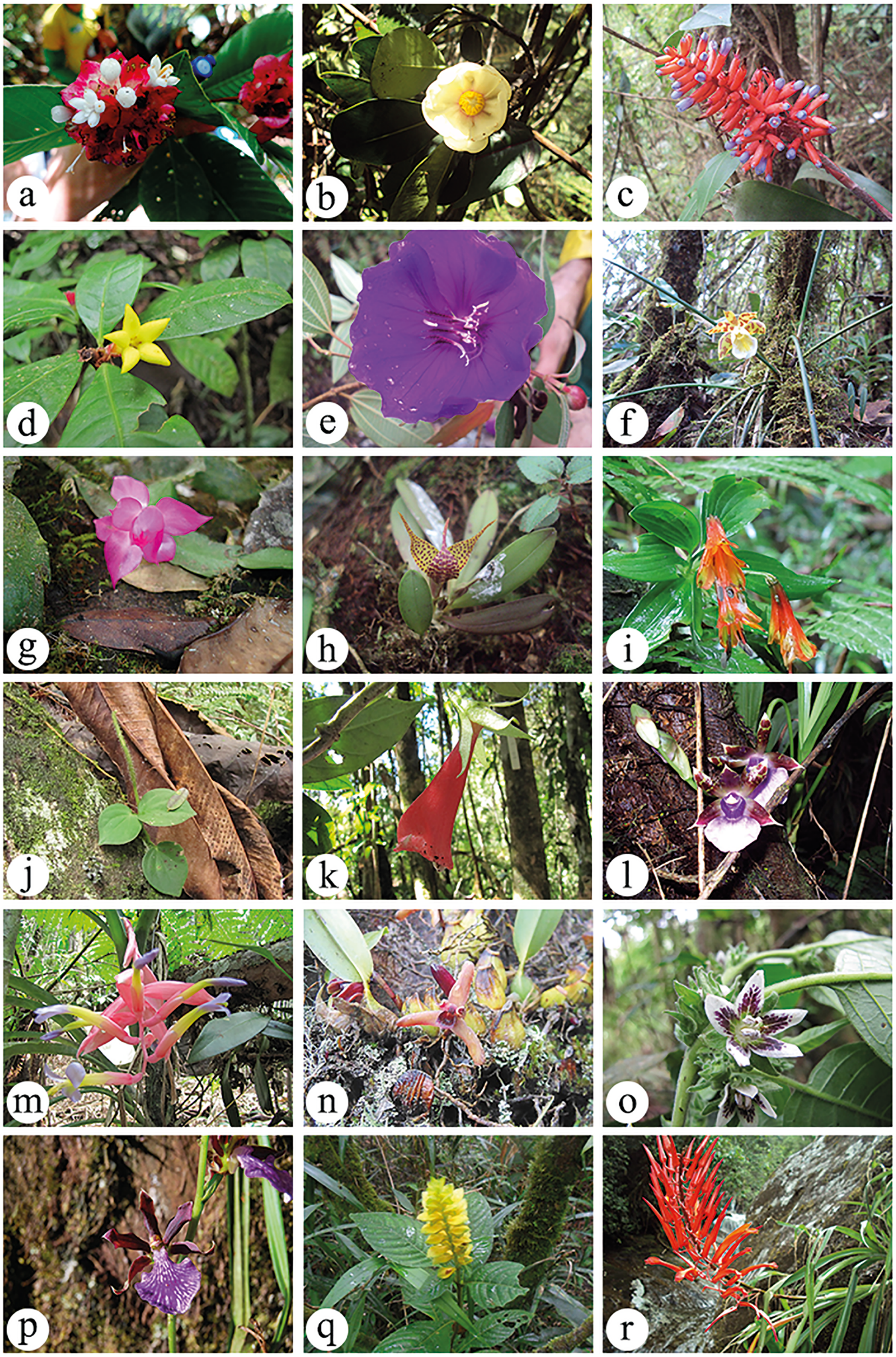

Figure 2. Sample of the vascular plants diversity collected in the Santa Marta valley, Caparaó National Park. (a) *Psychotria bracteocardia* (DC.) Müll.Arg. (Rubiaceae); (b) *Clusia criuva* Cambess. (Clusiaceae); (c) *Aechmea coelestis* (K.Koch) E.Morren (Bormeliaceae); (d) *Psychotria nuda* (Cham. & Schltdl.) Wawra (Rubiaceae); (e) *Pleroma foveolatum* (Naudin) Triana (Melastomataceae); (f) *Scuticaria hadwenii* (Lindl.) Planch. (Orchidaceae); (g) *Schlumbergera* cf. *kautskyi* (Horobin & McMillan) N.P.Taylor (Cactaceae); (h) *Dryadella crenulata* (Pabst) Luer (Orchidaceae); (i) *Alstroemeria cunha* Vell. (Alstroemeriaceae); (j) *Peperomia urocarpa* Fisch. & C.A.Mey. (Piperaceae); (k) *Nematanthus crassifolius* (Schott) Wiehler (Gesneriaceae); (l) *Zygopetalum maxillare* Lodd. (Orchidaceae); (m) *Billbergia euphemiae* E.Morren (Bromeliaceae); (n) *Maxillaria caparaoensis* Brade (Orchidaceae); (o) *Athenaea martiana* Sendtn. (Solanaceae); (p) *Zygopetalum mackayi* Hook. (Orchidaceae); (q) *Staurogyne anigozanthus* (Nees) Kuntze (Acanthaceae); (r) *Pitcairnia flammea* Lindl. (Bromeliaceae). Photos: Araujo, E.A.

Table 1. List of vascular plant species collected in the Santa Marta valley Caparaó National Park, organized by group and plant family, scored for category of threat (DD = Data Deficient; LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered; CR = Critically Endangered). * New records for the State of Espírito Santo; ** Possible new species that are under investigation. The acronym CAP (Herbarium "Capixaba"), where sterile specimens were deposited in a didactic collection, does not have a voucher number. The other with voucher were all deposited in herbaria VIES and CAP.

		Red lists		
GROUP / Family / *Species*	**Voucher**	**IUCN**	**CNCFlora**	**ES Red List**
ANGIOSPERMS				
Acanthaceae				
Aphelandra longiflora (Lindl.) Profice	Dias 666; Zorzanelli 25		LC	LC
Mendoncia velloziana Mart.	Dias 612			LC
Staurogyne anigozanthus (Nees) Kuntze	Araújo 154; Dias 747		NT	EN
Alstroemeriaceae				
Alstroemeria cf. cunha Vell.	Dias 589, 720, 765			
Amaryllidaceae				
Hippeastrum aulicum (Ker Gawl.) Herb.	Dias 727			NT
Annonaceae				
Guatteria pohliana Schltdl.	Araújo 119, 202, 204, 221		NT	
Aquifoliaceae				
Ilex cf. *chamaedryfolia* Reissek	Araújo 254, 272			
Ilex cf. *dumosa* Reissek	Araújo 265, 275			
Ilex cf. theezans Mart. ex Reissek	Araújo 267			
Araceae				
Anthurium gladiifolium Schott	Dias 706			
Anthurium scandens (Aubl.) Engl. *subsp*. scandens	Araújo 525			LC
Araliaceae				
Dendropanax cuneatus (DC.) Decne. & Planch.	Araújo 73; Campanharo 28		LC	
Schefflera calva (Cham.) Frodin & Fiaschi	Araújo 264, 488		LC	LC
Arecaceae				
Euterpe edulis Mart.	CAP		VU	VU
Geonoma schottiana Mart.	Araújo 28; Dias 762		LC	LC
Asteraceae				
Austroeupatorium inulaefolium (Kunth) R.M.King & H.Rob.	Araújo 144; Zorzanelli 557			
Baccharis oblongifolia (Ruiz & Pav.) Pers.	Araújo 490, 491			LC
Bidens cf. *segetum* Mart. ex Colla	Dias 616			
Cyrtocymura cf. scorpioides (Lam.) H.Rob.	Dias 755			
Exostigma rivulare (Gardner) G.Sancho	Araújo 148			
Mikania argyreiae DC.	Araújo 497		VU	LC
Mikania conferta Gardner	Dias 723			LC
Mikania cf. *hoffmanniana* Dusén	Araújo 499			
Mikania hirsutissima DC.	Araújo 146, 151			LC
Mikania lanuginosa DC.	Araújo 205			LC
Mikania lindbergii Baker	Dias 700		LC	LC
Mikania stylosa Gardner	Dias 702			
Mikania trinervis Hook. & Arn.	Araújo 489		LC	LC

continuation...

Piptocarpha leprosa (Less.) Baker	Araújo 173, 485			LC
Piptocarpha macropoda (DC.) Baker	Araújo 174, 492; Campanharo 13			LC
Piptocarpha ramiflora (Spreng.) Baker	Araújo 500, 501			LC
Verbesina cf. *glabrata* Hook. & Arn.	Araújo 362			
Vernonanthura discolor (Spreng.) H.Rob.	Araújo 494, 555; Pinto-Júnior 85			LC
Vernonanthura divaricata (Spreng.) H.Rob.	Araújo 161, 560			LC
Vernonanthura phaeoneura (Toledo) H.Rob.	Araújo 493			
Begoniaceae				
Begonia altamiroi Brade	Araújo 283		EN	DD
Begonia angularis Raddi	Araújo 147		LC	LC
Begonia convolvulacea (Klotzsch) A.DC.	Zorzanelli 525			LC
Begonia digitata Raddi	Dias 673; Zorzanelli 542			LC
Begonia cf. huegelii (Klotzsch) A.DC.	Zorzanelli 524			
Begonia integerrima Spreng.	Araújo 145, 156; Zorzanelli 735		LC	LC
Begonia valdensium A.DC.	Zorzanelli 717		LC	LC
Bromeliaceae				
Aechmea coelestis (K.Koch) E.Morren	Dias 619; Araújo 239, 523			LC
Aechmea lamarchei Mez	Zorzanelli 740			LC
Billbergia euphemiae E.Morren	Araújo 517; Zorzanelli 830			LC
Neoregelia farinosa (Ule) L.B.Sm.	Araújo 518			VU
Nidularium antoineanum Wawra	Zorzanelli 707			EN
Pitcairnia flammea Lindl.	Araújo 573			LC
Quesnelia kautskyi C.M.Vieira	Dias 771; Zorzanelli 556, 726		VU	LC
Tillandsia gardneri Lindl.	Araújo 550		LC	LC
Vriesea carinata Wawra	Araújo 524		LC	LC
Vriesea heterostachys (Baker) L.B.Sm.	Araújo 106; Dias 618, 732; Zorzanelli 706			LC
Vriesea paraibica Wawra	Dias 617			VU
Vriesea ruschii L.B. Sm.	Araújo 40			LC
Cactaceae				
Rhipsalis elliptica G.Lindb. ex K.Schum.	Zorzanelli 720	LC	LC	VU
Schlumbergera cf. *kautskyi* (Horobin & McMillan) N.P.Taylor	Araújo 569			
Campanulaceae				
Lobelia thapsoidea Schott	Araújo 143			LC
Siphocampylus aff. *longipedunculatus* Pohl	Araújo 155; Zorzanelli 725			
Cannaceae				
Canna cf. *paniculata* Ruiz & Pav.	Zorzanelli 722			
Celastraceae				
Maytenus longifolia Reiss. ex Loes.	Araújo 69			LC
Monteverdia cestrifolia (Reissek) Biral	Araújo 76, 135			

continuation...

Monteverdia schummaniana (Loes.) Biral	Araújo 33			
Salacia elliptica (Mart.) G.Don	Araújo 112			LC
Chloranthaceae				
Hedyosmum brasiliense Mart. ex Miq.	Araújo 51, 509; Campanharo 22			LC
Clethraceae				
Clethra scabra Pers.	Campanharo 21; Dias 879		LC	LC
Clusiaceae				
Clusia arrudea Planch. & Triana ex Engl.	Araújo 215			
Clusia organensis Planch. & Triana	Araújo 130, 531; Dias 697		LC	LC
Tovomitopsis saldanhae Engl.	Dias 770			CR
Commelinaceae				
Dichorisandra hexandra (Aubl.) C.B.Clarke	Araújo 60			LC
Cunoniaceae				
Lamanonia ternata Vell.	Araújo 164, 563; Campanharo 16			LC
Cyperaceae				
Scleria cf. *panicoides* Kunth	Zorzanelli 520			
Dichapetalaceae				
Stephanopodium organense (Rizzini) Prance	Araújo 541; Zorzanelli 554			
Elaeocarpaceae				
Sloanea hirsuta (Schott) Planch. ex Benth.	Araújo 111, 570; Dias 842		LC	LC
Sloanea sp.**	Araújo 213			
Ericaceae				
Gaylussacia cf. *martii* Meisn.	Araújo 564			
Euphorbiaceae				
Alchornea triplinervia (Spreng.) Müll. Arg.	Campanharo 14, 25			LC
Croton salutaris Casar.	Araújo 31, 32; Campanharo 15			LC
Tetrorchidium parvulum Müll. Arg.	Araújo 511; Zorzanelli 549			DD
Fabaceae				
Chaetocalyx scandens (L.) Urb.	Araújo 61	LC		
Copaifera trapezifolia Hayne	Campanharo 18			LC
Inga marginata Willd.	Dias 668	LC		LC
Inga platyptera Benth.	Araújo 559	EN	VU	EN
Inga schinifolia Benth.	Araújo 263, 466, 558			
Machaerium declinatum (Vell.) Stellfeld	Araújo 131			LC
Senna multijuga (Rich.) H.S.Irwin & Barneby	Araújo 15; Dias 667			LC
Gentianaceae				
Macrocarpaea glaziovii Gilg	Araújo 136; Zorzanelli 823			VU
Senaea janeirensis Brade	Araújo 522		EN	
Gesneriaceae				

continuation...

Nematanthus crassifolius (Schott) Wiehler	Dias 748, 836; Zorzanelli 739, 821		LC	LC
Paliavana prasinata (Ker Gawl.) Benth.	Araújo 138; Dias 691			LC
Sinningia cooperi (Paxton) Wiehler	Araújo 568		LC	EN
Sinningia magnifica (Otto & A.Dietr.) Wiehler	Dias 589B		LC	LC
Heliconiaceae				
Heliconia angusta Vell.	Zorzanelli 1587		LC	LC
Hypoxidaceae				
Hypoxis decumbens L.	Dias 677			LC
Lauraceae				
Aiouea saligna Meisn.	CAP			LC
Cinnamomum glaziovii (Mez) Kosterm.	Dias 662			
Cinnamomum triplinerve (Ruiz & Pav.) Kosterm.	CAP		LC	
Endlicheria paniculata (Spreng.) J.F.Macbr.	CAP			LC
Licaria bahiana Kurz	Araújo 376			LC
Nectandra aff. *barbellata* Coe-Teix.	CAP			
Nectandra aff. *debilis* Mez	CAP			
Nectandra oppositifolia Nees	CAP			LC
Nectandra psammophila Nees	CAP	EN		LC
Ocotea aciphylla (Nees & Mart.) Mez	Araújo 433	LC		LC
Ocotea aff. *bicolor* Vattimo-Gil	CAP			
Ocotea cernua (Nees) Mez	CAP			LC
Ocotea corymbosa (Meisn.) Mez	Araújo 217, 399			DD
Ocotea dispersa (Nees & Mart.) Mez	Araújo 424			LC
Ocotea aff. *floribunda* (Sw.) Mez	CAP			
Ocotea glaziovii Mez	CAP			LC
Ocotea indecora (Schott) Mez	CAP			LC
Ocotea leucoxylon (Sw.) Laness.	Araújo 428			LC
Ocotea longifolia Kunth	CAP			
Ocotea aff. *notata* (Nees & Mart.) Mez	CAP			
Ocotea aff. *nunesiana* (Vattimo-Gil) J.B. Baitello	CAP			
Ocotea aff. *nutans* (Nees) Mez	CAP			
Ocotea odorifera (Vell.) Rohwer	CAP		EN	EN
Ocotea silvestris Vattimo-Gil	CAP		LC	LC
Ocotea aff. *spixiana* (Nees) Mez	CAP			
Ocotea sulcata Vattimo-Gil	CAP			
Ocotea vaccinioides (Meisn.) Mez	Araújo 383			LC
Persea aff. *fusca* Mez	CAP			
Persea aff. *willdenovii* Kosterm.	CAP			
Rhodostemonodaphne macrocalyx (Meisn.) Rohwer ex Madriñán	Araújo 389, 415			LC
Loranthaceae				
Struthanthus salicifolius (Mart.) Mart.	Araújo 253			DD
Malpighiaceae				
Heteropterys aff. *rubiginosa* A.Juss.	Araújo 135			

continuation...

Malvaceae			
Triumfetta semitriloba Jacq.	Araújo 546; Zorzanelli 743		LC
Marcgraviaceae			
Marcgravia polyantha Delpino	Araújo 547		LC
Melastomataceae			
Henriettea cf. *glabra* (Vell.) Penneys, F.A. Michelangeli, Judd et Almeda	CAP		
Leandra acutiflora (Naudin) Cogn.	Dias 670		DD
Leandra amplexicaulis DC.	Araújo 288		LC
Leandra barbinervis (Cham. ex Triana) Cogn.	Araújo 317		DD
Leandra fallax (Cham.) Cogn.	Araújo 42		LC
Leandra melastomoides Raddi	Araújo 18; Dias 592, 699		LC
Leandra multiplinervis (Naudin) Cogn.	Araújo 20		DD
Leandra quinquedentata (DC.) Cogn.	Araújo 233, 256, 350		
Leandra xanthostachya Cogn.	Araújo 289		DD
Meriania tetramera Wurdack	Araújo 346; Campanharo 8	NT	LC
Miconia cf. *atlantica* Caddah & R. Goldenb.	CAP		
Miconia budlejoides Triana	Araújo 349		LC
Miconia chartacea Triana	Dias 739		LC
Miconia fasciculata Gardner	Araújo 327, 359; Dias 761	LC	LC
Miconia flammea Casar.	Araújo 234, 247, 250		LC
Miconia formosa Cogn.	Araújo 331		LC
Miconia goldenbergiana Caddah	CAP		
Miconia cf. *hirtella* Cogn.	Araújo 304		
Miconia ibaguensis (Bonpl.) Triana	Araújo 59; Zorzanelli 518		LC
Miconia laevigata (L.) D.Don	Zorzanelli 1591		
Miconia latecrenata (DC.) Naudin	Araújo 340		LC
Miconia aff. *lepidota* DC.	Araújo 123, 179		
Miconia cf. *paniculata* (DC.) Naudin	Araújo 13, 231		
Miconia ligustroides (DC.) Naudin	Araújo 291		DD
Miconia longicuspis Cogn.	Araújo 77, 85, 229, 310	NT	LC
Miconia molesta Cogn.	Araújo 196, 218, 259		
Miconia aff. *petroniana* Cogn. & Saldanha	CAP		
Miconia cf. *polyandra* Gardner	CAP		
Miconia pusilliflora (DC.) Naudin	Araújo 258, 352; Dias 675		LC
Miconia sellowiana Naudin	Araújo 158, 167, 187, 341, 356		LC
Miconia setosociliata Cogn.	Araújo 357, 372	VU	VU
Miconia tristis Spring	Araújo 128; Zorzanelli 550		
Miconia aff. *valentinensis* Bacci & R.Goldenb.	Araújo 292		
Ossaea angustifolia (DC.) Triana	Araújo 290		LC
Pleiochiton blepharodes (DC.) Reginato et al.	Dias 593	LC	LC
Pleroma arboreum Gardner	Campanharo 24		LC
Pleroma fissinervium Schrank et Mart. ex DC.	CAP		LC

continuation...

Pleroma fothergillii (Schrank et Mat. ex DC.) Triana	Araújo 293			LC
Pleroma foveolatum (Naudin) Triana*	Araújo 262			
Pleroma heteromallum D. Don (D.Don)	Araújo 62			LC
Tibouchina estrellensis (Raddi) Cogn.	Dias 682			LC
Meliaceae				
Trichilia elegans A.Juss.	Araújo 539			
Trichilia hirta L.	Araújo 540		LC	
Monimiaceae				
Macropeplus schwackeanus (Perkins) I.Santos & Peixoto	Araújo 199, 444, 445		VU	
Mollinedia argyrogyna Perkins	CAP	NT	LC	VU
Mollinedia gilgiana Perkins	Araújo 38, 440, 446; Dias 603, 760	CR	LC	LC
Mollinedia cf. *heteranthera* Perkins	CAP			
Mollinedia oligantha Perkins	Araújo 227; Dias 815			CR
Mollinedia puberula Perkins	CAP			VU
Mollinedia salicifolia Perkins	Araújo 562		NT	VU
Mollinedia schottiana (Spreng.) Perkins	Araújo 183; Dias676; Zorzanelli 527			LC
Moraceae				
Sorocea bonplandii (Baill.) W.C.Burger et al.	Araújo 17			LC
Myrtaceae				
Blepharocalyx salicifolius (Kunth) O.Berg	CAP		LC	LC
Calyptranthes brasiliensis Spreng.	CAP			LC
Calyptranthes pulchella DC.	CAP			LC
Campomanesia cf. *phaea* (O.Berg) Landrum	CAP			
Eugenia cf. *candolleana* DC.	CAP			
Eugenia cf. *capitulifera* O.Berg	CAP			
Eugenia involucrata DC.	CAP			LC
Eugenia leonorae Mattos	CAP		EN	
Eugenia nutans O.Berg	CAP		LC	LC
Eugenia cf. *pisiformis* Cambess.	CAP			
Eugenia ramboi D.Legrand	CAP			
Eugenia cf. *rostrata* O.Berg	CAP			
Marlierea cf. *regeliana* O.Berg	CAP			
Myrceugenia miersiana (Gardner) D.Legrand & Kausel	Dias 724	NT	LC	LC
Myrcia altomontana Sobral & Zorzanelli	Dias 710			
Myrcia bergiana O.Berg	Campanharo 12			LC
Myrcia cf. *bicolor* Kiaersk.	CAP			
Myrcia cf. *coelosepala* Kiaersk.	CAP			
Myrcia guianensis (Aubl.) DC.	Dias 751		LC	LC
Myrcia hartwegiana (O.Berg) Kiaersk.	Dias 754			LC
Myrcia lineata (O.Berg) Nied.	Araújo 375; Dias , 753; Zorzanelli 727	VU	EN	LC
Myrcia cf. *oligantha* O.Berg	CAP			

continuation...

Myrcia pubipetala Miq.	CAP	LC	
Myrcia retorta Cambess.	CAP		
Myrcia splendens (Sw.) DC.	Araújo 89, 557; Zorzanelli 552		LC
Myrcia subcordata DC.	Araújo 121, 201		
Myrciaria cf. *floribunda* (H.West ex Willd.) O.Berg	CAP		
Pimenta pseudocaryophyllus (Gomes) Landrum	Araújo 556		
Plinia rivularis (Cambess.) Rotman	Araújo 505		LC
Siphoneugena dussii (Krug & Urb.) Proença	CAP		LC
Nyctaginaceae			
Guapira graciliflora (Mart. ex Schmidt) Lundell	Dias 841		
Ochnaceae			
Ouratea grandiflora (A.DC.) Engl.	Araújo 214		
Ouratea parviflora (A.DC.) Baill.	Araújo 190		
Ouratea vaccinioides (A.St.-Hil. & Tul.) Engl.	Araújo 513		
Onagraceae			
Fuchsia regia (Vell.) Munz	Dias 590	LC	LC
Orchidaceae			
Anathallis sclerophylla (Lindl.) Pridgeon & M.W.Chase	Araújo 549		LC
Brasiliorchis cf. *picta* (Hook.) R.B.Singer et al.	Dias 768		
Brasiliorchis ubatubana (Hoehne) R.B.Singer et al.	Zorzanelli 532		LC
Cattleya cf. *coccinea* Lindl.	Dias 766		
Dryadella crenulata (Pabst) Luer	Araújo 207		
Elleanthus brasiliensis (Lindl.) Rchb.f.	Dias 826		LC
Epidendrum paranaense Barb.Rodr.	Araújo 84	LC	EN
Epidendrum saxatile Lindl.	Dias 813	LC	LC
Eurystyles actinosophila (Barb.Rodr.) Schltr.	Dias 686		LC
Gomesa forbesii (Hook.) M.W.Chase & N.H.Williams	Dias 820		LC
Gomesa cf. *recurva* R.Br.	Araújo 574		
Isochilus linearis (Jacq.) R.Br.	Zorzanelli 523		LC
Maxillaria caparaoensis Brade	Zorzanelli 546		VU
Pabstiella fusca (Lindl.) Chiron & Xim. Bols.	Zorzanelli 834		LC
Pabstiella pseudotrifida L. Kollmann & D. R. Couto	Dias 827		EN
Pabstiella punctatifolia (Barb.Rodr.) Luer	Dias 821		LC
Pogoniopsis nidus-avis Rchb.f. & Warm.	Dias 811		VU
Pogoniopsis schenckii Cogn.	Araújo 193	LC	VU
Prescottia stachyodes (Sw.) Lindl.	Dias 764		LC
Promenaea cf. *xanthina* (Lindl.) Lindl.	Araújo 191; Dias 854		

continuation...

Prosthechea cf. *bulbosa* (Vell.) W.E.Higgins	Dias 672		
Prosthechea pygmaea (Hook.) W.E.Higgins	Zorzanelli 541		LC
Scaphyglottis modesta (Rchb.f.) Schltr.	Zorzanelli 540		LC
Scuticaria hadwenii (Lindl.) Planch.	Araújo 244		VU
Zygopetalum maculatum (Kunth) Garay	Dias 767	LC	LC
Zygopetalum maxillare Lodd.	Araújo 575	LC	EN
Passifloraceae			
Passiflora mediterranea Vell.	Araújo 63		LC
Passiflora aff. *porophylla* Vell.	Dias 828		
Passiflora speciosa Gardner	Araújo 512, 551; Zorzanelli 544		LC
Pentaphylacaceae			
Freziera atlantica Zorzanelli & Amorim	CAP		CR
Peraceae			
Pera glabrata (Schott) Poepp. ex Baill.	Araújo 280, 545		LC
Pera heteranthera (Schrank) I.M.Johnst.	Araújo 544		LC
Phyllanthaceae			
Hyeronima alchorneoides Allemão	Campanharo 29		LC
Piperaceae			
Peperomia alata Ruiz & Pav.	Dias 615, 685; Zorzanelli 521		LC
Peperomia corcovadensis Gardner	Araújo 361; Zorzanelli 729		LC
Peperomia choroniana C.DC.	Araújo 514		EN
Peperomia mandioccana Miq.	Dias 728	LC	VU
Peperomia martiana Miq.	Dias 684		LC
Peperomia tetraphylla (G.Forst.) Hook. & Arn.	Araújo 195, 237; Dias 824		LC
Peperomia tetraphylla var. valantoides (Miq.) Yunck.	Dias 729		LC
Peperomia urocarpa Fisch. & C.A.Mey.	Araújo 37; Dias 611; Zorzanelli 517		LC
Piper aduncum L.	Zorzanelli 829		LC
Piper eucalyptophyllum C.DC.	Dias 604		LC
Piper cf. *lhotzkyanum* Kunth	Araújo 58		
Piper mollicomum Kunth	Araújo 516		LC
Piper richardiifolium Kunth	Araújo 515; Dias 607		LC
Piper strictifolium D.Monteiro & E.F.Guim.	Araújo 565		VU
Piper tectoniifolium Kunth	Araújo 21	LC	LC
Polygalaceae			
Polygala campestris Gardner	Araújo 576		
Primulaceae			
Cybianthus fuscus Mart.	CAP		LC
Cybianthus cf. *obovatus* (Mart.) Mart. ex Miq.	CAP		
Cybianthus peruvianus (A.DC.) Miq.	Araújo 118, 192, 487		LC
Myrsine gardneriana A.DC.	Araújo 109, 159		LC

continuation...

Myrsine hermogenesii (Jung-Mend. & Bernacci) M.F.Freitas & Kin.-Gouv.	CAP		LC
Myrsine lancifolia Mart.	Araújo 278, 278-B		LC
Myrsine umbellata Mart.	Zorzanelli 728		LC
Myrsine villosissima Mart.	CAP	EN	EN
Stylogyne warmingii Mez	CAP		LC
Proteaceae			
Roupala consimilis Mez ex Taub.	Araújo 178, 530		EN
Quiinaceae			
Lacunaria crenata (Tul.) A.C.Sm.	Araújo 521		DD
Rhamnaceae			
Reissekia smilacina (Sm.) Steud.	Araújo 64	LC	LC
Rhamnus sphaerosperma Sw.	Araújo 228, 486, 498	LC	LC
Rubiaceae			
*Amaioua intermedia Mart. ex Schult. & S*chult.f.	CAP		LC
Bathysa australis (A.St.-Hil.) K.Schum.	Araújo 98, 482; Dias 605	LC	LC
Bathysa cf. *nicholsonii* K.Schum.	Araújo 481		
Coccocypselum lanceolatum (Ruiz & Pav.) Pers.	Dias 703		
Cordiera cf. *longiflora* (K.Schum.) Kuntze	Araújo 273, 484		
Coussarea cf. *congestiflora* Müll.Arg.	CAP		
Coussarea cf. *nodosa* (Benth.) Müll.Arg.	CAP		
Emmeorhiza umbellata (Spreng.) K.Schum.	Araújo 152		LC
Hillia parasitica Jacq.	Araújo 39; Dias 743	LC	LC
Palicourea longipedunculata Gardner	Araújo 480; Campanharo 1		
Posoqueria cf. *acutifolia* Mart.	CAP		
Posoqueria cf. *latifolia* (Rudge) Schult.	Dias 878		
Psychotria bahiensis DC.	Araújo 81, 225, 242, 266, 475		LC
Psychotria bracteocardia (DC.) Müll. Arg.	Araújo 47		
Psychotria leiocarpa Cham. & Schltdl.	Araújo 474		LC
Psychotria nuda (Cham. & Schltdl.) Wawra	Araújo 12; Dias 595, 694; Zorzanelli 537		
Psychotria pallens Gardner	Araújo 71; Zorzanelli 522		
Psychotria ruelliifolia (Cham. & Schltdl.) Müll.Arg.	Araújo 82; Zorzanelli 745		
Psychotria suterella Müll.Arg.	Dias 610		LC
Psychotria vellosiana Benth.	Araújo 75, 78, 169, 170, 483		LC
Psychotria sp.**	Araújo 19		
Rudgea cf. *coronata* (Vell.) Müll.Arg.	CAP		
Rudgea cf. *jasminoides* (Cham.) Müll. Arg.	CAP		
Rudgea cf. *triflora* Benth.	Araújo 133		
Sabiaceae			
Meliosma sellowii Urb.	Araújo 548		LC
Salicaceae			

continuation...

Casearia arborea (Rich.) Urb.	Araújo 542, 543			LC
Sapindaceae				
Allophylus edulis (A.St.-Hil. et al.) Hieron. ex Niederl.	Araújo 72, 536, 537			LC
Allophylus racemosus Sw.	Araújo 538			LC
Cupania ludowigii Somner & Ferrucci	Araújo 535			LC
Paullinia carpopoda Cambess.	Araújo 125			LC
Thinouia mucronata Radlk.	Araújo 561			LC
Sapotaceae				
Micropholis crassipedicellata (Mart. & Eichler) Pierre	Araújo 211, 216	NT	LC	LC
Siparunaceae				
Siparuna brasiliensis (Spreng.) A.DC.	Zorzanelli 828		LC	LC
Smilacaceae				
Smilax staminea Griseb.	Araújo 571			
Solanaceae				
Athenaeae cuspidata (Witasek) I.M.C.Rodrigues & Stehmann	Dias 750			
Athenaeae martiana (Sendtn.) I.M.C.Rodrigues & Stehmann	Araújo 97, 120; Dias 749; Zorzanelli 831			
Athenaeae picta (Mart.) I.M.C.Rodrigues & Stehmann.	Zorzanelli 719			
Brunfelsia brasiliensis (Spreng.) L.B.Sm. & Downs	Araújo 189; Campanharo 27			LC
Capsicum mirabile Mart.	Dias 713			
Cestrum bracteatum Link & Otto	Dias 614, 692, 846; Zorzanelli 543			LC
Cestrum strigilatum Ruiz & Pav.	Dias 769			LC
Cestrum subpulverulentum Mart.	Dias 688			LC
Solanum campaniforme Roem. & Schult.	Dias 663			LC
Solanum cinnamomeum Sendtn.	Araújo 168,528; Campanharo 4; Dias 690	NT	LC	LC
Solanum didymum Dunal	Zorzanelli 528			LC
Solanum leucodendron Sendtn.	Araújo 163, 175, 529	NT	LC	LC
Solanum pseudoquina A.St.-Hil.	Campanharo 26		LC	LC
Solanum swartzianum Roem. & Schult.	Dias 722			LC
Solanum sp.**	Araújo 200			
Symplocaceae				
Symplocos estrellensis Casar.	Araújo 70, 132			
Theaceae				
Laplacea fruticosa (Schrad.) Kobuski	Araújo 532, 533	LC		LC
Thymelaeaceae				
Daphnopsis fasciculata (Meisn.) Nevling	Araújo 124			LC
Urticaceae				
Pilea hilariana Wedd.*	Araújo 235		NT	
Verbenaceae				
Lantana camara L.	Zorzanelli 545			LC
Vitaceae				

continuation...

Cissus cf. *tinctoria* Mart.	Araújo 65		
Vochysiaceae			
Vochysia angelica M.C.Vianna & Fontella	Araújo 53	EN	EN
Vochysia bifalcata Warm.	CAP		DD
Vochysia glazioviana Warm.	Araújo 519		
Vochysia cf. *oppugnata* (Vell.) Warm.	CAP		
Vochysia santaluciae M.C.Vianna & Fontella	Araújo 520	EN	EN
Winteraceae			
Drimys brasiliensis Miers	Araújo 203, 223; Dias 736	LC	LC
FERNS AND LYCOPHYTES			
Anemiaceae			
Anemia mandioccana Raddi	Araújo 27, 188; Dias 599; Zorzanelli 536		LC
Anemia phyllitidis (L.) Sw.	Araújo 26		LC
Aspleniaceae			
Asplenium gastonis Fée	Zorzanelli 733		
Asplenium cf. *harpeodes* Kunze	Zorzanelli 515		
Asplenium scandicinum Kaulf.	Araújo 572		
Blechnaceae			
Neoblechnum brasiliense (Desv.) Gasper & V.A.O. Dittrich	Zorzanelli 736		LC
Cyatheaceae			
Alsophila salvinii Hook.*	Araújo 502		DD
Alsophila setosa Kaulf.	Araújo 534, 552, 553		LC
Cyathea atrocastanea Labiack P.E. et Matos F.B.	Araújo 94, 96, 186		EN
Cyathea corcovadensis (Raddi) Domin	Araújo 29; Zorzanelli 553	LC	LC
Cyathea delgadii Sternb.	Araújo 90, 100, 113		LC
Cyathea dichromatolepis (Fée) Domin	Araújo 23, 25		LC
Cyathea phalerata Mart.	Araújo 114, 116, 186B		LC
Cyathea rufa (Fée) Lellinger	Araújo 171, 503, 504		LC
Dennstaedtiaceae			
Blotiella lindeniana (Hook.) R.M.Tryon	Araújo 209	LC	EN
Marattiaceae			
Eupodium kaulfussii (J.Sm.) J.Sm.	Araújo 22, 554; Zorzanelli 832		LC
Polypodiaceae			
Campyloneurum repens (Aubl.) C.Presl	Zorzanelli 519		
Cochlidium punctatum (Raddi) L.E.Bishop	Zorzanelli 715		LC
Microgramma percussa (Cav.) de la Sota	Zorzanelli 530		LC
Niphidium crassifolium (L.) Lellinger	Araújo 16		LC
Pleopeltis hirsutissima (Raddi) de la Sota	Zorzanelli 822		LC
Serpocaulon fraxinifolium (Jacq.) A.R. Sm.	Araújo 137		
Pteridaceae			
Pteris splendens Kaulf.	Zorzanelli 534		LC

results may thus contribute to real actions for the conservation of biodiversity in the park, given that listings are an important source of basic information for scientists and decision-makers (Ulloa Ulloa et al. 2017), in addition to providing relevant data for the "Flora do Espírito Santo" and "Catálogo de Plantas das Unidades de Conservação do Brasil" Projects (Dutra et al. 2015, Carrijo et al. 2020).

The CNP is one of the few large remnants in the Atlantic Forest, which thereby confers larger potential to keep high rates of biodiversity in relation to other smaller remnants, making it extremely important for species conservation (Oliveira et al. 2017). In a scenario in which protected areas within the Atlantic Forest are mostly small and disconnected (Joppa et al. 2008), our list demonstrates the importance of CNP for the conservation of species, since one of the great challenges for biodiversity conservation in Brazil is to create matrices of protected areas which are large enough for the actual conservation of biological diversity (Rylands & Brandon 2005).

We highlight herein the role played by the CNP in protecting species in montane environments of the Atlantic Forest, as shown in our study (Table 1). Protected areas are indeed the best strategy to reduce deforestation and the extinction of species in tropical regions (Joppa et al. 2008). For example, a recent study in Brazil has demonstrated that protected areas preserve a considerable share of known Brazilian biodiversity (Oliveira et al. 2017).

However, we have mentioned the need for conservation actions that can surpass the CNP limits and to which the importance of local communities, should be recognized to protect forest remnants and maintain the biodiversity in these areas. It is important to have a positive interaction between the protected area and its surrounding areas, since the maintenance of native vegetation close to it contributes to maintaining ecological processes and species richness in protected areas (DeFries et al. 2005). In certain occasions, pressure within the protected area's limits reflect the ones happening in its surrounding areas (Laurance et al. 2012). Thus, affirmative actions taken with surrounding communities of the CNP are essential, aiming to reduce treats such illegal hunting and extraction of native species, especially endangered ones (e.g. *E. edulis*).

The botanical families that have presented the highest richness in our study are also the most rich ones across the Atlantic Forest mountains (Amorim et al. 2009, Pifano et al. 2010, Coelho & Amorim 2014, Meireles et al. 2014, BFG 2018, Dutra et al. 2015, Zorzanelli et al. 2017). In addition to these families, the most representative genera (*Miconia*) in our research usually present high numbers of species in montane and upper montane forests, being the main taxa in these formations (Oliveira-Filho & Fontes 2000). For example, Melastomataceae species are common to the Atlantic Forest, rainforests and more elevated forests (Goldenberg et al. 2012). We highlight the *Miconia* genus, represented in the Santa Marta Valley by almost half of known *Miconia* species known to Espírito Santo (23/55 spp. - 42%) (Bacci et al. 2016). This number shows the significance of the elevation gradient for the wealth and abundance of plant groups, and also reinforces the need to consider it when proposing biodiversity conservation measures for mountains in the Atlantic Forest as center of diversity.

The occurrence of species classified into different endangered degrees enables a basis for conservation actions for these species. According to Moraes et al. (2014), the conservation process of a species categorized as endangered begins with its inclusion on a Red List, and after that moment collective efforts must be made to protect these species. We suggest the adoption of the list of threatened species created by this study as a possibility to guide a better zoning of the park, subsidizing protection actions for the area as a whole.

Our results have indicated high diversity of vascular plants in the Santa Marta valley, municipality of Ibitirama, with presence of species threatened, new records, occurrence of possible new species and the presence of recently described species. These data contribute to the knowledge of the Caparaó National Park Flora, mainly for the Capixaba portion where further research to prospect biological data should be encouraged. Moreover, these results highlight the role played by protected areas, showing that they are an essential strategy for protecting diversity from to extinction threats. As such, the CNP plays an important role for species preservation in montane and upper montane forests in the Brazilian southeast and for biodiversity in the Atlantic rainforest, and efforts must be maintained to mitigate existing conflicts within the territorial limit of the protected area.

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Author Contributions

Eduardo Alves Araújo: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Sustanis Horn Kunz: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to critical revision, adding intellectual content.

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Conflicts of interest

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

Ethics

The authors declares that the research did not involve humans or clinical trials in this manuscript.

Data availability

The authors inform that all data are available in the SISBIO Database because it was carried out within a federal protected area (National Park).

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Inventory

Eunotia (Bacillariophyceae) from a subtropical stream adjacent to Iguaçu National Park, Brazil, with the proposition of a new species

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Abstract: *Eunotia* species from a stream located in the surrounding area of the Iguaçu National Park conservation unit were analyzed. Samples were monthly collected from September 2012 to August 2013. The periphytic material, manually obtained by squeezing aquatic macrophytes, was oxidized and analyzed under light and scanning electron microscopy. Twenty-one infrageneric taxa of *Eunotia* were identified, measured, and illustrated. *Eunotia caniculoides* sp. nov. is proposed as a new species, and eight taxa are new records for Paraná state.
Keywords: *Bacillariophyta; diatoms; periphyton; southern Brazil; taxonomy.*

Eunotia (Bacillariophyceae) de um riacho subtropical adjacente ao Parque Nacional do Iguaçu, Brasil, com a proposta de uma espécie nova

Resumo: Espécies de *Eunotia* Ehrenberg de um ambiente lótico localizado em área adjacente à unidade de conservação Parque Nacional do Iguaçu foram analisadas. As coletas foram realizadas mensalmente de setembro de 2012 a agosto de 2013. O material perifítico obtido do espremido manual de macrófitas aquáticas foi oxidado e analisado sob microscopia óptica e eletrônica de varredura. Vinte e um táxons de *Eunotia* foram identificados, medidos e ilustrados. *Eunotia caniculoides* sp. nov. é proposta como uma nova espécie e oito táxons constituem novos registros para o estado do Paraná.
Palavras-chave: *Bacillariophyta; diatomáceas; perifíton; sul do Brasil; taxonomia.*

Introduction

The genus *Eunotia* Ehrenberg is characterized by the valve isopolarity, apical axis asymmetry, uniseriate striae, short raphe system, usually restricted to the valve apices and the presence of rimoportulae (Kociolek & Spaulding 2003, Furey 2011). The extant species are found in freshwater environments (Round et al. 1990) but some fossil *Eunotia* have already been described from marine sediments in New Zealand (Novitski & Kociolek 2005). The genus includes species with ecological optimum in the epiphyton and metaphyton of dystrophic to oligotrophic waters, being valuable indicators of acidic and low-conductivity conditions (Round et al. 1990, Van Dam et al. 1994, Cantonati & Lange-Bertalot, 2011, Cox 2015).

Eunotia is one of the most common and species-rich diatom genera in the Neotropics (Patrick 1940a, 1940b, Hustedt 1952a, 1952b, 1965, Metzeltin & Lange-Bertalot 1998, 2007, Sala et al. 2002a, 2002b, Dunck et al 2016, Faustino et al. 2016, Costa et al. 2017), with remarkable diversity in Brazilian acidic environments (Ferrari et al. 2007, Bicca et al. 2011, Canani & Torgan 2013, Dunck et al. 2013a, 2013b, 2016, Cavalcante et al. 2014). In the state of Paraná, the genus were reported by Ludwig & Valente-Moreira (1989), Fürstenberger & Valente-Moreira (2000), Tremarin et al. (2008), Faria et al. (2010), Bertolli et al. (2010), Santos et al. (2011), Bartozek et al. (2013), Marra et al. (2016), Silva-Lehmkuhl et al. (2019), and Bartozek et al. (2020) recently proposed a new species. Based on a bibliographic check-list of freshwater diatom studies developed in the state of Paraná from 1954 to 2009, Tremarin et al. (2009) listed 110 *Eunotia* infrageneric taxa, and Cavalcante et al. (2014) mention about 245 taxa registered to Brazil.

The purpose of the present publication was to investigate the species diversity of *Eunotia* from a stream located in the surrounding area of the Iguaçu National Park conservation unit. A new species, *Eunotia caniculoides*, was formally described. The distinctive characters between similar taxa were discussed and taxonomical notes were provided. Distribution in the state of Paraná and ecological informations were also added.

Material and Methods

The present study was carried out on the Tenente João Gualberto stream, located in a conservation unit area adjacent to the Iguaçu National Park, in the municipality of São Miguel of Iguaçu (25º28’36.3”S; 54º19’40.9”W), the western region of the state of Paraná. According to Horton-Strahler criteria (Horton 1945, Strahler 1952), the stream is a major tributary of the Baixo Iguaçu Basin, having its source and part of its course among agricultural fields (Figure 1). The regional climate is Cfa type, subtropical humid mesotherm, with well-defined summer and winter periods, and rains distributed during the year (Alvares et al. 2013).

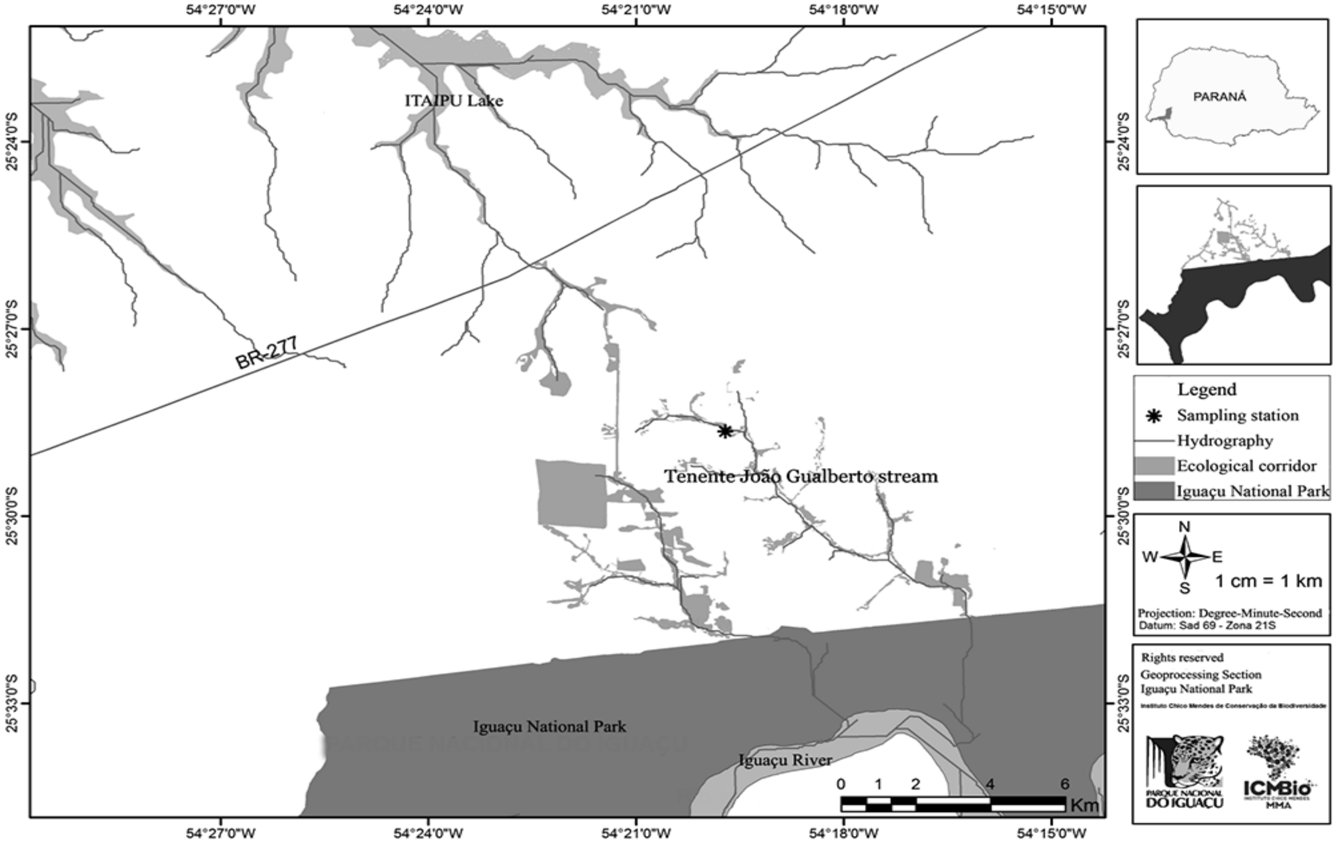


Figure 1. Location of the sampling station in the Tenente João Gualberto stream, São Miguel do Iguaçu, PR, Brazil.

Diatom samples were collected monthly, from September 2012 to August 2013. Limnological variables as dissolved oxygen (mg L^{-1}), pH, water temperature (ºC), conductivity (mS cm^{-1}), and turbidity (NTU) were measured in the field using a Horiba U-5000 multiparameter probe. Nutrient analysis of organic nitrogen – N_{org}. (mg L^{-1}), ammoniac nitrogen – NH_4^+ (mg L^{-1}), nitrate – NO_3^- (mg L^{-1}), nitrite – NO_2^- (mg L^{-1}), orthophosphate – PO_4^{-3} (mg L^{-1}) e total phosphorous – TP (mg L^{-1}, were carried out at the Limnological Laboratory of the Research Group on Fishery Resources and Limnology (GERPEL), from UNIOESTE, campus Toledo, according to American Public Health Association (2005). Weekly precipitation (mm) data were provided by Paraná Meteorological System (SIMEPAR). Minimum and maximum values of limnological data were described for each identified *Eunotia* species.

Periphytic samples were removed by manually squeezing the aquatic macrophyte *Eleocharis minima* Kunth (Cyperaceae) and were preserved in Transeau solution, in 1:1 proportion (Bicudo & Menezes 2017). The organic matter was eliminated by oxidation with $KMnO_4$ and HCl according to Simonsen (1974) technique modified by Moreira-Filho & Valente-Moreira (1981). Cleaned material was dried onto glass coverslips and mounted on slides with Naphrax® (IR= 1.74). The taxonomic analysis was performed using an Olympus BX60 light microscope equipped with a DP71 image capture camera. Subsamples from cleaned samples were mounted on aluminum stubs and coated with gold. Micrographs were taken with JEOL JSM 6360 scanning electron microscope (SEM), operated at15 kV and 8mm working distance, housed in the Electron Microscopy Center from the Federal University of Paraná. Slides and samples were deposited in the UNOPA Herbarium, UNIOESTE, campus Cascavel and registered as shown in Table 1.

Morphological terminology follows Barber & Haworth (1981) and Round et al. (1990). The classification system was based on Cox (2015).

Every *Eunotia* species was measured at the valve apical length, transapical width and striae density in the central part of the valve. The ecological information cited for identified taxa corresponds to the abiotic parameters measured in the samples collected during this study.

Constancy is a measure of species occurrence (C) and was expressed as follows: constant (C ≥ 70%), common (30% ≥ C ≤ 70%), sporadic (10% ≥ C ≤ 30%) and rare (C ≤ 10%) (Dajoz 2005).

Results

Twenty-one taxa were measured and illustrated. Eight *Eunotia* taxa are new records for the state of Paraná: *E. georgii* Metzeltin & Lange-Bertalot, *E. karenae* Metzeltin & Lange-Bertalot, *E. guianensis* (Ehrenberg) De Toni, *E. juettnerae* Lange-Bertalot, *E. pileus* Ehrenberg, *E. pseudosudetica* var. *rotundata* Cavalcante, Tremarin & T.Ludwig, *E. sedina* Lange-Bertalot, Bak & Witkowski, and *E. tropico-arcus* Metzeltin & Lange-Bertalot. *Eunotia caniculoides* sp. nov. is formally proposed as a new species and are compared with close taxa (Table 2). The occurrence of species in the samples as well as ecological and environmental data from the sampling are listed in Table 3.

Bacillariophyceae Haeckel
Eunotiophycidae D.G. Mann
Eunotiales P.C. Silva
Eunotiaceae Kützing
Eunotia Ehrenberg

Table 1. Herbarium vouchers (UNOPA), date, geographic coordinates, and collector of samples

UNOPA	Sampling date	Geographic coordinates	Collector
3943	20/IX/2012	25º28'36.3"S 54º19'40.9"W	Servat, L.C
3962	29/X/2012	25º28'36.3"S 54º19'40.9"W	Servat, L.C
3976	13/XI/2012	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4035	18/XII/2012	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4044	15/I/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4057	06/II/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4081	11/III/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4093	04/IV/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4102	09/V/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4116	06/VI/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4126	16/VII/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C
4136	12/VIII/2013	25º28'36.3"S 54º19'40.9"W	Servat, L.C

Eunotia ambivalens Lange-Bertalot & Tagliaventi. In: Lange-Bertalot (ed.), Diatoms of Europe 6, p. 49, pl. 11, figs 1–11, pl. 12, figs 1–6, pl. 13, figs. 1–11, 2011.

Figures 2a–2d, 4a.

Valves slightly arched, 77.6–177.3 µm long and 4.2–5.6 µm wide. Dorsal margin convex, ventral margin concave. Apices rounded. Terminal nodules near the apices. Striae parallel, 11–12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM. In SEM: presence of short marginal spines, ca. 41 areolae in 10 µm.

Frequency of occurrence: common (Table 3).

Paraná State citations: cited as *E. bilunaris* var. *linearis* (Okuno) Lange-Bertalot & Nörpel-Schempp: Tremarin et al. (2009), Faria et al. (2010), Santos et al. (2011)

Taxonomic remarks: *Eunotia ambivalens* distinguishes from *E. bilunaris* by slightly arched valves, almost parallel margins, ends not protracted, lower striae density (11.5–13/10 µm vs 13–17/10 µm), and by the presence of marginal spines (Figure 3a) only discernible in SEM (Lange-Bertalot et al. 2011). The species "locus typicus" is Albania, Flower Lake, Lura Mountais, but it was also observed in oligotrophic alkaline or slightly acidic waters with low to moderate conductivity (Lange-Bertalot 2011).

Eunotia bilunaris (Ehrenberg) Schaarschmidt. In: Kanitz, Magyar Novenytani Lapok 5: 159, 1880.

Basionym: *Synedra bilunaris* Ehrenberg. Abh. Königl. Akad. Wiss. Berl. 1831: 87, 1832.

Figures 2e–2j, 4b–c.

Table 2. Comparison of morphometric data and morphological features of *E. caniculoides* sp. nov., *E. canicula*, *E. intricans* and *E. sioliopsis*

Feature	*E. caniculoides*	*E. canicula*	*E. intricans*	*E. sioliopsis*
Length (µm)	30–58.5	20–35[2] 23.2–25[3]	17–48[1]	20–40[4]
Width (µm)	3.5–4.9	3.5–4.0[2] 3.5–5.2[3]	4–5[1]	3.5–4.5[4]
Striae in 10 µm	10–14 (center) 13-14 (ends)	14–15[2] (center) 16–22[3] (center)	11–13 (center)[1] 18 (ends)[1]	16–17 (center)[4] 18 (ends)[1]
Areolar density in the central area in 10 µm	38–40	> 35[2]	39–42[1]	Up to 30[4]
Valve symmetry	Dorsal margin slightly convex, almost straight in the larger valves; ventral margin straight to subtly concave	Dorsal margin convex, ventral margin straight to slightly concave, with valve mantle thickened at the proximal raphe ends[2] Dorsal margin convex, ventral margin concave[3]	Dorsal margin moderately convex to almost straight in the longer valves; ventral margin straight or almost straight[1]	Dorsal margin convex; ventral margin straight to slightly concave, with valve mantle thickened at the proximal raphe end[4]
Valve apices	Apices acutely rounded, nose-like, quite protracted, differentiated from the main body and deflected to the ventral side	Apices acutely rounded, tapered, subrostrate, slightly delimited by a change in the slope of the dorsal margin, appearing nose-like[2]	Apices rounded, shortly nose-like, not flexed to the ventral side[1]	Apices rounded, nose-like, deflected to the ventral side[4]
Rimoportula	At one end, at the center of the apex	At one end, at the center of the apex[2]	At one end, at the center of the apex[1]	No described

Lange-Bertalot & Metzeltin (2009)[1], Furey et al. (2011)[2], Fontana & Bicudo (2012)[3], Moser et al. (1998) [4]

Valves arched tapering toward apices, 16.2–98.7 µm long and 3.1–4.6 µm wide. Dorsal margin convex, ventral margin concave sometimes with median swelling (Figure 2e-2j). Apices rounded to acutely rounded. Terminal nodules near the apices. Distal raphe end strongly curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel, denser near the apices, 12–15 in 10 µm in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four open bands perforated by a linear row of pores.

Frequency of occurrence*:* constant (Table 3).

Paraná State citations: Tremarin et al. (2009), Faria et al. (2010), Silva et al. (2010), Santos et al. (2011), Marra et al. (2016), Bartozek et al. (2013). Sometimes cited as *Eunotia curvata* (Kützing) Lagerstedt var. *curvata* and as *Eunotia lunaris* (Ehrenberg) Grunow var. *lunaris* (Tremarin et al. 2009).

Taxonomic remarks*: Eunotia bilunaris* is a very common periphytic species, broadly distributed in the state of Paraná, and frequently registered in Europe, Africa, Asia, North and South America (Costa et al. 2017). According to Lange-Bertalot et al. (2011), the species have a heterogeneous concept and a confusing taxonomy due to their wide morphological variability, mostly related to shape and dimensions. Specimens with uncommon morphology are common and have already been formally described as infraspecific taxa. The material analysed showed a wide morphological and metric variation of the valves, corroborating with the observations of Krammer & Lange-Bertalot (1991) and Tavares & Valente-Moreira (2000).

Eunotia botulitropica C.E. Wetzel & L.F. Costa. In: Costa et al., Bibliotheca Diatomologica 64: 14, pl. 58, figs 11–46, pl. 59, figs 1–3, pl. 61, figs 1–3, 2017.

Figures 2p–2t, 4d–4f.

Frustule rhomboid in lateral view. Valves dorsiventral, sometimes heteropolar, 11.1–22.5 µm long and 2.5–3.7 µm wide. Dorsal margin convex, ventral margin straight to slightly concave. Apices rounded to obtusely rounded . Terminal nodules near the apices. Distal raphe end curved onto the valve face. One small rimoportula per valve, near the apex. Striae parallel, 14–19 in 10 µm in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four open bands perforated by one row of pores.

Frequency of occurrence*:* common (Table 3).

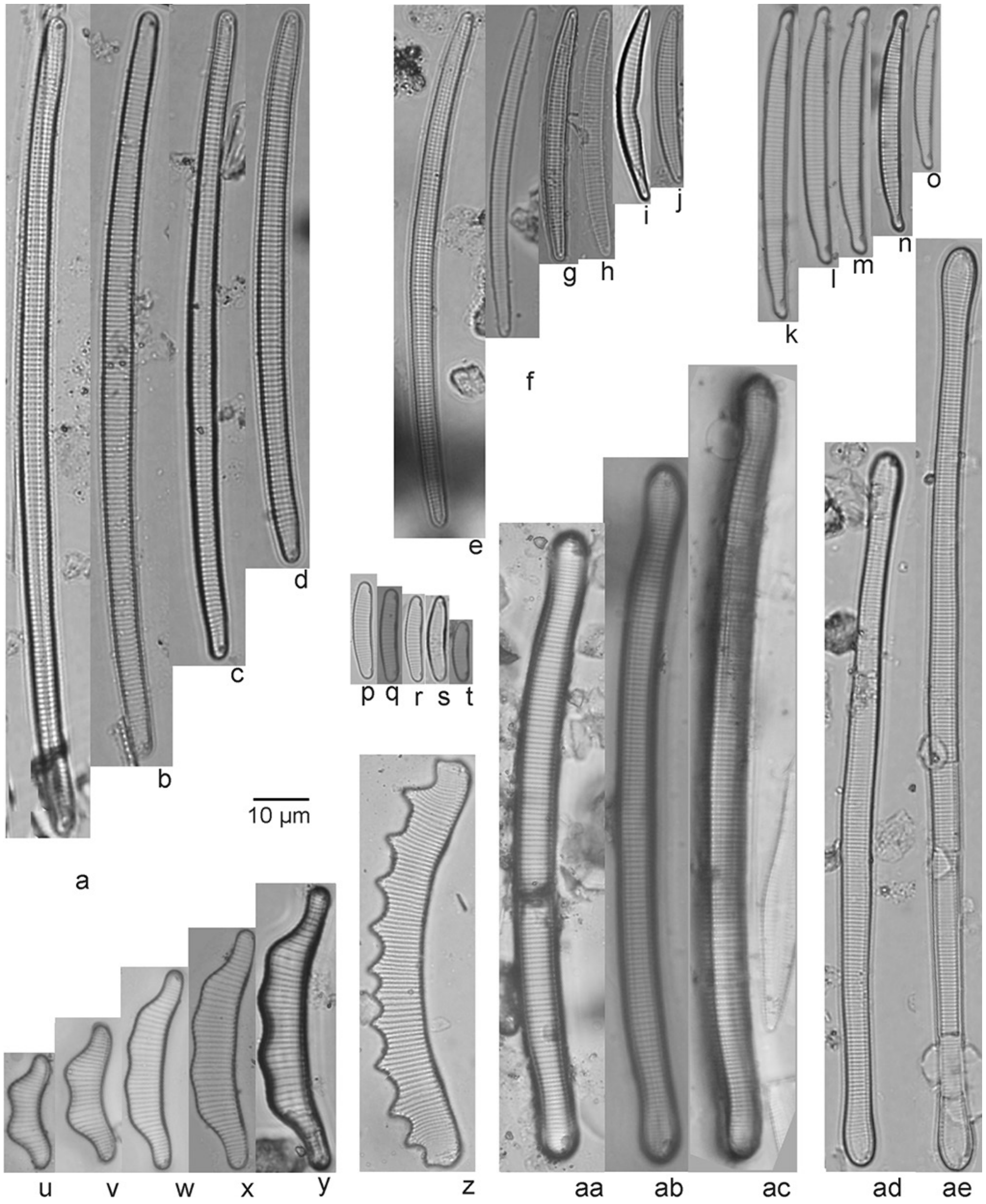
Paraná State citations*:* cited as *E. intermedia* (Krasske ex Hustedt) Nörpel & Lange-Bertalot) in Tremarin et al. (2008, fig. 28).

Taxonomic remarks*: Eunotia botulitropica* was recently described based on samples collected in reservoirs from São Paulo state. According to Costa et al. (2017), *E. botulitropica* differs from *E. botuliformis* Wild, Nörpel & Lange-Bertalot by its "narrower valve, lower striae density, rounded and more tapered ends" (Lange-Bertalot et al. 2011). However, the measurements and number of striae overlap, and *E. botuliformis* can be separated by the parallel valvar margins and the regular distribution of the striae along the valve. *Eunotia botulitropica* shows rhomboid girdle view, resembling *E. rhomboidea* Hustedt, but the latter is clearly a heteropolar species and presents larger valves (Costa et al. 2017).

Eunotia caniculoides Favaretto, Tremarin, T.Ludwig & Bueno sp. nov.

Table 3. Occurrence of *Eunotia* species, ecological data and environmental data by sampling in Tenente João Gualberto stream, Paraná, Brazil

UNOPA	**3943**	**3962**	**3976**	**4035**	**4044**	**4057**	**4081**	**4093**	**4102**	**4116**	**4126**	**4136**
Sampling date	**20/ IX/2012**	**29/ X/2012**	**13/ XI/2012**	**18/ XII/2012**	**15/ I/2013**	**06/ II/2013**	**11/ III/2013**	**04/ IV/2013**	**09/ V/2013**	**06/ VI/2013**	**16/ VII/2013**	**12/ VIII/2013**
Occurrence in samples												
Eunotia ambivalens		x		x				x	x	x	x	x
Eunotia bilunaris	x	x					x	x		x	x	x
Eunotia botulitropica	x	x					x	x		x	x	x
Eunotia caniculoides	x	x			x		x	x	x	x	x	x
Eunotia desmogonioides			x						x	x		x
Eunotia georgii		x										
Eunotia guianensis	x		x	x				x				
Eunotia juettnerae	x		x	x				x		x	x	
Eunotia karenae	x	x				x		x	x		x	
Eunotia longicamelus	x	x	x	x	x			x	x	x	x	x
Eunotia meridiana	x	x			x			x		x		x
Eunotia minor	x	x		x				x				
Eunotia monodon	x				x		x	x	x		x	
Eunotia pileus	x	x	x		x	x			x	x	x	
Eunotia pseudosudetica var. *pseudosudetica*	x	x		x				x		x		
Eunotia pseudosudetica var. *rotundata*	x	x	x					x		x		x
Eunotia rabenhorstii var. *monodon*	x	x	x			x	x			x	x	x
Eunotia rabenhorstii var. *triodon*	x	x	x			x	x	x		x	x	x
Eunotia sedina	x	x					x	x	x	x	x	x
Eunotia tropico-arcus								x		x	x	
Eunotia yanomami						x			x		x	
Riches of Eunotia species	16	15	8	6	5	5	7	16	9	15	14	11
Ecological data												
Water Temperature (°C)	19.39	22.46	23.58	25.38	24.07	23.26	23.32	22.12	15.39	16.93	17.07	13.46
Conductivity (ms.cm^{-1})	0.025	0.023	0.031	0.03	0.012	0.025	0.022	0.018	0.017	0.018	0.019	0.020
pH	6.14	5.41	5.6	6.5	6.21	5.08	6.56	6.33	8.1	6.73	7.69	5.74
Turbidity (NTU)	3.74	4.29	14.5	9.36	7.59	5.77	1.42	2.61	0.76	2.11	1.37	1.17
Dissolved oxygen (mg.L^{-1})	11.05	0.09	1.49	1.44	4.43	2.56	3.29	11.88	16.49	22.89	9.83	5.85
Ammoniac nitrogen (mg.L^{-1})	0.1405	0.183	0.138	0.218	0.178	0.3105	0.373	0.223	0.018	0.0755	0.058	0.0605
Nitrite (mg.L^{-1})	0.0028	0.0026	0.0117	0.0026	0.0096	0.0053	0.0001	0.0001	0.0008	0.0005	0.0008	0.00001
Nitrate (mg.L^{-1})	0.3775	0.365	0.22125	0.12125	0.115	0.15875	0.1025	0.07125	0.09	0.05875	0.1025	0.115
Total phosphorous (mg.L^{-1})	0.0198	0.0523	0.019	0.0048	0.0157	0.0157	0.0123	0.0115	0.009	0.0065	0.004	0.0057
Orthophosphate (mg.L^{-1})	0.0057	0.054	0.004	0.0073	0.0065	0.0115	0.0082	0.0057	0.0065	0.0057	0.009	0.0032
Environmental data												
Depth (m)	0.16	0.21	0.17	0.18	0.24	0.22	0.22	0.22	0.20	0.22	0.27	0.23
Flow ($m^3.s^{-1}$)	0.16	0.20	0.19	0.18	0.37	0.26	0.35	0.27	0.24	0.24	0.37	0.23
Weekly precipitation (mm)	21.4	77.6	15.8	63	0	64.2	84.4	32.2	53.2	23.4	0	0



Figures 2. *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. LM. a–d. *Eunotia ambivalens*. e–j. *Eunotia bilunaris*. k–o. *Eunotia caniculoides* sp. nov. p–t. *Eunotia botulitropica.* u–y. *Eunotia longicamelus.* z. *Eunotia georgii*. aa–ac. *Eunotia karenae*. ad–ae. *Eunotia desmogonioides*. Scale: 10 µm.

Figures 2k–2o, 5a–5d.

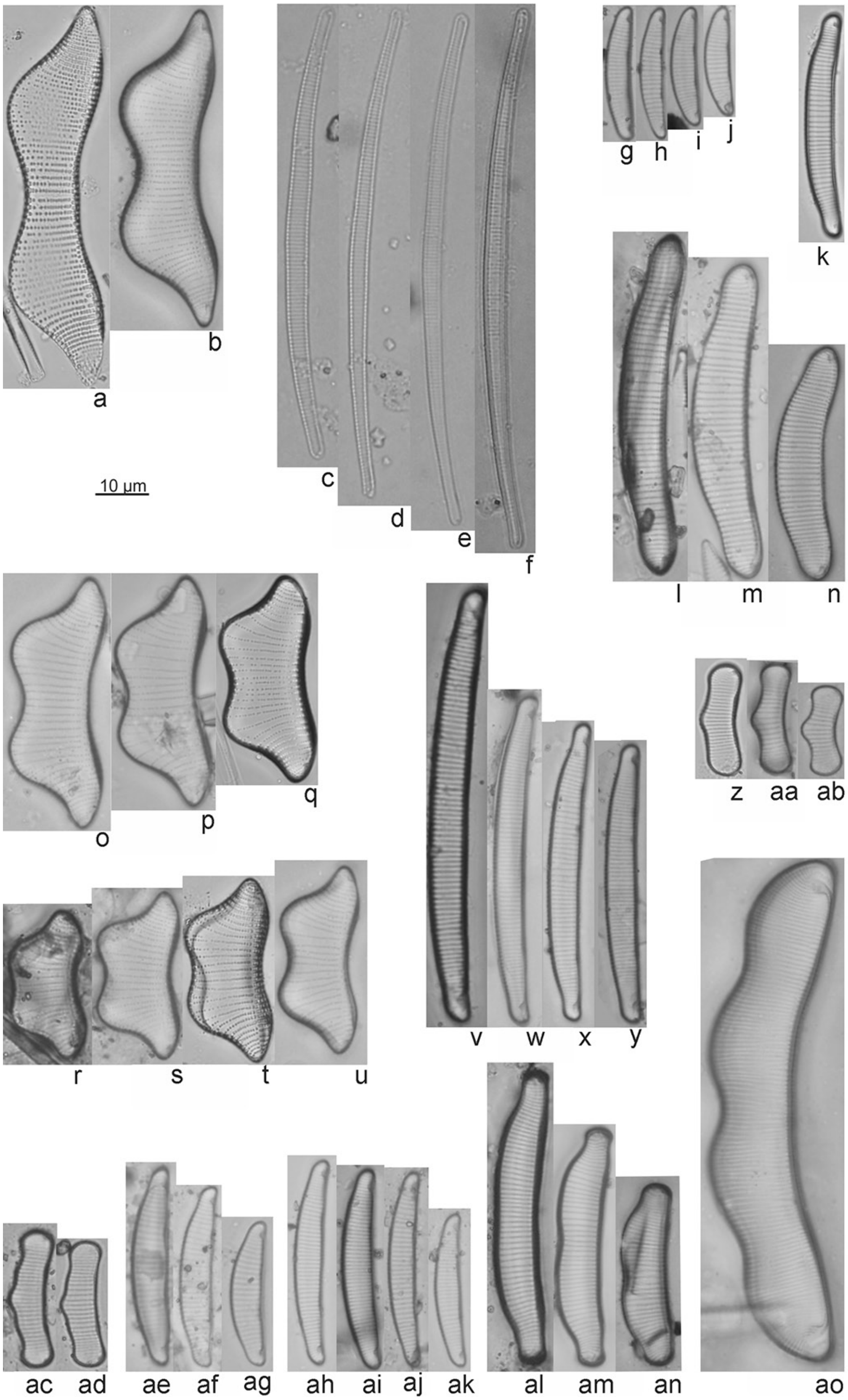
Valves dorsiventral, 30.0–58.5 µm long and 3.5–4.9 µm wide. Dorsal margin slightly convex, to almost straight in the larger valves; ventral margin straight to subtly concave. Apices acutely rounded, nose-like, quite protracted from the main body, deflected to ventral side. Pseudosepta in the valve ends. Terminal nodules ventral, somewhat apart from the apices. Distal raphe ends simple, not reaching the valve center. Raphe fissure obliquely curved toward ventral mantle, with proximal ends dilated in pore. One small rimoportula per valve, at the center of the apex. Striae parallel to radiate, denser near the apices, 10–14 in 10 µm in the middle region of the valve. Areolae round, 38–40 in 10 µm, not visible in LM. Cingulum composed of four open bands perforated by a single linear row of pores. On the mantle, at the middle of raphe branch, 3 areolae between valve face margin and raphe fissure; 2 to 3 areolae between the raphe branch and lower mantle margin.

Holotype (here designated): BRAZIL, Paraná: São Miguel do Iguaçu, Tenente Gualberto stream, 20 september 2012, *L.C. Servat* (holotype UNOPA 3943!, depicted in Figure 2n.

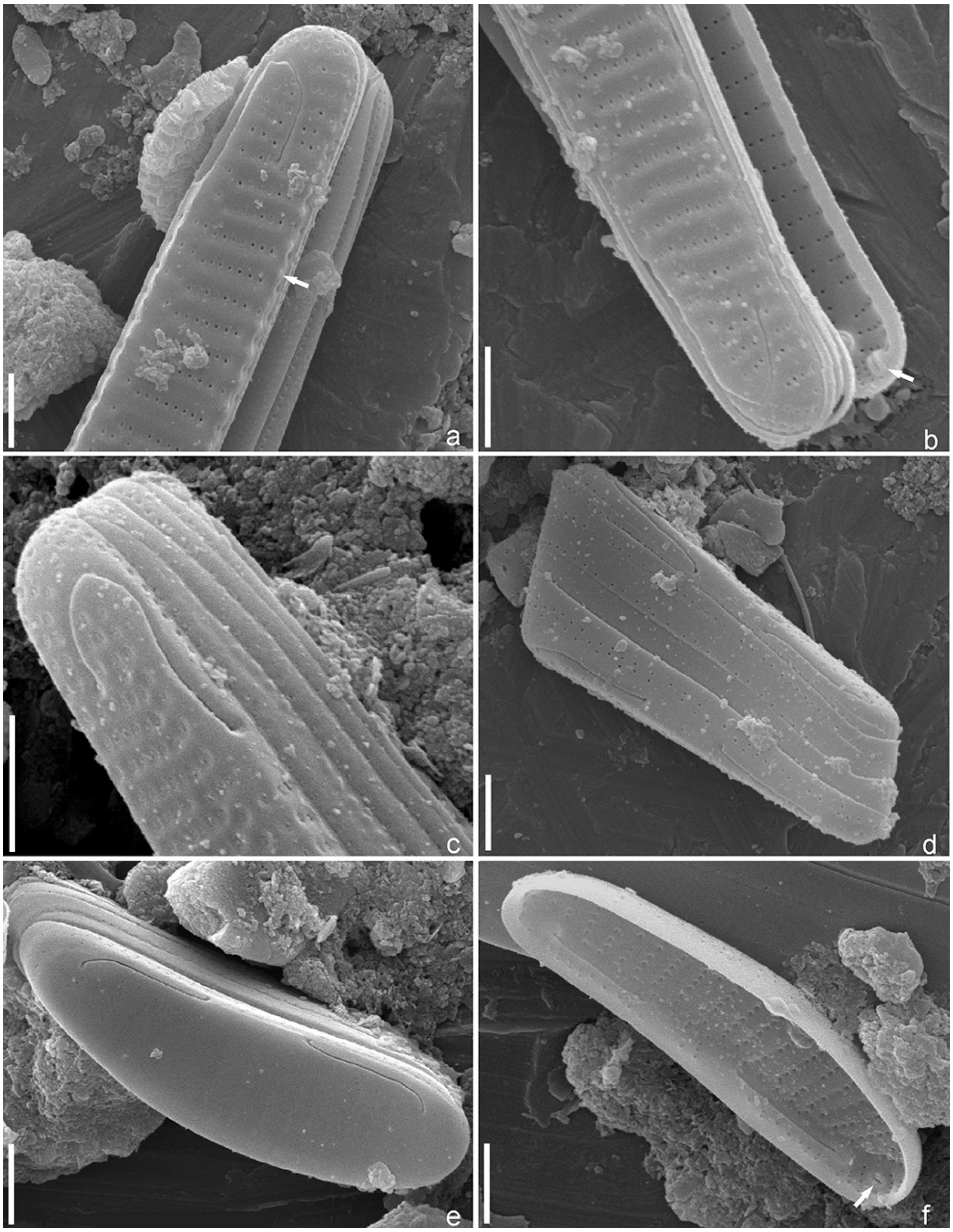
Etymology: The epithet refers to the general similarities to *Eunotia canicula* Furey, Lowe & Johansen (2011: 57, pl. 30, figs. 39-46).

Frequency of occurrence*:* constant (Table 3).

Taxonomic remarks: *Eunotia canicula* Furey, Lowe & Johansen is similar to *E. caniculoides*, but the valve outline and ends shape are quite different. *Eunotia canicula* has dorsal margin more convex and higher striae density (14–15 in 10 µm). *E. caniculoides* shows longer protracted ends when compared to *E. canicula* (Furey et al. 2011).



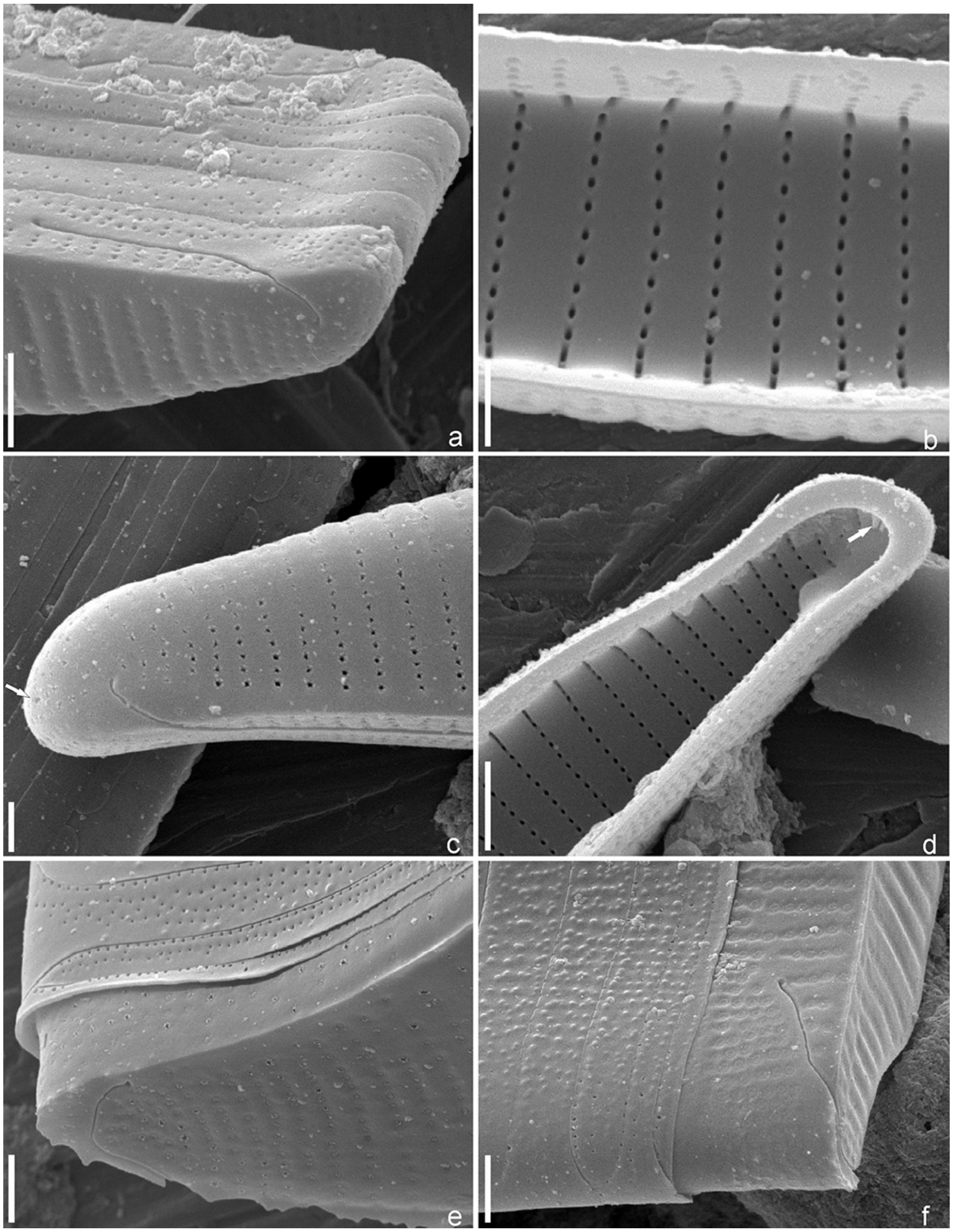
Figures 3. *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. LM. a–b. *Eunotia guianensis.* c–f. *Eunotia juettnerae.* g–j. *Eunotia meridiana*. k. *Eunotia minor*. l–n. *Eunotia monodon.* o–u. *Eunotia pileus.* v–y. *Eunotia pseudosudetica* var. *rotundata*. z–ab. *Eunotia rabenhorstii* var. *monodon*. ac–ad. *Eunotia rabenhorstii* var. *triodon*. ae–ag. *Eunotia pseudosudetica* var. *pseudosudetica.* ah–ak. *Eunotia sedina.* al–an. *Eunotia tropico-arcus*. ao. *Eunotia yanomami.* Scale: 10 µm.



Figures 4. *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. SEM. a. *E. ambivalens* - external view of valve apex showing the raphe and striae pattern. Note the marginal spines (arrow). b. *E. bilunaris* - external and internal view of valve apex showing the raphe, striae pattern and rimoportula (arrow). c. *E. bilunaris* - tilted frustule showing the raphe and girdle bands. d. *E. botulitropica* - girdle view of frustule. e. *E. botulitropica* - external view of valve showing the raphe fissures. f. *E. botulitropica* – internal view of valve. Note the rimoportula (arrow). Scales: 2 µm.

Fontana & Bicudo (2012) found *E. canicula* in the Paranapanema river watershed, state of São Paulo, but the population is clearly distinct from *E. caniculoides*, and the striae density are even much higher (16–22/10 µm).

Eunotia caniculoides is similar by the valvar contour and nose-like apices to *E. intricans* Lange-Bertalot & Metzeltin, described from a lake in Panamá (Lange-Bertalot & Metzeltin 2009), and to *E. sioliopsis* Moser, Lange-Betalot & Metzeltin, proposed based on samples collected in New Caledonia. *E. intricans* has similar dimensions (length 17–48 µm, width 4.0–5.0 µm) and striae density (11–13 in 10 µm) (Table 2) but the apices are more rounded, less protracted, and not flexed to ventral side as in *E. caniculoides* (Lange-Bertalot & Metzeltin 2009). *E. sioliopsis* shows similar dimensions and apices shape, but striae and areolae densities are lower and dorsal margins are more convex (see Table 2). Raphe distal ends in *E. sioliopsis* and in *E. intricans* are shorter in valve face and longer in the mantle; and the terminal nodules are less proeminent (Lange-Bertalot & Metzeltin 2009, Moser et al. 1998), when compared to *E. caniculoides*. Also, in



Figures 5. *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. SEM. a. *E. caniculoides* - tilted frustule showing the raphe and girdle bands. b. *E. caniculoides* - striae in the median region of valve in internal view. c. *E. caniculoides* - external view of apex. Note the rimoportula opening (arrow). d. *E. caniculoides* - internal view of apex showing the rimoportula (arrow). e. *E. guianensis* - external view of apex showing the raphe. f. *E. longicamelus* - girdle view of frustule showing the raphe and bands. Scales: 1 µm (Figs 5b, 5c), 2 µm (Figs 5a, 5d–5f).

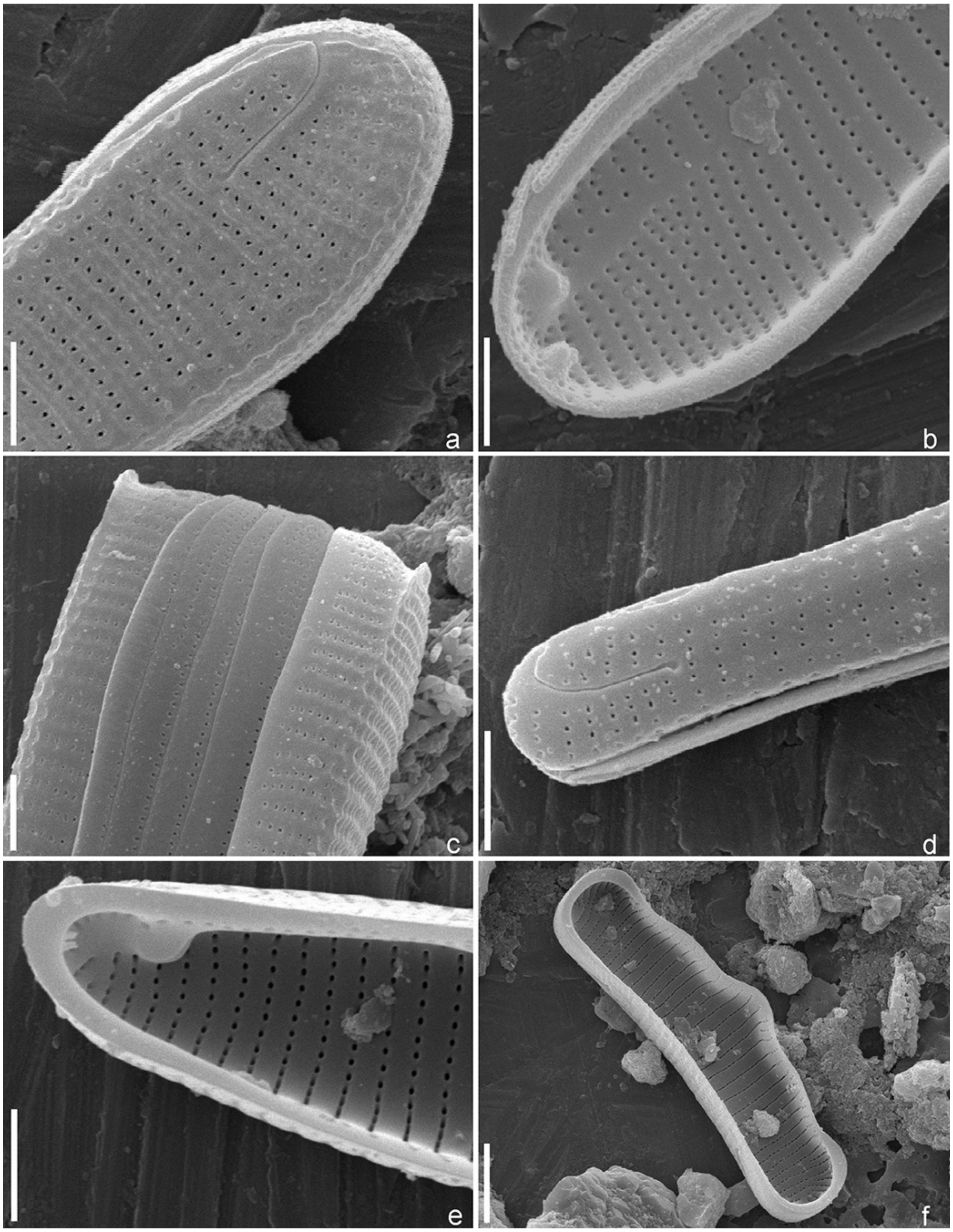
E. sioliopsis, the areola number in the middle of the raphe branch in the mantle, between the raphe and the mantle margin is higher (4 to 5) (Moser et al. 1998), and the stability of this character in the epitheca had been reported to the genus by Mayama (2001).

Morphometric data and morphological features of closer species are compared in Table 2. *Eunotia caniculoides* occurred regularly along the sampling period, except in February and November.

Eunotia desmogonioides Metzeltin & Lange-Bertalot. In: Lange-Bertalot, H. (ed.), Iconographia Diatomologica 11: pl. 5, figs 1–7, pl. 6: figs 1–4, 2002.

Figures 2ad–2ae, 6a–6c.

Valves linear, sometimes sinuous, 129.9–177.7 µm long and 4.7–6.5 µm wide. Dorsal margin slightly convex, ventral margin almost straight. Apices capitate-rounded. Terminal nodules in the apices. Distal raphe



Figures 6. *Eunotia* of a subtropical stream adjacent to Iguaçu National Park, Brazil. SEM. a. *E. desmogonioides* - external view of apex showing the raphe. b. *E. desmogonioides* - internal view of apex showing the rinoportula and helictoglossa. c. *E. desmogonioides* - detail of apex of frustule showing the valvar mantle and girdle bands. d. *E. juettnerae* - external view of apex showing the raphe. e. *E. pseudosudetica* var. *pseudosudetica* - internal view of apex showing the rimoportula and helictoglossa. f. *E. rabenhorstii* var. *triodon* - internal view of valve. Scales: 5 µm (Fig. 6f), 2 µm (Figs 6a–6e).

end strongly curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel, 14–15 in 10 µm in the middle region of the valve. Areolae rounded, not visible in LM. Cingulum composed of four to five open bands perforated by rows of pores.

Frequency of occurrence: common (Table 3).

Paraná State citations: Marra et al. (2016), Silva-Lehmkuhl et al. (2019); cited as *Eunotia rabenhorstiana* (Grunow) Hustedt): Tremarin et al. (2009).

Taxonomic remarks: *Eunotia rabenhorstiana* (Grunow) Hustedt resembles *E. desmogonioides*, but differs by the terminal nodules more distant from the apices, by the shorter raphe fissures in the valve face, by the presence of rimoportula at two valve poles, and marginal spines (Metzeltin & Lange-Bertalot 1998, 2002).

Eunotia georgii Metzeltin & Lange-Bertalot. In: Lange-Bertalot, H. (ed.), Iconographia Diatomologica 5, p. 61-62; pl. 41, fig. 1-7, pl. 42, fig. 7-8, 1998.

Figure 2z.

Valves arched, 76.2 µm long and 7.7 µm wide. Dorsal margin convex with twelve undulations, ventral margin concave. Apices obtusely rounded. Terminal nodules near the apices. Striae parallel, denser near the apices, 12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: rare (Table 3).

Paraná State citation: first record.

Taxonomic remarks*:* *E. georgii* resembles *E. muelleri* Hustedt, but the latter distinguishes by the wider and more rounded ends, nodules closer to the ends and lower number of dorsal undulations. Metzeltin & Lange-Bertalot (1998) stated that the terminal raphe fissure of *E. muelleri* extends along the valvar face reaching the dorsal margin and might be an important feature to analyse.

Eunotia guianensis (Ehrenberg) De Toni, Syll. Alg. 2: 792, 1892.

Basionym: *Himantidium guianense* Ehrenberg, Abh. Königl. Akad. Wiss. Berlin: 417, 2/1, fig. 4, (1841) 1843.

Figures 3a–3b, 5e.

Valves subtly arched, 31.5–73.1 µm long and 10.2–12.7 µm wide. Dorsal margin convex, with two undulations; ventral margin concave. Apices attenuate to acutely rounded, slighly deflected to the dorsal side. Terminal nodules near the apices. Distal raphe end curved onto the valve face, almost reaching the dorsal margin. Rimoportula not visible in LM. Striae parallel to radiate, not equidistant, denser near the apices, 7–12 in 10 µm in the middle region of the valve. Areolae rounded, 15–18 in 10 µm. Cingulum composed of six open bands perforated by several rows of pores.

Frequency of occurrence*:* common (Table 3).

Paraná State citation*:* first record.

Taxonomic remarks: the smaller valves of *Eunotia guianensis* resemble *E. pileus* Ehrenberg and *E. ventriosa* var. *brevis* (Patrick) Metzeltin & Lange-Bertalot. *E. pileus* has more rounded ends and terminal raphe fissures straight. The population analyzed here is similar to *Eunotia guianensis* registered by Metzeltin & Lange-Bertalot (1998:286, fig.26:1-7) in "Brasilien, Lago Calado". Registered before as part of *E. didyma* Hustedt ex Zimmermann population illustrated by Bicudo et al. (1995, fig. 24).

Eunotia juettnerae Lange-Bertalot. In: Lange-Bertalot (ed.), Diatoms of Europe 6: 127, pl. 26, figs 1–17, pl. 27, figs 1–6, 2011

Figures 3c–3f, 6d.

Valves arcuate, gradually tapering towards the apices, 51.7–121.9 µm long and 2.4–3.7 µm wide. Dorsal margin convex, ventral margin concave. Apices rounded, slightly deflected to dorsal side. Distal raphe fissure curved extending to the middle of the valve. Striae parallel, denser near the apices, 15–19 in 10 µm in the middle region of the valve. Areolae rounded, ca. 50 in 10 µm. Rimoportula and areolae not visible in LM.

Frequency of occurrence*:* common (Table 3).

Paraná State citation*:* first record.

Taxonomic remarks: The studied population of *Eunotia juettnerae* has morphology and measurements (20.9–99.7 µm long, 2.5–3.1 µm wide, 17–20 striae in 10 µm) similar to those recorded in specimens from southeastern Brazil observed by Costa et al. (2017). *Eunotia naegelii* has narrower valves (1.5–3.0 µm) and higher striae density (17–27 in 10 µm) and *E. bilunaris* differs on broader valves (3.5–5.5 µm) and lower striae density (13–17 in 10 µm) (Lange-Bertalot et al. 2011).

Eunotia karenae Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 18: 105, pl. 48, figs 1–4, 2007.

Figures 2aa–2ac.

Valves arched, 118.3–148.6 µm long and 6.2–7.6 µm wide. Dorsal margin convex, ventral margin concave. Apices subcapitate, slightly deflected to the dorsal side. Terminal nodules thickened, near the apices. One rimoportula per valve, near the apex. Striae parallel, denser near the apices, 10–12 in 10 µm in the middle region of the valve. Areolae visible in LM, 23–26 in 10 µm.

Frequency of occurrence: common (Table 3).

Paraná State citation*:* first record.

Taxonomic remarks: The overlapped length measure and valve contour of the neotropical *E. karenae* (length: 128–200 µm) and the similar *E. glacialifalsa* Lange-Bertalot (length: 60–150 µm) represent a difficulty to separate both. *E. glacialifalsa*, a holartic species, is narrower (5–6 µm), and has lower striae density (8.5–10 in 10 µm), according to (Metzeltin & Lange-Bertalot et al. 2007). *Eunotia glacialis* F.Meister has wider valves (7–12 µm) and *E. glacispinosa* Lange-Bertalot & Cantonati possess polar spines (Lange-Bertalot 2011), characteristics that distinguish them from *E. karenae.*

Eunotia longicamelus L.F.Costa, D.C.Bicudo & C.E.Wetzel, Bibliotheca Diatomologica 64: 32, pl. 73, figs 1–17, pl. 74, figs 1–8, pl. 75, figs 1–5, pl. 76, figs 1–4, 2017.

Figures 2u–2y, 5f.

Valves arched, 22.6–73.5 µm long and 5.0–9.6 µm wide. Dorsal margin convex, with two to four undulations; ventral margin concave. Apices rostrate to subcapitate, slightly deflected to the dorsal side. Terminal nodules in the apices. Distal raphe end curved onto the valve face, reaching the dorsal margin. Striae parallel to radiate, denser near the apices, 8–15 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM. Cingulum composed of six open bands perforated by several rows of pores.

Frequency of occurrence*:* constant (Table 3).

Paraná State citations*:* Silva-Lehmkuhl et al. (2019); cited as *E. camelus* var. *denticulata* Grunow in Moreira-Filho & Kutner (1962); cited as *E. camelus* in Tremarin et al. (2009), Bertolli et al. (2010), Faria et al. (2010), Silva et al. (2010), Santos et al. (2011), Bartozek et al. (2013), Marra et al. (2016).

Taxonomic remarks: *Eunotia longicamelus* is easily separated from *E. camelus* Ehrenberg bydorsal margin with two to four undulations and less concconcave ventral margin. *Eunotia camelus* has two evident undulations subdivided into two more smooth ones, and ventral margin more concave (Costa et al. 2017). The specimen shown in Figure 2y slightly differs from the others (figs 2u-2x) in the sub-capitate apices and irregular striae density pattern. Although similar to *Eunotia karveerensis* (Gandhi) Gluschenko & Kulikovskiy (= *E. camelus* var. *karveerensis* Gandhi) (Glushchenko & Kulikovskiy 2017), the valves are much more curved and narrower (6-7 µm and 4.5-5.5 µm in the humps) than *E. longicamelus*. The authors registered a morphologically diverse population occurring in southeast Asia, western India and Ceylon.

Eunotia meridiana Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 5: 67, pl. 59, figs 7–10, 1998.

Figures 3g–3j.

Valves subtly arched, 14.7–37.7 µm long and 3.7–5.4 µm wide. Dorsal margin convex, ventral margin linear to slightly concave.

Apices attenuate-rounded. Terminal nodules in the ventral margins. Striae parallel to radiate, denser near the apices, 14–17 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: common (Table 3).

Paraná State citations: Tremarin et al. (2008, 2009), Santos et al. (2011), Marra et al. (2016).

Eunotia minor (Kützing) Grunow in Van Heurck, Syn. Diat. Belg., pl. 33: figs 20, 21, 1881.

Figure 3k.

Valves dorsiventral, 23.5–59.0 µm long and 4.0–5.2 µm wide. Dorsal margin straight, slightly undulate; ventral margin straight to subtly concave. Apices nose-like. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 12–17 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: common (Table 3).

Paraná State citations: Tremarin et al. (2009), Santos et al. (2011),Silva et al. (2010, Marra et al. 2016), Faria et al. (2019), Silva-Lehmkuhl et al. (2019).

Taxonomic remarks: it is a species with complex taxonomy and we based the identification on Lange-Bertalot (2011).

Eunotia monodon Ehrenberg, Abh. K. Akad. Wiss. Berlin, p. 414, pl. 2, fig. 7, 1841 (1843).

Figures 3l–3n.

Valves arched, 44.5–80.7 µm long and 7.9–10.8 µm wide. Dorsal margin convex, ventral margin concave. Apices rounded, differentiated from the main body. Terminal nodules in the apices. Striae parallel to radiate, 9–12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: common (Table 3).

Paraná State citations: Tremarin et al. (2009), Marra et al. (2016); cited as *E. monodon* var. *major* (W. Smith) Hustedt - Tremarin et al. (2009), Procopiak et al. (2006), Faria et al. (2010).

Taxonomic remarks: *E. maior* (W. Smith) Rabenhorst is easily distinguished from *E. monodon* by the clearly set off capitate ends and by the more convex dorsal margin, but parallel to the ventral margin. *Eunotia monodon* usually has dorsal margin more strongly convex and ventral concave, but not parallel, rounded apices, slightly differentiated from the main body (Patrick & Reimer 1966).

Eunotia pileus Ehrenberg, Ber. K. Akad. Wiss. Berlin 1841: 414 , pl. 2/1, fig. 5, 1843.

Figures 3o–3u.

Valves subtly arched, 24.8–43.9 µm long and 11.6–13.1 µm wide. Dorsal margin convex, with two undulations; ventral margin concave. Apices obtusely rounded to cuneate, weakly deflected to the dorsal side. Terminal nodules in the apices. Rimoportula not visible in LM. Striae parallel to radiate, not equidistant, denser near the apices, 7–10 in 10 µm in the middle region of the valve. Areolae rounded, 15–19 in 10 µm.

Frequency of occurrence: common (Table 3).

Paraná State citation: first record.

Taxonomic remarks: see *Eunotia guianensis* for comments.

Eunotia pseudosudetica Metzeltin, Lange-Bertalot & García-Rodríguez var. *pseudosudetica.* In: Lange-Bertalot (ed.), Iconographia Diatomologica 15: 57, pl. 24, figs 15–18, 2005.

Figures 3ae–ag, 6e.

Valves dorsiventral, 27.2–38.0 µm long and 4.8–6.6 µm wide. Dorsal margin convex, ventral margin straight to subtly concave. Apices nose-like, differentiated from the main body. Terminal nodules near the apices. Distal raphe end short, obliquely curved onto the valve face. One rimoportula per valve, near the apex. Striae parallel to radiate, denser near the apices, 11–15 in 10 µm in the middle region of the valve. Areolae rounded, ca. 38 in 10 µm.

Frequency of occurrence: common (Table 3).

Paraná State citations: Bartozek et al. (2013),Tremarin et al. (2009), Marra et al. (2016), Silva-Lehmkuhl et al. (2019).

Taxonomic remarks: The population of *Eunotia pseudosudetica* analyzed here is similar to type material from Uruguay (Metzeltin et al. 2005) and to southern Brazilian specimens described and illustrated by Cavalcante et al. (2014).

Eunotia pseudosudetica var. *rotundata* Cavalcante, Tremarin & T. Ludwig. In: Cavalcante et. al., Biota Neotropica 14(3): 10, figs 67–74, 80–83, 2014.

Figures 3v–3y.

Valves dorsiventral, 52.0–63.3 µm long and 5.0–6.0 µm wide. Dorsal margin slightly convex, ventral margin subtly concave to almost straight. Apices rounded, subtly nose-like. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 11–16 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: common (Table 3).

Paraná State citation: first record.

Taxonomic remarks: *Eunotia pseudosudetica* var. *rotundata* was originally found in macrophyte samples collected in the state of Santa Catarina. The more rounded ends and less pronounced dorsiventrality distinguish this variety from the typical (Cavalcante et al. 2014).

Eunotia rabenhorstii var. *monodon* Cleve & Grunow. In: Van Heurck, Syn. Diat. Belg., pl. 35, fig. 12B, 1881.

Figures 3z–3ab.

Valves arched, 17.2–32.7 µm long and 6.2–8.5 µm wide. Dorsal margin convex with one undulation in the middle of the valve, ventral margin concave. Apices broadly rounded. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 12–15 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence: common (Table 3).

Paraná State citations: Tremarin et al. (2009), Marra et al. (2016), Silva-Lehmkuhl et al. (2019), Faria et al. (2010, 2019).

Taxonomic remarks: The pronounced dorsal median undulation distinguishes *E. rabenhorstii* var. *monodon* from *E. rabenhorstii* var. *triodon* characterized by three dorsal undulations (Van Heurck, 1881).

Eunotia rabenhorstii var. *triodon* Cleve & Grunow. In: Van Heurck, Syn. Diat. Belg., pl. 35, fig. 12A, 1881.

Figures 3ac–3ad, 6f.

Valves slightly arched, 22.9–32.7 µm long and 6.5–8.5 µm wide. Dorsal margin convex with three undulations being the median undulation more pronounced than the others; ventral margin concave. Apices broadly rounded. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 12–14 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM. In SEM, 40 areolae in 10 µm.

Frequency of occurrence: constant (Table 3).

Paraná State citations: Tremarin et al. (2009), Bartozek et al. (2013).

Eunotia sedina Lange-Bertalot, Bak & Witkowski. In: Lange-Bertalot (ed.), Diatoms of Europe 6: 215, pl. 47, figs 1–17, 2011.

Figures 3ah–3ak.

Valves dorsiventral, 22.1–40.5 µm long and 4.7–5.5 µm wide. Dorsal margin convex, ventral margin straight to slightly concave. Apices rounded, subtly nose-like, undifferentiated to the main body. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 12–14 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence*:* common (Table 3).

Paraná State citation: first record.

Taxonomic remarks*:* the analyzed population was similar to that described by Lange-Bertalot et al. (2011). *E. sedina* resembles *Eunotia sioliopsis* Moser, Lange-Bertalot & Metzeltin, but the later differs by narrower valves (length 20–40 µm, width 3.5–4.5 µm) and higher striae density (16–17 in 10 µm) (Moser et al. 1998).

Eunotia tropico-arcus Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 18: 123, pl. 74, figs 1–10, 2007.

Figures 3al–3an.

Valves dorsiventral, 34.3–56.1 µm long and 7.6–8.5 µm wide. Dorsal margin convex with a median depression; ventral margin slightly concave. Apices truncate, differentiated to the main body. Terminal nodules in the apices. Striae parallel to radiate, denser near the apices, 11–12 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence*:* sporadic.

Paraná State citation*:* first record.

Taxonomic remarks*: Eunotia tropico-arcus* resembles *E. bidens* Ehrenberg, *E. praerupta* Ehrenberg and *E. arcus* Ehrenberg. However, *E. bidens* has more pronounced dorsal median depression and *E. praerupta* has dorsal margin strongly convex and higher valve width (10–18 µm) (Metzeltin & Lange-Bertalot 2007, Lange-Bertalot et al. 2011). Finally, *E. arcus* differs in having more arched valves, concave ventral margin and wider apices (Metzeltin & Lange-Bertalot 2007).

Eunotia yanomami Metzeltin & Lange-Bertalot. In: Lange-Bertalot (ed.), Iconographia Diatomologica 5: 86, pl. 34, figs 1–6, pl. 35, figs 1–6, pl. 36, figs 1–13, pl. 37, figs 7–8, 1998.

Figure 3ao.

Valves arched, 67.0–95.3 µm long and 10.8–13.2 µm wide. Dorsal margin convex with two undulations; ventral margin concave. Apices cuneate, rounded. Terminal nodules near the apices. Striae parallel to radiate, denser near the apices, 15–16 in 10 µm in the middle region of the valve. Rimoportula and areolae not visible in LM.

Frequency of occurrence*:* sporadic (Table 3).

Paraná State citations*:* Marra et al. (2016), Silva-Lehmkuhl et al. (2019); cited as *Eunotia zygodon* Ehrenberg in Tremarin et al. (2008).

Taxonomic remarks*:* Metzeltin & Lange-Bertalot (1998) described *E. yanomami* based on Brazilian samples. This species resembles *E. zygodon* Ehrenberg, however, *E. zygodon* has a trilobate terminal nodule (Patrick & Reimer 1966) and smaller valves (lectotype: length 53.3 µm and width 10 µm) (Reichardt 1995). Ferrari et al. (2007) commented that further studies would be needed in order to conclude on the best distinguishing features between the two species.

The more common taxa in the studied river, occurring in at least 50% of the samples, were: *E. bilunaris, E. ambivalens, E. longicamelus, E. karenae, E. minor, E. pseudosudetica* var. *rotundata, E. monodon, E. juettnerae, E. pileus, E. rabenhorstii* var. *monodon, E. rabenhorstii* var. *triodon, E. botulitropica* and *E. sedina.* 66.6% of identified species were widely distributed in the study area and considered of common occurrence, 19.04% were constant, 9.52% were sporadic and 4.76% were rare. In general, the high frequencies of occurrence of the periphytic *Eunotia* community were in the spring period (76%) and in October (71%).

Discussion

The number of taxa found in the present study (21) can be considered high when compared to *Eunotia* studies carried out in other Brazilian regions that used a similar methodology, obtaining samples by squeezing an aquatic macrophyte. We highlight Ludwig & Valente-Moreira (1989) that identified 24 *Eunotia* and Santos et al. (2011) with 18 species. Other studies about diatom flora based on samples collected in the state of Paraná included 44 taxa (Tremarin et al. 2008), 16 taxa (Marra et al. 2016), 13 taxa (Bichoff et al. 2016), 10 taxa (Fürstenberger & Valente-Moreira 2000), and 8 taxa (Bertolli et al. 2010). Bicudo et al. (1995) identified 14 taxa on samples from Mato Grosso wetland, Cavalcante et al. (2014) found 12 taxa in the state of Santa Catarina, and Ferrari et al. (2007) registered 23 species in amazonian water bodies.

Despite numerous investigations of diatom diversity in the state of Paraná, the taxonomic study of the *Eunotia* based on 12 samples from the Tenente João Gualberto stream resulted in eight new citations for the State, and *Eunotia caniculoides* was proposed as new. The more common taxa in the studied river, occurring in at least 50% of the samples, were: *E. bilunaris, E. ambivalens, E. longicamelus, E. karenae, E. pseudosudetica* var. *rotundata, E. monodon, E. juettnerae, E. pileus, E. rabenhorstii* var. *monodon, E. rabenhorstii* var. *triodon, E. botulitropica* and *E. sedina.* 66.6% of identified species were widely distributed in the study area and considered of common occurrence, 19.04% were constant, 9.52% were sporadic and 4.76% were rare. In general, the high frequencies of occurrence of the periphytic *Eunotia* community were in the spring period (76%) and in October (71%).

Species of Eunotiaceae are influenced by pH and prefer acidic water (Patrick & Reimer 1966, Cantonati & Lange-Bertalot 2011). They are typical components of the diatom flora from tropical and subtropical areas, frequently associated with acid waters and oligotrophic or dystrophic habitats (Metzeltin & Lange Bertalot 1998, Kociolek & Spaulding 2003, Furey et al. 2011, Burliga et al. 2013). During this study period, the annual pH mean value was predominantly acidic (6.3), an environmental condition that may have favored the species richness of *Eunotia*. The months of September and October 2012, April and June 2013, with greater richness of *Eunotia* taxa, showed higher concentrations of dissolved oxygen (11.05 – 22.89 $mg.L^{-1}$). Of the months cited, those of 2012 had higher concentrations of TP and NO_3^- (0.0198-0.0523 $mg.L^{-1}$ and 0.365-0.3775 $mg.L^{-1}$ respectively), and lower depth and flow values (0.16-0.21 m and 0.16-0.20 $m^3.s^{-1}$ respectively).

In conclusion, *Eunotia* is of great taxonomic complexity and studies are still needed, leading to more complete knowledge of species diversity and distribution in Brazil. In addition, taxonomic knowledge of *Eunotia* species is essential due to their already known ecological preferences, being useful information for ecological researches aimed at the conservation and management of aquatic environments.

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Author Contributions

Norma Bueno: contributed in the concept and design of the study.

Cinthia Favaretto: contributed in the data collections.

Cinthia Favaretto, Priscila Tremarin, Thelma Ludwig, Gabriela Medeiros, Norma Bueno: contributed to data analysis and interpretation; critical revision and manuscript preparation, all 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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