

Erratum: Type of shelter and first description of the echolocation call of disk-winged bat (*Thyroptera devivoi*)

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Abundance and richness of Arctiinae moths throughout the night in a Cerrado area

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Abstract: The main goal of this work was to investigate how the abundance and richness of Arctiinae moths varies over time, during the night. Specifically, we analyzed the following questions: (1) Is there a relationship between Arctiinae abundance and richness with the temperature and relative humidity? (2) What are the hours of activity of each species of moth? (3) Does the species composition differ over night? (4) Is it necessary to sample this group of moths throughout the night to have a representative sample of the species? We sampled the moths in Emas National Park (17°49'-18°28'S and 52°39'-53°10'W), Brazil. We selected seven sampling points in an area of savanna. At each sampling point, we collected the Arctiinae moths with a light trap (with a 15-W black light fluorescent light bulb), reflected in a white cloth (2 x 3 m) extended vertically. We sampled the moths in seven consecutive nights (one night in each sampling point, from December 13 to December 19, 2012, from 7 p.m. until 7 a.m.). We divided the samplings in twelve periods over the night, with an hour each. At each period of time, we measured the temperature and the relative humidity with a digital termohygrometer. We sampled 149 individuals belonging to 17 species of Arctiinae moths. Most species (70.5%) were active only for one or two hours at night. The species differed in terms of time activity. The higher abundance occurred at 8 p. m. (44 individuals), followed by 38 individuals at 9 p. m. and 23 at 10 p. m. The species richness was also higher in the early hours of the night. The temperature was the only variable that showed a positive and significative relationship with the Arctiinae moth abundance. The species richness was not influenced neither by the temperature nor by the relative air humidity. The possible causes of the peak of abundance and species richness in specific hours of the night are discussed. **Keywords:** Lepidoptera; nocturnal activity; relative humidity; temperature; temporal partition.

Abundância e riqueza de mariposas Arctiinae ao longo da noite em uma área de Cerrado

Resumo: Este trabalho teve como objetivo principal investigar como a abundância e a riqueza de mariposas Arctiinae variam temporalmente, ao longo do período noturno. Especificamente, analisamos as seguintes questões: (1) existe relação entre a abundância e a riqueza de Arctiinae e a umidade relativa do ar e a temperatura? (2) qual é o horário de atividade de cada espécie de mariposa? (3) a composição de espécies difere ao longo da noite? (4) é necessário amostrar esse grupo de mariposas ao longo de toda a noite para ter uma amostra representativa das espécies? Realizamos as amostragens no Parque Nacional das Emas (17°49'-18°28'S e 52°39'-53°10'W), Brasil. Selecionamos sete pontos amostrais em uma área de campo cerrado. Em cada ponto, coletamos as mariposas Arctiinae utilizando uma fonte luminosa (lâmpada UV de 15 W) refletida em um pano branco (2 x 3 m) estendido na vertical. Coletamos as mariposas durante sete noites consecutivas (uma noite por ponto, entre os dias 13 e 19 de dezembro de 2012, das 19:00 até as 07:00). Dividimos o período noturno em doze faixas de uma hora. Medimos a temperatura e a umidade relativa do ar com auxílio de um termohigrômetro digital. Amostramos 149 indivíduos pertencentes a 17 espécies de mariposas Arctiinae. A maioria das espécies (70,5%) esteve ativa apenas por uma ou duas horas durante a noite. As espécies diferiram em termos de horário de atividade. O horário das 20 h apresentou uma maior abundância (44 indivíduos), seguido do horário das 21 (38) e das 22 h (23). A riqueza também foi maior nas primeiras horas da noite. Somente a temperatura foi positivamente correlacionada com a abundância de mariposas. A riqueza de mariposas não foi influenciada nem pela temperatura nem pela umidade relativa do ar. As possíveis causas das diferenças em termos de horários de atividade são discutidas..

Palavras-chave: Lepidoptera; atividade noturna; umidade relativa; temperatura; partição temporal.

Introduction

Patterns of abundance and species richness vary both spatially and temporally (Silva et al. 2011). Several factors may influence these patterns, such as climate, the distribution of resources and the intra and interspecific interactions (Wolda 1978, Pinheiro et al. 2002). In the tropics, the abundance of insects in mainly determined by abiotic factors, such as temperature, air humidity, precipitation and photoperiod (Wolda 1988, Axmacher et al. 2009).

Generally, environments with precipitation and temperature uniformly distributed throughout the year, present a low temporal variation in the abundance and richness of species. In a humid forest in Ecuador, for example, the abundance of Arctiinae moths did not differ among the seasons (Hilt et al. 2007). However, in environments with two well-defined seasons (hot and cold or rainy and dry), there is usually a significant temporal variation in abundance and richness of butterflies are related to temperature, being higher in the warmer period of the year (Ribeiro et al. 2010). In Caatinga biome, Sphingidae moths are more abundant in the rainy season, being practically absent in the dry season (Gusmão & Creão-Duarte 2004).

The Cerrado biome presents a bimodal climate in relation to the distribution of rainfall periods and temperatures (Ramos-Neto & Pivello 2000, Silva et al. 2008). During the months of April to September (dry season), monthly rainfall ranges from 49 to 79 mm, and the monthly temperatures ranges from 15 to 19°C (Ministério da Agricultura 1992, Ramos-Neto & Pivello 2000). From October to March (rainy season), the monthly rainfall ranges from 111 to 197 mm and the monthly temperatures ranges from 19.4 to 22.5°C (Ministério da Agricultura 1992, Ramos-Neto & Pivello 2000). Several families of insects show a seasonal pattern in their abundances, being significantly higher in the rainy season (Pinheiro et al. 2002, Oliveira & Frizzas. 2008). However, Lepidoptera larvae show population peaks in the dry season (Morais et al. 1999) and adult lepidopterans do not show difference in abundance throughout the year (Pinheiro et al. 2002, Oliveira & Frizzas 2008).

Most studies on the temporal variation of diversity focus on the fluctuations in richness and abundance throughout the year, with few studies dealing with the daily variation of these two parameters (ex. Scherrer et al. 2013, Camargo et al. 2016). Studies on the daily activity of species are important to answer technical questions (such as to know the best period for sampling a specific species), as well as for scientific questions, such as niche temporal partition and co-occurrence patterns.

The order Lepidoptera (butterflies and moths) has more than 160 thousand described species (Van Nieukerken et al. 2011). This taxon has great relevance in terrestrial ecosystems, acting mainly as herbivores and pollinators (Scoble 1995). Among lepidopterans, the Arctiinae subfamily is one of the most diverse (Heppner 1991, Weller et al. 2009). Arctiinae is composed by approximately 11,000 species of moths distributed worldwide (Scoble 1995, Weller et al. 2009). Approximately six thousand species are found in the Neotropical region (Heppner 1991) and 1,400 species were sampled in Brazil (Ferro & Diniz 2010). In the Cerrado biome, there are records of 723 species (Ferro et al. 2010), and in Emas National Park, around 150 species were already registered (Moreno & Ferro 2016, Moreno et al. 2016). Arctiinae represents one of the most nocturnal lepidopterans used as bioindicators because it responds quickly to environmental changes, is usually abundant, is easily sampled with light traps and its taxonomy is relatively well known (Kitching et al. 2000). Although these moths form a diverse and relatively well studied group, little is known about basic aspects of their natural history in the Neotropical region, such as larvae diet and time of activity of adults.

The main objective of this work was to investigate how the abundance and species richness of Arctiinae moths vary temporally, during the night. Specifically, we analyzed the following questions: (1) is there a relationship between the abundance and the species richness with the relative air humidity and with the temperature? (2) what is the time of activity of each specie of moth? (3) does the species composition differ over the night? (4) is it necessary to sample this group of moths throughout the night to have a representative sample of the species?

Material and Methods

We sampled the moths in Emas National Park (ENP), located in Brazil, State of Goiás, between Mineiros and Chapadão do Céu cities (17°49'-18°28'S and 52°39'-53°10'W). We selected seven sampling points (Table 1, Figure 1) in an area of "campo cerrado", one of the

Sampling points	Sampling date	Geographic coordinates	Altitude (m)	Average temperature (°C)	Average relative humidity (%)
1	13/12/2012	S 17° 55'26.4" W 53° 00'20.3"	871	19.9	86
2	14/12/2012	S 17° 55'35.8" W 53° 00'19.3"	870	20.7	89
3	15/12/2012	S 17° 55'44.1" W 53° 00'17.5"	871	20.0	88
4	16/12/2012	S 17° 55'54.1" W 53° 00'15.7"	863	18.4	88
5	17/12/2012	S 17° 56'02.0" W 53° 00'15.1"	875	20.7	86
6	18/12/2012	S 17° 56'15.0" W 53°00'14.3"	865	20.5	84
7	19/12/2012	S 17° 56'24.9" W 53°00'13.8"	865	19.1	89

Table 1. Sampling dates, geographical coordinates, altitudes, and climatic conditions of the seven sample points of this study (Emas National Park, GO, Brazil).



Figure 1. Map showing the points in which we sampled the Arctiinae moths. Each point was sampled at one different night. A - Location of sampling points (circle) in Brazil. B - Location of the sampling points (circle) in the state of Goiás (gray). C - Location of the sampling points (circle) in the Emas National Park (gray). D - Location of the 7 sampling points (circles) in an area of "campo cerrado" of the Emas National Park (gray)

physiognomies of Cerrado biome. In each sampling point, we sampled the Arctiinae moths with a light trap (with a 15-W black light fluorescent light bulb), reflected in a white cloth $(2 \times 3 \text{ m})$ extended vertically.

We sampled the moths during seven consecutive nights (one point per night, from December 13 to December 19, 2012) of new moon phase, because in this moon phase the nights are darker, and therefore the attraction radius of the light trap is higher (Yela & Holyoak 1997). The samplings were performed in December because it is summer in Brazil, the nights are not so cold, and the chances to collect moths in an open area such as campo cerrado are higher than in colder nights. We divided the night sampling in twelve periods of one hour each. The night of sampling started at 7 p. m. and ended at 7 a.m. of the next day. We measured the temperature and the relative humidity in each of the 12 sampling periods with a digital termohigrometer. The sampled moths were sorted to the lowest taxonomic level possible by comparison with the reference collection (UFG Zoological Collection), with the published literature (Watson & Goodger 1986, Piñas-Rubio et al. 2000, Piñas-Rubio & Manzano 2003), and with the types at V. O. Becker collection (Camacan, Brazil). All individuals were deposited in the UFG Zoological Collection (Goiânia, GO).

We tested the relationship between Arctiinae abundance and Arctiinae species richness with the climate variables through multiple regressions. In order to test the existence of a concentrate pattern on the peaks of abundance and richness of moths over the night, we performed a circular analysis (Rayleigh test) (Zar 1996). We performed the multiple regressions in the R program (R Core Team 2018) and the circular analysis in the Oriana program (Kovach Computing Services 2011).

Results

We sampled 149 individuals belonging to 17 species of Arctiinae moths in the seven nights of sampling. The most abundant specie was *Phoenicoprota baeri* (Rothschild, 1911), with 70 individuals, followed by *Leucanopsis annulosa* (Walker, 1855) (22), *Phoenicoprota* sp.1 (19), and *Agylla* sp. 1 (six individuals).

Most species (12 species, 70.5%) was active only for one or two hours over the night (Table 2). Three species (17.5%) were active for four or five hours, and only two species (12%) were active for nine or ten hours over the night.

The species differed in terms of activity time. *Dycladia lucetius* (Stoll, 1781), *Utetheisa ornatrix* (Linnaeus, 1758), *Agylla argentifera* (Walker, 1866), *Pheia seraphina* (Herrich-Schaffer, 1854) and *Pheia albisigna* (Walker, 1854) were present in the early evening only. *Amaxia dyuna* Schaus, 1896, *Idalus citrina* Druce, 1890, *Melese dorothea* (Stoll, 1782), *Aclytia heber* (Cramer, 1780) and *Cosmosoma restrictum* Butler, 1876 occurred at the end of the night only. *Phoenicoprota baeri* Rothschild, 1911 and *Lophocampa annulosa* (Walker, 1855) were active throughout the night (Table 2).

The Arctiinae abundance was higher at the beginning of the night (Figure 2, Table 2). The circular analysis confirmed that the abundance is concentrated over time (r = 0.70, Z = 73.11, p < 0.001). The hour with the highest abundance was 8 p. m (44 individuals), followed by 9 p. m. (38) and 10 p. m. (23 individuals).

The species richness was also higher at the first hours of the night (Figure 2, Table 2), which was also concentrated over time (r = 0.62, Z = 19.35, p < 0.001). The hour with the highest richness was 10 p. m. (10 species), followed by 8 p. m (eight species) and 9 p. m. (five species).

The relative humidity of the air ranged from 78 to 90% during the seven nights of samplings (Table 3). However, the average humidity per hour practically did not vary (87-88%) (Table 3, last column). The temperature, on the other hand, varied from 15 to 23.9°C throughout the seven nights of samplings (Table 4). The mean temperature per hour ranged from 18.2 to 20.7°C, showing a decreasing trend as the night progressed (Table 4, last column).

There was a positive and significative relationship between the moth abundance and the temperature (R^2 =0.09, F=4.009, p=0.01). However, there was no significant relationship between moth abundance and relative humidity (R^2 =0.002, F=1.67, p=0.62). The Arctiinae species richness was neither influenced by temperature (R^2 =0.04, F=3.05, p=0.08) nor by the relative humidity (R^2 =0.001, F=0.12, p=0.72).

Discussion

We sampled only 11.4% of the Arctiinae species already registered in Emas National Park (Moreno & Ferro 2016). However, our sampling effort was much lower than the study of Moreno & Ferro (2016). In this study, we sampled two species of Arctiinae that were new records to Emas National Park: *Agylla argentifera*, and *Phoenicoprocta* sp. 2 (Moreno et al. 2014, Moreno & Ferro 2016, Moreno et al. 2016). The majority of the Arctiinae moths sampled belong to the tribe Arctiini, a common pattern for this group in Cerrado biome (Moreno et al. 2014, Moreno & Ferro 2016, Moreno et al. 2016).

Arctiinae abundance and species richness were higher at the beginning of the night. These results are in agreement with the study of Scherrer et al. (2013), who also observed higher abundances and richness of Arctiinae in the first hours of the night. However, the richness of Sphingidae moths were practically constant throughout the night and their abundance was higher in the beginning (7-9 p.m.), in the middle (0-2 a.m.) and at the end (4-5 a.m.) of the nocturnal period (Camargo et al. 2016). Thus, it is important to search for the hours of activity of the interest taxa to improve the chances of sampling a higher quantity of individuals and species. If our effort was to sample only in the first hours of the night (Scalercio et al. 2008), we would not have collected

Table 2. Number of individuals of Arctinae sampled throughout the night (from 7)	p.m. to 7 a.m. $\mathbf{\tilde{p}}$) in Emas National Park	, GO, Brazil
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	7	8	9	10	11	0	1	2	3	4	5	6	Total
	p.m.	p.m.	p.m.	p. m.	p.m.	a. m.	a.m.	a.m.	a.m.	a.m.	a.m.	a.m.	Total
Arctiini													
Callimorphina													
Utetheisa ornatrix (Linnaeus, 1758)		1		2									3
Ctenuchina													
Aclytia heber (Cramer, 1780)										1			1
Pseudosphex nivaca (Jones, 1914)				1				1					2
Euchromiina													
Cosmosoma restrictum Butler, 1876										1			1
Dycladia lucetius (Stoll, 1781)		2		1									3
Pheia albisigna (Walker, 1854)			2										2
Pheia seraphina (Herrich-Schaffer, 1854)		2		1									3
Phoenicoprota baeri Rothschild, 1911	1	26	28	6	2	2	1	2		1		1	70
Phoenicoprota sp. 1	1	7	5	5			1						19
Phoenicoprota sp. 2		2	2										4
Phaegopterina													
Amaxia dyuna Schaus, 1896											2		2
Idalus carinosa (Schaus, 1905)		1	1	2								1	5
Idalus citrina Druce, 1890											1	3	4
Lophocampa annulosa (Walker, 1855)		3		2	1		2	1	1	6	3	3	22
Melese dorothea (Stoll, 1782)											1		1
Lithosiini													
Lithosina													
Agylla argentifera (Walker, 1866)				1									1
Agylla sp . 1				2			1		2	1			6
Total abundance	2	44	38	23	3	2	5	4	3	10	7	8	149
Total richness	2	8	5	10	2	1	4	3	2	5	4	4	17



Figure 2. Abundance (bars) and richness (line) of Arctiinae moths throughout the night. The samples were performed in an area of savanna in Emas National Park (GO, Brazil), from December 13 to December 19, 2012

almost 30% of the species and 25% of the individuals of this study. Thus, in response to our fourth question, we suggest that rapid inventories or characterization of nocturnal Arctiinae fauna should be carried out throughout the night to raise the chances to capture the majority of species that fly in different periods.

There was a positive (even though low) and significative relationship between the moths' abundance and the temperature. This relationship was also observed in butterflies of Brazilian Atlantic Forest (Ribeiro et al. 2010), in butterflies of Ecuadorian Amazon (Checa et al. 2009), adult lepidopterans from Cerrado biome (Oliveira & Frizzas 2008), Noctuidae and Geometridae moths from Czech Republic (Holyoak et al. 1997) and insects in general (Silva et al. 2011). The temperature is one of the main abiotic factors that influence insects in a direct (i.e. survivorship, time of development, fecundity, longevity, color patterns, and movement) and in an indirect way (i.e. abundance, occurrence, nutritional quality and defense of their hosts, as well as the abundance and occurrence of their natural enemies) (Checa et al. 2009, Ribeiro et al. 2010). The relative humidity of the air, however, was not related to the abundance or richness of moths. A possible explanation for this result is that the humidity did not vary much throughout the night (average relative humidity varied only 1% per hour).

More than 70% of the species were active for a very short period of time (one to two hours). A similar result was found by Scherrer et al. (2013), who observed that 80% of Arctiinae species were active for a maximum of three hours. The species of moths also differed in terms of time of activity. Some of them were observed at the beginning of the night only, some at the end, and others throughout the night. These differences in terms of activity times can be explained by intrinsic (such as sensory system, thermoregulation mechanism, body size, sexual and nutritional status of species) or by extrinsic factors (such as competition, predation and temperature). The temporal escape of natural enemies was the variable that best explained the seasonal pattern of abundance of lepidopteran caterpillars in the Cerrado biome (Morais et al. 1999). Likewise, we point out that the pressure of predation by bats may be one

Hour	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Average temperature
7	22.1	21.3	22.6	21.8	23.5	23.9	22.9	22.6
8	21.3	21.3	23.3	18.2	19.9	23.5	21.3	21.3
9	20.9	20.9	22.9	18.1	19.7	23.1	19.5	20.7
10	19.7	20.9	23	19.2	20.6	21.4	20.3	20.7
11	19.6	20.5	22.1	18	20.8	22.2	20	20.5
0	19.3	20.5	22	18	22.6	19.5	19.3	20.2
1	19.1	20.3	17.3	18	22.1	19.0	19.5	19.4
2	19.8	20.5	15.2	19	23.1	18	19	19.2
3	19.7	20	16.8	17	20.7	18.2	18	18.6
4	19	21.5	18.5	16	20	18.5	16.1	18.5
5	18	20.2	18.2	17	20	18.9	15.1	18.2
6	20.2	20.2	18.6	21	15	18.5	18	18.6

Table 3. Variation of the relative humidity throughout the night at the sampling points, located in a savanna area of the Emas National Park, GO, Brazil. The sample period was from December 13 to December 19, 2012.

Table 4. Variation of temperature throughout the night at the sampling points, located in a savanna area of the Emas National Park, GO, Brazil. The sample period was from December 13 to December 19, 2012.

Hour	Point 1	Point 2	Point 3	Point 4	Point 5	Point 6	Point 7	Average temperature
7	22.1	21.3	22.6	21.8	23.5	23.9	22.9	22.6
8	21.3	21.3	23.3	18.2	19.9	23.5	21.3	21.3
9	20.9	20.9	22.9	18.1	19.7	23.1	19.5	20.7
10	19.7	20.9	23	19.2	20.6	21.4	20.3	20.7
11	19.6	20.5	22.1	18	20.8	22.2	20	20.5
0	19.3	20.5	22	18	22.6	19.5	19.3	20.2
1	19.1	20.3	17.3	18	22.1	19.0	19.5	19.4
2	19.8	20.5	15.2	19	23.1	18	19	19.2
3	19.7	20	16.8	17	20.7	18.2	18	18.6
4	19	21.5	18.5	16	20	18.5	16.1	18.5
5	18	20.2	18.2	17	20	18.9	15.1	18.2
6	20.2	20.2	18.6	21	15	18.5	18	18.6

of the main biotic factors responsible for the time of activity observed in adult tiger moths, even though many Arctiinae species developed ways to avoid the predation by bats (Barber & Conner 2007, Corcoran et al. 2009). Some species of insectivorous bats, which are important predators of moths (Barber & Conner 2007, Corcoran et al. 2009), have their peak of activity at the end of the night (Emrich et al. 2014). We observed an opposite pattern for Arctiinae, which were more abundant in the early evening. Bats produce ultrasound when they are hunting for their prey. In response, several Arctiinae species also emit ultrasound to confuse or to alert their natural enemies about their toxicity (Conner 1999, Hristov & Conner 2005, Barber & Conner 2007, Corcoran et al. 2009). However, we do not know if the majority of the Neotropical Arctiinae species emitted effective sounds to be protected against the hunting of bats. Thus, the period of time in which Arctiinae forage might reflect their avoidance to be found by their natural enemy. As the 12 genera sampled in our study vary in terms of size, color pattern, and phylogenetic proximity, we expect that there will also be variation in terms of chemical and acoustic defense mechanisms against predators and that some species will use the temporal predatory escape strategy

in daily periods. In addition to predation, the temperature, as discussed above, significantly influences various aspects of the moths'life history, such as defense (as it can accelerate or delay the development time), locomotion pattern (through thermoregulation) and population dynamics (Checa et al. 2009, Ribeiro et al. 2010).

The niche temporal partition can facilitate the coexistence of species by reducing overlapping resources, decreasing competition. As Devries et al. (2008) suggested, we agree that it is unlikely that the food resource (nectar) for most adults is a limiting factor. Thus, competition is not the main reason these species forage in distinct periods of the night. However, the use of flowers as a nectar resource for Arctiinae species is poorly known. We suggest that more studies on basic biology should be performed for this group of species, mainly in tropical environments.

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Author Contributions

Carolina Moreno: contributed with data acquisition; data analyses; manuscript preparation.

Ângela S. Barbosa: contributed with data acquisition; data analyses; manuscript preparation.

Viviane G. Ferro contributed with data acquisition; data analyses; 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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Cecidomyiidae (Diptera, Insecta): richness of species and distribution in Brazil

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Abstract: Most Neotropical species of Cecidomyiidae (Diptera) have been described from Brazil, but a list of species with occurrence in the country has never been published. Little is known about their distribution and richness in the Brazilian phytogeographic domains. Additionally, a list of host plant species has never been gathered. The present study aims to fill these knowledge gaps and provides an overview of this family in Brazil. For this, data were obtained mainly from the literature, but also from the Cecidomyiidae collection of Museu Nacional and two herbaria (RB and R). Based on the site "Flora do Brasil 2020", botanical names were updated and plant species origin and distribution were verified. A total of 265 gall midge species have been recorded in Brazil, most from the Atlantic Forest (183), followed by Cerrado (60), and Amazon Forest (29). The other phytogeographic domains shelter from five to ten species. Phytophagous gall midges occur on 128 plant species of 52 families, almost all native, being 43 endemic to Brazil (21 endemic to Atlantic Forest, five to Cerrado, and one to Amazon). Although, the taxonomical knowledge is focused on the Atlantic Forest, each domain has its own fauna composition and these informations can be useful for environmental conservational purposes. About 58% of the Brazilian fauna are known only from the type-locality. In order to fill these gaps, it is necessary and important to collect in uninvestigated areas. *Keywords: Phytogeographic domains; host plants; endemism; taxonomical knowledge.*

Cecidomyiidae (Diptera, Insecta): riqueza de espécies e distribuição no Brasil

Resumo: A maioria das espécies neotropicais de Cecidomyiidae (Diptera) foi descrita do Brasil, mas uma lista das espécies com ocorrência no país nunca foi publicada. Pouco se sabe sobre sua distribuição e riqueza nos domínios fitogeográficos brasileiros. Adicionalmente, uma lista das espécies de plantas hospedeiras nunca foi elaborada. O presente estudo visa preencher estas lacunas de informação e fornecer um panorama geral desta família no Brasil. Para tal, dados foram obtidos principalmente da literatura, mas também da coleção de Cecidomyiidae do Museu Nacional e de dois herbários (RB e R). Com base no site "Flora do Brasil 2020", os nomes botânicos foram atualizados e a origem e distribuição das espécies vegetais foram verificadas. Um total de 265 espécies de cecidomiídeos é assinalado para o Brasil, a maioria da Mata Atlântica (183), seguida pelo Cerrado (60) e Floresta Amazônica (29). Os outros domínios fitogeográficos abrigam de cinco a dez espécies. Os cecidomiídeos fitófagos estão associados a 128 espécies de plantas de 52 famílias, quase todas nativas, sendo 43 endêmicas do Brasil (21 endêmicas da Mata Atlântica, cinco do Cerrado e uma da Floresta Amazônica). Embora o conhecimento taxonômico se concentre na Mata Atlântica, cada domínio tem sua própria composição faunística e estas informações podem ser úteis para a conservação ambiental. Cerca de 58% da fauna brasileira é conhecida apenas da localidade-tipo. Para preencher estas lacunas, é necessário e importante coletar em áreas não investigadas.

Palavras-chave: Domínios fitogeográficos; plantas hospedeiras; endemismo; conhecimento taxonômico.

Introduction

Cecidomyiidae are one of the most speciose families of Diptera, with more than 6,500 species. They are cosmopolitan and known mainly as gall-inducers (Gagné & Jaschoff 2017). Most species have been described from the Holarctic Region, while the Neotropical fauna comprises less than 10% of the known species. This low richness reflects the scarcity of taxonomic studies in this region. Most species have been described from Brazil. Nevertheless, a list of Brazilian species has never been published.

Brazil comprises six phytogeographic domains: Amazon Forest, Atlantic Forest, Caatinga, Cerrado, Pampa, and Pantanal, which greatly differ from each other in flora composition, and consequently they shelter different assemblages of gall-inducing species. The richness of cecidomyiid species by domain is still unknown, as well as the number of gall-inducing, predaceous, inquilinous, fungivorous, and freeliving phytophagous species. Most of them appear to have a restricted distribution, but there are several locality records scattered in the literature.

Additionally, a list of host plant species has never been elaborated and many botanical names need to be updated. The main goals of the this study are: 1) to present a general overview of the richness of this family in Brazil, 2) to provide for the first time a list of gall midges species with occurrence in Brazil as well as in each phytogeographic domain, 3) to fill a knowledge gap about the distribution of this group, and 4) to provide for the first time a list of host plant species in Brazil.

Materials and Methods

The last version of the world catalog of Cecidomyiidae written by Gagné & Jaschhof 2017 was used as starting point. Using the find tool, all species with records in Brazil were retrieved. Papers with the original description of each species were consulted to obtain more detailed data on its occurrence localities. Furthermore, a literature survey was performed on the database "Web of Science" using "Cecidomyiidae" and "Brazil/Brasil" as key words in order to verify the gall midge species described after 2017. Based on the site "Flora do Brasil 2020", botanical names were updated and plant species origin and distribution were verified. Synonyms were provided in brackets after the correct names to allow linking of retrieved data to the original publications. Additionally, all insect gall inventories of Brazil were also consulted to recover information about locality records of the gall midge species, based on host plant species and gall morphology, whenever possible, or on gall-inducer identification. Data on phytogeographic domains were obtained using maps of IBGE 2004 or directly from the literature. In some cases, domains were not determined because data on localities were insufficient. When gall midge species were recorded in localities occupied by two different domains, both were considered as part of their distributional area.

Besides, the Collection of Cecidomyiidae (Diptera) of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ) was examined to aggregate unpublished data of species occurrence. Simultaneously, the Jardim Botânico do Rio de Janeiro herbarium (RB) and the Museu Nacional herbarium (R) were consulted in a search of galled exsiccates. This procedure was adopted since galls are extended phenotypes of the gall-inducing insects (Stone & Schönrogge 2003), so their presence on the host plants indicates the gall-inducing species' presence. Data on localities were retrieved from labels and new records were established by comparison with the literature. The geographic distribution of all gall-inducing species was updated. Data on Brazilian localities were detailed, including states and municipalities. To discriminating Rio de Janeiro and São Paulo states from Rio de Janeiro and São Paulo municipalities, the word "state" was used whenever necessary.

Results

In Brazil, 265 species of Cecidomyiidae of 93 genera have been recorded. It corresponds to about 43% of the Neotropical fauna richness; 226 are gall-inducing (about 85%), 15 are predaceous, 11 are fungivorous, nine are inquilinous, three are free-living species, and one is kleptoparasite in spider webs. Phytophagous gall midges are collectively associated with 52 plant families, 105 genera, and 128 determined species. Among these hosts, only five are exotic, one is naturalized and all others are native to Brazil (Table 1). Futhermore, ten gall midge species are associated with hosts identified only in family, totaling six families, and 37 with hosts identified only in genus, totaling 35 genera. Additionally, host plants of six cecidomyiid species are unknown. Besides, the identification of some hosts are doubtful, e.g.: "Mikania cf. biformis", "? Smilax sp.", "Guarea sp. poss. guidonia (L.)", "Guapira pernambucensis (Casar.) Lundell (possibly Guapira opposita (Vell.) Reitz", "poss Smilax sp.", and the record of Youngomyia pouteriae on Pouteria torta (Mart.) Radlk. (Sapotaceae) corresponds to a misidentification of the gall-inducing species.

Three incongruities were also observed between the host plant and gall-inducing species geographic distributions, namely: 1) *Kielmeyera rosea* Mart. & Zucc. (Calophyllaceae) x *Arcivena kielmeyerae* Gagné, 1984, 2) *Guapira pernambucensis* (Casar.) Lundell (Nyctaginaceae) x *Bruggmannia chapadensis* Proença & Maia, 2018 and 3) *Urvillea uniloba* Radlk. (Sapindaceae) x *Neolasioptera urvilleae* (Tavares, 1909). Finally, 27 botanical names were uptaded.

Most gall-inducing species are monophagous (about 90%), but oligophagous and polyphagous species have been reported. Oligophagous species are represented by at least 15 gall midge species, 11 of them occur on two or three plant species of the same genus and four on two or three genera of the same family. The number of hosts of some gall midge species could not be determined, since the level of plant identification does not allow it. This is the case of five gall midge species, four of them have been associated with an identified plant species plus a non identified congeneric host, and the other with an identified species plus a morphospecies of the same plant family. A single cecidomyiid species is polyphagous, occurring in plants of different families.

Fabaceae, Asteraceae, and Myrtaceae are the plant families with the greatest richness of gall midge species (28, 26 and 25), followed by Nyctaginaceae (16). They together host about 36% of the Brazilian fauna richness. Among these families, Myrtaceae exhibit the highest average of gall midge species by host plant species, 1.8, while Fabaceae have a similar value to Asteraceae (1.2 and 1.3, respectively). The average number in Nyctaginaceae was not stablished, because most plants were not identified in species (Table 2). All other families shelter from nine to one gall midge species, but most of them (23) (about 44%) shelter a single gall-inducer. The average of gall midge species by host plant species was 1.0 in 33 families. This is the most frequent value (Table 2). *Eugenia* L. (Myrtaceae) and *Mikania* Wild. (Asteraceae) host 13 and 12 gall midge species, respectively, followed by *Guapira*

Amaranhaceae Alternanthera philozeroides (Mart, JGrisch. + A. aquatica (D. native to Brazil Pacoli) Chodat native to Brazil 1 Anacardiaceae Mangiferia indica L. (cultivated plant) 1 Annonaceae Duguetia furfuraceae (A. S. 1-III.) Saff. native to Brazil 1 Arainaceae Didymaparate mornotom (Aubi) Deene. & Planch native to Brazil 1 Asclepiadaceae Peplonia asteria (VeII.) Fontella & E. A. Schwarz endemic to Brazil 1 Asternecae Ageratum conzoides L. native to Brazil 1 Baccharis largers preg. (= B. schulte: Blake) + B. endemic to Brazil 1 Baccharis preudomyriacephala Malag. endemic to Atlantic Forest 1 Baccharis rigges Spreng. (= B. srimera (Less) DC.) native to Brazil 1 Chromolene odorati (L.) N. King & H. Koh. + Éuptaorium sp. native to Brazil 1 Expensatific L. Computer Spreng. native to Brazil 1 1 Hypocharis chillensis (Kuhth) Britton native to Brazil 1 1 Lessingianthus warningiams (Baker) H. Roh. endemic to Brazil 1 1 Hypocharis chillensis (Kuhth) Br	Family	Species	Origin	Number of gall midge species
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Protium heptaphyllum (Aubl.) Marchand native to Brazil 4 Protium icicariba (DC.) Marchand endemic to Atlantic Forest 2 Cactaceae Hylocereus setaceus (Salm-Dyck) R. Bauer (= Selenicereus setaceus (Salm-Dyck) Berg) native to Brazil 1 Calophyllaceae Calophyllum brasiliense Cambess. native to Brazil 5 Kielmeyera rosea Mart. & Zucc. endemic to Cerrado 1	Burseraceae	Protium brasiliense (Spreng.) Engl.	endemic to Brazil	1
Protium icicariba (DC.) Marchandendemic to Atlantic Forest2CactaceaeHylocereus setaceus (Salm-Dyck) R. Bauer (= Selenicereus setaceus (Salm-Dyck) Berg)native to Brazil1CalophyllaceaeCalophyllum brasiliense Cambess.native to Brazil5Kielmeyera rosea Mart. & Zucc.endemic to Cerrado1		Protium heptaphyllum (Aubl.) Marchand	native to Brazil	4
Cactaceae Hylocereus setaceus (Salm-Dyck) R. Bauer (= Selenicereus setaceus (Salm-Dyck) Berg) native to Brazil 1 Calophyllaceae Calophyllum brasiliense Cambess. native to Brazil 5 Kielmeyera rosea Mart. & Zucc. endemic to Cerrado 1		Protium icicariba (DC.) Marchand	endemic to Atlantic Forest	2
Calophyllaceae Calophyllum brasiliense Cambess. native to Brazil 5 Kielmeyera rosea Mart. & Zucc. endemic to Cerrado 1	Cactaceae	Hylocereus setaceus (Salm-Dyck) R. Bauer (= Selenicereus setaceus (Salm-Dyck) Berg)	native to Brazil	1
Kielmeyera rosea Mart. & Zucc. endemic to Cerrado 1	Calophyllaceae	Calophyllum brasiliense Cambess.	native to Brazil	5
		Kielmeyera rosea Mart. & Zucc.	endemic to Cerrado	1 Continue

Table 1. List of host plant species (organized by family), their origin and richness of gall midges (Diptera, Cecidomyiidae) by species in Brazil.

Maia, V.C. . et al.

Continuation			
Caryocaraceae	Caryocar brasiliense Cambess.	native to Brazil	1
Celastraceae	Monteverdia obtusifolia (Mart.) Biral (=Maytenus obtusifolia Mart.)	endemic to Brazil	2
Chrysobalanaceae	Couepia ovalifolia (Schott) Benth. ex Hook.f.	endemic to Atlantic Forest	2
Clusiaceae	Clusia fluminensis Planch. & Triana	endemic to Atlantic Forest	1
	Clusia hilariana Schltdl.	endemic to Atlantic Forest	1
	Clusia lanceolata Cambess.	endemic to Atlantic Forest	1
	Clusia sp.	-	1
Combretaceae	Combretum leprosum Mart.	native to Brazil	1
Convolvulaceae	Jacquemontia holosericea (Weinm.) O' Donell	native to Brazil	1
Dilleniaceae	Davilla rugosa Poir	native to Brazil	1
	Doliocarpus dentatus (Aubl.) Standl.	native to Brazil	1
Erythroxylaceae	Erythroxyum ovalifolium Peyr.	endemic to Atlantic Forest	3
	<i>Erythroxylum suberosum</i> A. StHil.	native to Brazil	1
Euphorbiaceae	Croton floribundus Spreng	native to Brazil	2
	Croton hemiargyreus Müll. Arg.	endemic to Atlantic Forest	1
	Dalechampia ficifolia Lam.	endemic to Brazil	1
	Manihot esculenta Crantz (= Manihot utilissima Pohl.) + Manihot caerulescens + Manihot sp.	native to Brazil	1
	Manihot esculenta Crantz (= Manihot utilissima Pohl.)	native to Brazil	1
	Microstachys corniculata (Vahl) Griseb. (=Sebastiana glandulosa (Mart.) Pax.)	native to Brazil	2
Fabaceae	Aeschynomene denticulata Rudd.	native to Brazil	1
	Aldina heterophylla Spruce ex Benth.	endemic to Amazon Forest	1
	Andira fraxinifolia Benth	endemic to Brazil	1
	Andira humilis Mart. ex Benth.	endemic to Brazil	1
	Andira vermifuga (Mart.) Benth.	native to Brazil	1
	Andira sp.	-	1
	Bauhinia brevipes Vogel	native to Brazil	2
	Bauhinia cupulata Benth.	native to Brazil)	1
	Bauhinia rufa (Bong.) Steud.	native to Brazil	1
	Dalbergia ecastophyllum (L.) Taub. + Dalbergia frutescens (Vell.) Britton	native to Brazil native to Brazil	1
	Dalbergia sp.	=	1
	Inga edulis Mart.	native to Brazil	2
	Inga vera Will. (= Inga spuria Humb. & Bonpl. ex Willd.) + Inga punctata Will. (= Inga leptoloba Schltdl.)	native to Brazil native to Brazil	1
	Machaerium hirtum (Vell.) Stellfeld + Machaerium sp. +	native to Brazil	1
	Machaerium macaense C. V. Mendonça, A. M. G. Azevedo & H. C. Lima	endemic to Atlantic Forest)	1
	Machaerium sp.	-	2
	Mimosa caesalpiniifolia Benth.	endemic to Brazil	1
	Mimosa tenuiflora (Willd.) Poir. (= Mimosa hostilis Benth.)	native to Brazil	2
	Parkia pendula (Willd.) Benth. ex Walp.	native to Brazil	1
	Senna bicapsularis (L.) Roxb.	exotic	1
	Swartzia langsdorffii Raddi	endemic to Atlantic Forest	1
	Undetermined	-	3
Lamiaceae	Hyptis sp.	-	1
	Melissa officinalis L.	exotic	1
Lauraceae	Ocotea pulchella (Nees & Mart) Mez	native to Brazil	1
	undetermined	-	1
Loranthaceae	Psittacanthus dichroos (Mart.) Mart.	endemic to Brazil	1

Loranthaceae

Continue...

Continuation...

	Struthanthus taubatensis Eichler (= S. maricensis Rizzini ex Profice	endemic to Brazil	1
	Struthanthus sp.	-	1
Lythraceae	Cuphea carthagenensis (Jacq.) J. F. Macbr	native to Brazil	1
Malpighiaceae	Banisteriopsis membranifolia (A. Juss.) B. Gates	endemic to Brazil	1
	Byrsonima sericea DC.	native to Brazil	2
	Diplopteryx pubipetala (A. Juss.) W. R. Anderson & C. C. Davis	native to Brazil	1
	Heteropterys nitida (Lam.) DC.	native to Brazil	1
	Heteropterys sp.	-	1
	Pterandra pyroidea A. Juss.	endemic to Cerrado	1
	Tetrapterys phlomoides (Spreng.) Nied.	native to Brazil	1
	Undetermined	-	1
Malvaceae	Undetermined	-	1
Melastomataceae	Clidemia sp.	-	1
	Leandra ionopogon (Mart.) Cogn.	native to Brazil	1
	Marcetia sp.	-	1
	Miconia cinnamomifolia (DC.) Naudin	endemic to Atlantic Forest	1
	Miconia cf. cinnamomifolia	_	1
	Miconia theaezans (Bonnl.) Cogn.	native to Brazil	1
	Ossapa sp	-	1
	Plaroma candollaanum (Mart. ex DC.) Triana (= Tibouching		1
	candolleana (Mart. ex DC.) Cogn.)	endemic to Cerrado	1
	<i>Tibouchina</i> sp.	-	1
Meliaceae	<i>Guarea macrophylla</i> Vahl	native to Brazil	2
	<i>Guarea</i> sp. poss. <i>guidonia</i> (L.) Sleumer (= <i>Guarea trichilioides</i> L.)	-	1
Moraceae	Coussapoa sp.	-	1
	Ficus sp.	-	2
	Maclura tinctoria (L.) D. Don ex Steud. (= Chlorophora tinctoria (L.) Gaudich. ex B.D. Jackson) (Moraceae)	native to Brazil	1
	Sorocea bonplandii (Baill.) W. C. Burger et al. (= Sorocea ilicifolia Miq.)	native to Brazil	1
Myrsinaceae	Myrsine sp.	-	1
Myrtaceae	Eugenia astringens Cambess (=E. rotundifolia Casar = Eugenia umbelliflora O. Berg.) (Myrtaceae)	endemic to Atlantic Forest	4
	Eugenia copacabanensis Kiaersk.	endemic to Atlantic Forest	3
	<i>Eugenia hiemalis</i> Cambess. (= <i>Eugenia multiflora</i> Cambess.) + undetermined Myrtaceae	native to Brazil	1
	<i>Eugenia punicifolia</i> (Kunth.) DC. (= (<i>E. ovalifolia</i> Cambess.) + <i>Eugenia</i> sp.	endemic to Brazil	1
	Eugenia uniflora L.	native to Brazil	4
	Myrcia ovata Cambess.	endemic to Atlantic Forest)	1
	Myrcia retorta Cambess	endemic to Brazil	1
	Myrciaria delicatula (DC.) O. Berg	native to Brazil	1
	Myrciaria floribunda (H. West ex Willd.)	native to Brazil	2
	Myrciaria tenella (DC.) O.Berg	native to Brazil	1
	Neomitranthes obscura (DC.) N. Silveira	endemic to Atlantic Forest	3
	Psidium cattleyanum Sabine	endemic to Brazil	2
	Undetermined	-	1
Nyctaginaceae	Guapira opposita (Vell.) Reitz	native to Brazil	7
	Guapira pernambucensis (Casar.) Lundell (possibly Guapira opposita (Vell.) Reitz)	endemic to Brazil	1
			a

Continuation...

	Guapira sp.	-	1
	Neea spp.	-	7
Ochnaceae	Ouratea spectabilis (Mart) Engl.	endemic to Cerrado	1
Olacaceae	Heisteria acuminata (Humb. & Bonpl.) Engl. (=Heisteria cyanocarpa Poepp.)	native to Brazil	1
	Ximenia americana L.	native to Brazil	1
Onagraceae	Ludwigia sp.	-	1
Orchidaceae	Cattleya spp. + Epidendrum spp. + Laelia spp.	-	1
Piperaceae	Piper sp.	-	3
Poaceae	Paspalum conjugatum P. J. Bergius	native to Brazil	1
Polypodiaceae	Microgramma vacciniifolia (Langsd. & Fisch.) Copel.	native to Brazil	1
Pontederiaceae Q	Eichhornia azurea (Sw.) Kunth	native to Brazil	1
Ranunculaceae	Clematis sp.	=	1
Rosaceae	Spiraea salicifolia L.	exotic	1
Rubiaceae	Borreria palustris (Cham. & Schltdl.) Bacigalupo & E.L.Cabral (= Diodia gymnocephala (DC.) K.Schum.)	native to Brazil	1
	B. verticillata (L.) G.Mey + Borreria sp.	native to Brazil	1
	Psychotria sp.	-	1
	Rubia sp.	-	1
	Undetermined	-	3
Rutaceae	Citrus sp.	-	1
Sapindaceae	Matayba guianensis Aubl.	native to Brazil	1
	Paullinia weinmanniifolia Mart.	endemic to Atlantic Forest	1
	Paullinia weinmanniifolia Mart. + Matayba guianensis Aubl.	endemic to Atlantic Forest native to Brazil	1
	<i>Serjania</i> sp.	-	1
	Urvillea uniloba Radlk.	native to Brazil	1
Sapotaceae	Manilkara subsericea (Mart.) Dubard	endemic to Atlantic Forest	1
	Pouteria caimito (Ruiz & Pav.) Radlk (=Pouteria caimito var. laurifolia (Gomes) Baehni)	native to Brazil	2
	Pouteria torta (Mart.) Radlk.	native to Brazil	1
	Pouteria venosa (Mart.) Baehni	native to Brazil	1
	Sideroxylon obtusifolium (Roem. and Schult.) T. D. Penn	native to Brazil	1
Smilacaceae	Smilax oblongifolia Pohl ex Griseb	endemic to Brazil	1
	Poss on Smilax sp.	-	1
	Smilax rufescens Griseb.	endemic to Brazil)	1
	?Smilax sp	-	1
Solanaceae	Physalis angulata L.	native to Brazil	1
	Solanum sp.	-	1
Sterculiaceae	<i>Waltheria indica</i> L.	native to Brazil	1
	Sterculia sp.	-	1
Styracaceae	Styrax sp.	-	5
Urticaceae	Cecropiae sp	-	1
Verbenaceae	Aegiphila integrifolia (Jacq.) Moldenke (= Aegiphila arborescens (Aubl.) J. F. Gmel.)	native to Brazil	1
	Lantana camara L.	naturalized	1
	Lantana sp.	-	3
	Stachytarpheta cayennensis (Rich.) Vahl. + Stachytarpheta sp.	native to Brazil	1
Unknown	-	-	6

Unknown

Table 2. Richness of host plant species and gall midge species (Diptera, Cecidomyiidae) by vegetable family, and average of gall midge species by host plant in each family in Brazil.

Family	Number of	Number of gall	Average		
	host species	midge species	number		
Amaranthaceae	2	1	0.5		
Anacardiaceae	2	2	1.0		
Annonaceae	1	1	1.0		
Araliaceae	1	1	1.0		
Asclepiadaceae	1	1	1.0		
Asteraceae	21	26	1.3		
Bignoniaceae	1	1	1.0		
Boraginaceae	3	2	0.7		
Burseraceae	3	7	2.3		
Cactaceae	1	1	1.0		
Calophyllaceae	2	6	3.0		
Caryocaraceae	1	1	1.0		
Celastraceae	1	2	2.0		
Clusiaceae	4	4	1.0		
Chrysobalanaceae	2	2	2.0		
Combretaceae	1	1	1.0		
Convolvulaceae	1	1	1.0		
Dilleniaceae	2	2	1.0		
Erythroxylaceae	2	4	2.0		
Euphorbiaceae	7	8	1.1		
Fabaceae	24	28	1.2		
Lamiaceae	2	2	1.0		
Lauraceae	2	2	1.0		
Lythraceae	1	1	1.0		
Loranthaceae	3	3	1.0		
Malpighiaceae	?	8	?		
Malvaceae	1	1	1.0		
Melastomataceae	9	9	1.0		
Meliaceae	2	3	1.5		
Moraceae	4	5	1.2		
Myrsinaceae	1	1	1.0		
Myrtaceae	14	25	1.8		
Nyctaginaceae	?	16	?		
Ochnaceae	1	1	1.0		
Olacaceae	2	2	1.0		
Onagraceae	1	1	1.0		
Orchidaceae	?	1	?		
Piperaceae	1	1	1.0		
Poaceae	1	1	1.0		
Polypodiaceae	1	1	1.0		
Ponteridaceae	1	1	1.0		
Ranunculaceae	1	1	1.0		
Rosaceae	1	1	1.0		
Rubiaceae	6	7	1.2		
Rutaceae	1	, 1	1.0		
Sanindaceae	4	5	1.0		
Sapotaceae	5	6	1.2		
Smilacaceae	4	4	1.2		
Solanaceae		т 2	1.0		
Sterculiaceae	2	2	1.0		
Styracaceae	∠ ?	2 5	1.0		
Urticaceae	1	5	1.0		
Verhengeege	5	6	1.0		
verbenaceae	5	0	1.2		

Aubl. (Nyctaginaceae) with 9. *Guapira opposita* (Vell.) Reitz, *Mikania glomerata* Spreng., and *Calophyllum brasiliense* Cambess (Calophyllaceae) highlight as plant species with the greatest number of gall midge species (seven, six and five, respectively).

The gall-inducers are represented by 80 genera. Among them, Lopesia Rübsaamen, 1909, Asphondylia Loew, 1850, and Clinodiplosis Kieffer 1895 are the most speciose, with 25, 23 and 20 species, respectively (Table 3). Predators are represented by four genera, Aphidoletes Kieffer, 1904, Diadiplosis Felt 1911, Feltiella Rübsaamen, 1910, and Lestodiplosis Kieffer, 1894, being Diadiplosis the most speciose, with ten species, while the others comprise three (Lestodiplosis) or one species (Aphidoletes and Feltiella). The first genus feeds on aphids, the second on scale insects (Coccoidea), the third on mites and the fourth mostly on other cecidomyiids, but also on mites. They have been used as biological control agents of some plant pests. Fungivorous species are represented by five genera, Dichodiplosis Rübsaamen, 1910, Haplusia Karsch, 1877, Mycodiplosis Rübsaamen, 1895, Stomatosema Kieffer, 1904, and Termitomastus Silvestri, 1901. Three of them comprise a single species, while Haplusia comprises two and Stomatosema six. Inquilines are represented by six genera, Clinodiplosis Kieffer, 1894, Contarinia Rondani, 1860, Dialeria Tavares 1918, Meunieriella Kieffer, 1909, Neolasioptera Felt, 1908 and Trotteria Kieffer, 1902. Among them, Dialeria and Trotteria include exclusively inquilinous species. Five of them comprise a single inquilinous species in Brazil, while Meunieriella comprises four. All were recorded in galls of other Cecidomyiidae. Although these cecidomyiids have been reported as inquilines, they are probably kleptoparasites, according to Luz and Mendonça-Júnior (2019). Free-living species are less common, being represented by three genera, Clinodiplosis, Lopesia, and Prodiplosis Felt, 1908, each with a single species. Their larvae feed on plant reproductive organs. And only one species, Didactylomyia longimana (Felt, 1908), is reported as the kleptoparasite in literature.

Most species of gall midges (about 90%) have been recorded exclusively in Brazil, while only 26 (about 10%) occur in other countries. The Atlantic Forest is the phytogeographic domain with the greatest richness of species, 183, followed by Cerrado (60 species), Amazon (29 species), Pampa (10 species), Caatinga (8 species), and Pantanal (5 species) (Table 3). These values correspond to about 69%, 23%, 11%, 4%, 3%, and 2% of the Brazilian fauna of Cecidomyiidae. Fourty-one cecidomyiid species are associated with 43 endemic Brazilian plant species. Among them, 21 hosts are endemic to Atlantic Forest, five to Cerrado, and one to Amazon. No hosts were endemic to Caatinga, Pampa and Pantanal (Table 4). Two-hundred thirty five gall midge species (235) are known from a single domain: 157 from Atlantic Forest, 35 from Cerrado, 17 from Amazon Forest, 8 from Pampa, 4 from Caatinga, and 3 from Pantanal. The others have been reported in two (23 species) or three domains (seven species). Onehundred and thirty-seven species (about 52% of the Brazilian fauna) are known only from the type-locality.

A list of gall midge species with occurrence in Brazil is presented below in alphabetical order. Data on their food habit, geographic distribution and host plant are added. Botanical names were updated (synomyms found in publications are given in brackets). The origin of each host plant and its occurrence in Brazilian phytogeographic domains are also provided. These last two informations are restricted to hosts identified at specific level. References are added in chronological order.

Table 3. Richness of gall midge species (Diptera	a, Cecidomyiidae) by Brazilian phytogeo-graphic domain.
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Genera	Number of gall midge species						
(n=93)	Brazil	Amazon	Atlantic Forest	Caatinga	Cerrado	Pampa	Pantanal
Alexomyia	1	1	0	0	0	0	0
Alycaulus	4	1	2	0	1	0	0
Anadiplosis	4	0	4	1	0	0	0
Anasphondylia	1	0	1	0	0	0	0
Andirodiplosis	1	0	1	0	1	0	0
Anisodiplosis	1	0	1	0	1	0	0
Aphidoletes	1	0	0	0	0	0	0
Apodiplosis	1	0	1	0	0	0	0
Arcivena	1	0	0	0	1	0	0
Arrabidaeamyia	1	0	1	0	0	0	0
Asphondylia	23	2	15	0	7	2	0
Asteromyia	1	0	1	0	0	0	0
Autodiplosis	1	0	1	0	0	0	0
Baccharomyia	2	0	2	0	0	0	0
Brethesiamyia	1	0	0	0	1	0	0
Brugmannia	14	2	11	0	1	1	0
Bruggmanniella	11	0	8	0	4	1	0
Burseramyia	1	0	1	0	0	0	0
Cerciplanus	2	0	0	0	2	0	0
Cleitodiplosis	1	0	1	0	0	0	0
Clinodiplosis	20	2	14	0	3	0	0
Clusiamvia	2	0	2	0	0	0	0
Compsodiplosis	2	0	1	0	0	1	0
Contarinia	2	1	2	0	1	0	0
Contodiplosis	3	0	3	0	0	0	0
Cordiamvia	- 1	0	1	0	0	0	0
Costadinlosis	1	0	1	0	0	0	0
Couridinlosis	- 1	0	1	0	1	0	0
Dactylodinlosis	4	1	3	ů 0	0	0	0
Dasineura	11	0	9	ů 0	2	0	0
Diadinlosis	10	0	7	ů 0	-	0	0
Dialeria	1	0	1	1	0	0	0
Dichodinlosis	1	0	1	0	0	0	0
Didactylomvia	1	0	0	0	1	0	0
Elachypalpus	1	0	0	ů 0	0	1	0
Enihormomvia	1	0	1	ů 0	0	0	0
Eugeniamvia	2	0	2	0	0	1	0
Feltiella	1	0	0	1	0	0	0
Fernandesia	1	0	1	0	0	0	0
Frauenfeldiella	1	1	1	ů 0	0 0	0	0
Geraldesia	1	0	1	0	0	0	0
Gnesiodinlosis	1	0	1	0	0	0	ů 0
Guareamvia	1	0	1	0 0	0	0	0
Guarenhila	1	0	0	0	0	1	0
Hanlopalnus	1	1	0	0	0	0	0
Hanlusia*	2	1	0	0	0	0	0

Continue...

Cecidomyiidae (Diptera, Insecta) in Brazil

continuation							
Houardodiplosis	1	0	1	1	0	0	0
Iatrophobia	1	1	1	0	0	0	0
Jorgenseniella	1	0	1	0	0	0	0
Lestodiplosis	3	0	3	0	0	0	0
Liodiplosis	3	0	3	0	0	0	0
Lopesia	26	7	18	2	14	0	0
Machaeriobia	2	0	2	0	1	0	0
Macroporpa	2	2	0	0	0	0	0
Manilkaramyia	1	0	1	0	0	0	0
Mayteniella	1	0	1	0	0	0	0
Megaulus	1	1	0	0	0	0	0
Metasphondylia	1	0	1	0	0	0	0
Meunieriella	4	0	3	0	1	0	0
Mikaniadiplosis	1	0	1	0	0	0	0
Mycodiplosis	1	0	0	0	0	0	0
Myrciamvia	2	0	1	0	1	0	0
Myrciariamyia	3	0	1	0	2	0	0
Neolasioptera	9	0	7	0	0	1	1
Neomitranthella	1	0	1	0	0	0	0
Novocalmonia	2	0	2	0	0	0	0
Ouradiplosis	1	1	0	0	0	0	0
Parametasphondvlia	1	0	0	0	1	0	0
Parazalepidota	1	0	1	0	0	0	0
Parkiamvia	1	1	0	0	0	0	0
Paulliniamvia	1	0	1	0	0	0	0
Perasphondvlia	2	1	1	0	0	0	0
Pisphondvlia	1	0	1	0	0	0	0
Primadiplosis	1	0	1	0	0	0	0
Proasphondvlia	3	0	3	0	0	0	0
Procontarinia	1	0	1	0	0	0	0
Prodinlosis	1	0	0	0	0	0	0
Rhoasphondvlia	1	0	1	0	0	0	0
Rochadinlosis	1	0	1	0	1	0	0
Schismatodiplosis	1	1	1	0	0	0	0
Schizomvia	8	0	3	1	4	0	0
Smilasiontera	1	0	1	0	0	0	0
Sphaeramvia	1	0	1	0	0	0	0
Sphaerodiplosis	1	0	0	0	0	0	0
Stephomvia	6	0	6	0	1	0	0
Stomatosema	6	0	0	0	4	0	4
Styraxdiplosis	2	0	2	1	0	0	0
Termitomastus	1	0	0	0	1	0	0
Trotteria	1	0	1	0	0	0	0
Uleella	1	0	1	0	0	0	0
Uleia	1	1	0	0	0	0	0
Youngomyia	2	0	1	0	1	0	0
Zalepidota	3	0	2	0	0	1	0
Total	261	29	178	8	57	10	5

	F 1	E.L	F . J		N
Host Plant	Endemic to	Endemic to	Endemic to	Endemic to Cerrado	Number of gain
	Drazli	Amazon Forest	Atlantic Forest		
Alaina neterophylia	X	λ	-	-	1
	A V	-	-	-	1
Anaira jraxinijolia	X	-	-	-	1
Baccharis lateralis	Х	-	-	-	1
Baccharis	Х	-	Х	-	1
pseudomyriocephala					
Banisteriopsis	Х	-	-	-	1
	V		V		1
Clusia juminensis	A	-	A V	-	1
Clusia nilariana	X	-	Χ	-	1
	X	-	-	-	1
Couepia ovalifolia	X	-	X	-	2
Croton hemiargyreus	X	-	Х	-	1
Dalechampia ficifolia	Х	-	-	-	1
Eremanthus	Х	-	-	-	1
erythropappus					
Erythroxylum	Х	-	Х	-	3
ovalifolium	37		37		4
Eugenia astringens	Х	-	Х	-	4
Eugenia	Х	-	Х	-	3
copacabanensis	••				
Eugenia punicifolia	Х				1
Guapira	Х	-	Х	-	1
pernambucensis	37			V	1
Kielmeyera rosea	Х	-	-	Х	1
Lessingianthus	Х	-	-	Х	1
warmingianus					
Machaeriobia	Х		Х		1
machaeri	V		V		1
Maniikara subsericea	Λ	-	Х	-	1
Miconia	Х	-	Х	-	1
Milani a tainania	V		V		1
Mikania trinervis	Λ	-	Λ	-	1
Mimosa	Х	-	-	-	1
Caesaipiniijoita Montovordia					
Monteverata	Х	-	-	-	2
Oblusijolla Munoja ovata	v		v		1
Myrcia ovala Munoia notonta		-	Λ	-	1
Myrcia reioria		-	- V	-	1
Neomitranines obscura	A V	-	Λ	-	5
Ocolea notala	A V	-	-	- V	1
Ourated speciabilis	A V	-	-	Λ	1
Pepionia asteria	Λ	-	Χ	-	1
Paulinia	Х	-	Х	-	2
weinmanniijoita Diamanni ann dalla muun	V				1
Pieroma canaoileanum	A V	-	-	Х	1
Protium brasiliense	A V	-	-	-	1
Protium icicariba	X	-	Х	-	2
Pstatum cattleyanum	X	-	-	-	2
Psittacanthus dichroos	X	-	-	-	1
Pieranara pyroidea	X	-	-	Х	1
Smilax oblongifolia	X	-	-	-	l
Smilax rufescens	Х	-	-	-	1
Struthanthus	Х	-	-	-	-
taubatensis	37				1
Swartzia langsdorffii	Х	-	X	-	1

Table 4. Richness of gall midge species (Diptera: Cecidomyiidae) by endemic plant species in Brazilian phytogeographic domains. There is no endemic host plants in the Caatinga, Pampa and Pantanal until the current moment.

Cecidomyiidae (Diptera, Insecta) in Brazil

Family			Number of gal	ll midge species		
гашпу	Amazon Forest	Atlantic Forest	Caatinga	Cerrado	Pampa	Pantanal
Amaranthaceae	0	0	0	0	0	0
Anacardiaceae	0	2	0	0	0	0
Annonaceae	0	0	0	1	0	0
Araliaceae	0	0	0	1	0	0
Asclepiadaceae	0	1	0	0	0	0
Asteraceae	3	19	0	6	1	0
Bignoniaceae	0	1	0	0	0	0
Boraginaceae	1	2	0	1	0	0
Burseraceae	2	4	0	4	0	0
Cactaceae	0	1	0	0	0	0
Calophyllaceae	5	5	0	6	0	0
Caryocaraceae	0	0	0	0	0	0
Celastraceae	0	2	0	0	0	0
Chrysobalanaceae	0	2	0	0	0	0
Clusiaceae	1	3	0	0	0	0
Combretaceae	0	1	1	0	0	0
Convolvulaceae	Ő	1	0	0	0	ů 0
Dilleniaceae	0	1	0	1	Ő	ů 0
Erythroxylaceae	Õ	4	ů 0	1	Ő	Ő
Euphorbiaceae	1	4	0	2	0	ů
Fabaceae	2	15	4	10	0	1
Lamiaceae	0	2	4 0	1	0	0
Lauraceae	1	2	0	0	0	0
Lauraceae	1	2	0	0	0	0
Lorantilaceae	0	5	0	0	0	0
Malnichiaaaaa	1	1	0	5	0	0
Malpignaceae	1	4	0	5	0	0
Malastamataaaaa	0	2	0	0	0	0
Melasiomataceae	0	2	0	3	0	0
Menaceae	0	2	0	0	1	0
Moraceae	1	3	0	0	1	0
Myrsinaceae	0	0	0	0	1	0
Myrtaceae	0	21	0	3	1	0
Nyctaginaceae	1	14	0	2	0	0
Ochnaceae	0	0	0	1	0	0
Olacaceae	1	l	0	0	0	0
Onagraceae	0	1	0	0	0	0
Orchidaceae	0	0	0	0	0	0
Piperaceae	0	l	0	l	l	0
Poaceae	0	1	0	0	0	0
Polypodiaceae	0	1	0	0	0	0
Pontederiaceae	0	0	0	1	0	0
Ranunculaceae	0	0	0	0	0	0
Rosaceae	0	0	0	0	0	0
Rubiaceae	0	8	0	0	0	0
Rutaceae	0	0	0	0	0	0
Sapindaceae	1	2	0	1	1	0
Sapotaceae	0	5	0	1	0	0
Smilacaceae	0	1	0	1	1	0
Solanaceae	1	0	0	0	0	0
Sterculiaceae	1	0	0	1	0	0
Styracaceae	0	2	1	0	0	0
Urticaceae	1	0	0	0	0	0

Table 5. Richness of gair muge species (Dipiera, Ceciuomynuae) by vegetable faimly in Diazman phytogeographic doma	5. Kichness of gail midge species (Diplera, Cecidomvildae) by vegetable family in Brazilian phytogeographic dop
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Verbenaceae

New records are indicated by an asterisk. The number in brackets after the locality represents the voucher number of the plant species.

1. *Alexomyia ciliata* Felt, 1921a (gall-inducer). Distribution: Brazil: Pará (Amazon Forest). Host plant: unkown. Refs.: Felt 1921a, Gagné 1994, Gagné & Jaschhof 2017.

2. Alycaulus globulus Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Poço das Antas, Rio de Janeiro (Grumari), Parque Nacional do Itatiaia, Reserva Biológica União; São Paulo state: Bertioga (Atlantic Forest). Host plant: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* DC. (Asteraceae). Refs.: Gagné et al. 2001, Oliveira & Maia 2005, Maia et al. 2008, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017, Maia & Siqueira 2020.

3. *Alycaulus hexadentatus* Urso-Guimarães, 2018a (gall-inducer). Distribution: Brazil: São Paulo state: Altinópolis (Cerrado). Host plant: *Smilax oblongifolia* Pohl ex Griseb (Smilacaceae) (endemic to Brazil) (Caatinga and Cerrado). Refs.: Urso-Guimarães 2018a, Ribeiro et al. 2019.

4. *Alycaulus mikaniae* Rübsaamen, 1915 (gall-inducer). Distribution: Brazil: Amazon (Amazon Forest). Host plant: *Mikania* sp. (Asteraceae). Refs.: Rübsaamen 1915, Gagné 1994, Gagné & Jaschhof 2017.

5. *Alycaulus trilobatus* Möhn, 1964a (gall-inducer). Distribution: El Salvador; Colombia; Brazil: São Paulo state: Bertioga (Atlantic Forest). Host plants: *Mikania micrantha* Kunth (native to Brazil) (Amazon Forest, Cerrado, Atlantic Forest, and Pampa) and *M. cordifolia* (L.f.) Willd. (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pampa) (Asteraceae). Refs.: Möhn 1964a, Gagné 1994, Maia et al. 2008, Gagné & Jaschhof 2017.

6. Anadiplosis caetetensis Tavares, 1920a (gall-inducer). Distribution: Brazil: Bahia: Caetité (Atlantic Forest, Caatinga). Host plant: undetermined Fabaceae. Refs.: Tavares 1920a, Gagné 1994, Gagné & Jaschhof 2017.

7. Anadiplosis procera Tavares, 1920a (gall-inducer). Distribution: Brazil: Bahia: Salvador (Itaparica) (Atlantic Forest). Host plant: undetermined Fabaceae. Refs.: Tavares 1920a, Gagné 1994, Gagné & Jaschhof 2017.

8. Anadiplosis pulchra Tavares, 1916 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Machaerium* sp. (Fabaceae). Refs.: Tavares 1916, Gagné 1994, Gagné & Jaschhof 2017.

9. Anadiplosis venusta Tavares, 1916 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Machaerium* sp. (Fabaceae). Refs.: Tavares 1916, Gagné 1994, Gagné & Jaschhof 2017.

10. *Anasphondylia myrtacea* Tavares, 1920b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: undetermined Myrtaceae. Refs.: Tavares 1920b, Gagné 1994, Gagné & Jaschhof 2017.

11. Andirodiplosis bahiensis Tavares, 1920c (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest); São Paulo state: Luiz Antônio (Cerrado). Host plant: Andira sp. (Fabaceae). Refs. Tavares 1920c, Gagné 1994, Saito & Urso-Guimarães 2012, Gagné & Jaschhof 2017.

12. Anisodiplosis waltheriae Maia, 2005 (gall-inducer). Distribution: Brazil: Minas Gerais: Aimorés (Atlantic Forest); Mato Grosso: Chapada dos Guimarães (Cerrado). Host plant: Waltheria indica L. (Sterculiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Maia & Fernandes, 2005a, Almeida et al. 2006, Gagné & Jaschhof 2017. Proença & Maia 2020.

13. *Aphidoletes aphidimyza* (Rondani, 1847) (predator of aphids: Hemiptera). Distribution: Widespread Palearctic, Hawaii, widespread Nearctic, Chile, New Zealand, and Brazil (unstated locality). Refs.: Gagné & Jaschhof 2017.

14. *Apodiplosis praecox* Tavares, 1922 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Psychotria* sp. (Rubiaceae). Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017.

15. Arcivena kielmeyerae Gagné, 1984 (gall-inducer). Distribution: Brazil: São Paulo state: Mogi Guaçu (Cerrado). Host plant: Kielmeyera rosea Mart. & Zucc. (Calophyllaceae) (endemic to Cerrado). Refs.: Gagné 1984, 1994, Gagné & Jaschhof 2017.

16. Arrabiadaeamyia serrata Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Grumari, Mangaratiba), Reserva Biológica União, Maricá, Araruama, Arraial do Cabo, São João da Barra (Atlantic Forest). Host plant: Fridericia conjugata (Vell.) L. G. Lohmann (Bignoniaceae) (=Arrabidaea conjugata Mart.) (native to Brazil) (Amazon Forest, Cerrado, Atlantic Forest, and Pantanal). Refs.: Monteiro et al. 1994, Maia 2001a, b, Oliveira & Maia 2005, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017, Maia & Siqueira 2020.

17. Asphondylia bahiensis Tavares, 1917a (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: undetermined Rubiaceae. Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

18. Asphondylia borreriae Rübsaamen, 1905a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro, Maricá, Saquarema, Cabo Frio, Carapebus, Arraial do Cabo (Atlantic Forest). Host plants: *Borreria* sp. and *B. verticillata* (L.) G. Mey. (Rubiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs. Rübsaamen 1905a, Gagné 1994, Monteiro et al. 1994, Maia 2001b, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

 Asphondylia canastrae Urso-Guimarães & Amorim, 2002 (gallinducer). Distribution: Brazil: Minas Gerais: Delfinópolis (Cerrado).
 Host plant: *Hyptis* sp. (Lamiaceae). Refs. Urso-Guimarães & Amorim 2002, Urso-Guimarães et al. 2003, Gagné & Jaschhof 2017.

20. Asphondylia cipo Urso-Guimarães, 2018b (gall-inducer). Distribution: Brazil: Minas Gerais: Santana do Riacho (Cerrado). Host plant: *Lessingianthus warmingianus* (Baker) H. Rob. (Asteraceae) (endemic to Cerrado). Refs.: Urso-Guimarães, 2018b

21. Asphondylia communis Maia & Couri, 1992 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Maricá, Arraial do Cabo (Ilha do Cabo Frio), São João da Barra (Atlantic Forest). Host plant: Ximenia americana L. (Olacaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs. Maia & Couri 1992, Monteiro et al. 1994, Maia 1999a, Maia 2001b, Maia & Souza 2013, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

22. Asphondylia cordiae Möhn, 1959 (gall-inducer). Distribution: El Salvador and Brazil: Minas Gerais: Lagoa Santa (Cerrado); Espírito Santo: Anchieta-Piúma (Atlantic Forest) Rio de Janeiro state: Rio de Janeiro, Maricá, Reserva Biológica União, Saquarema, Araruama, Arraial do Cabo, Quissamã, Carapebus, Campos de Goitacazes, São João da Barra (Atlantic Forest); São Paulo state: Bertioga, Ubatuba (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest); Rio Grande do Sul: Porto Alegre (Atlantic Forest). Host plant: *Cordia dentata* Poir. (exotic) (no records in Brazil), *C. alba* (Jacq.) Roem. & Schult. (exotic) (no records in Brazil), and *Varronia curassavica* Jacq. (*= Cordia verbenacea* DC. *= Cordia curassavica* (Jacq.) Roem. & Schult.) (Boraginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pampa). Refs.: Möhn 1959, Gagné 1994, Maia 2001b, Maia et al. 2008, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018, Maia & Siqueira 2020, Maia & Flor 2020.

23. Asphondylia fructicola Maia, 2009 (gall-inducer). Distribution: Brazil: Pará: Oriximiná (Porto Trombetas) (Amazon Forest). Host plant: Solanum sp. (Solanaceae). Refs.: Maia et al. 2009, Gagné & Jaschhof 2017.

24. Asphondylia glomeratae Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro, Parque Nacional do Itatiaia, Valença (Atlantic Forest); Minas Gerais: Viçosa (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* (Asteraceae). Refs.: Gagné et al. 2001, Maia et al. 2008, Proença & Maia 2012, Maia & Proença 2016, Maia & Mascarenhas 2017, Gagné & Jaschhof 2017.

25. Asphondylia gochnatiae Maia, 2008 (gall-inducer). Distribution: Brazil: Minas Gerais: Luz (Cerrado). Host plant: Moquiniastrum polymorphum (Less.) G. Sancho (=Gochnatia polymorpha (Less.) Cabrera) (Asteraceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia et al. 2008, Gagné & Jaschhof 2017.

26. Asphondylia maricensis Maia & Couri, 1992 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Struthanthus taubatensis* Eichler (= S. *maricensis* Rizzini ex Profice (Loranthaceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia & Couri 1992, Maia 2001b, Gagné & Jaschhof 2017.

27. Asphondylia microcapillata Maia, 2005 (gall-inducer). Distribution: Brazil: Minas Gerais: Três Marias (Cerrado). Host plant: *Bauhinia brevipes* Vogel (Fabaceae) (native to Brazil) (Amazon Forest, Cerrado, and Atlantic Forest). Refs.: Maia & Fernandes, 2005b, Gagné & Jaschhof 2017.

28. Asphondylia moehni Skuhravá, 1989 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa), Canela, Santa Tereza (Atlantic Forest); São Paulo state: Ubatuba, Bertioga (Atlantic Forest); Rio de Janeiro state: Rio de Janeiro (Grumari), Parque Nacional do Itatiaia (Atlantic Forest). Host plants: *Mikania guaco* Kunth (native to Brazil) (Amazon), *M. glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest), and *Mikania cf biformis* (Asteraceae). Refs.: Möhn 1973, Gagné 1994, Oliveira & Maia 2005, Maia et al. 2008, Maia & Mascarenhas 2017, Gagné & Jaschhof 2017, Goetz et al. 2018.

29. Asphondylia parva Tavares, 1917a (gall-inducer). Distribution: Brazil: Bahia: Madre de Deus (Atlantic Forest). Hos plant: undetermined Rubiaceae. Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

30. Asphondylia peploniae Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Carapebus (Atlantic Forest). Host plant: Peplonia asteria (Vell.) Fontella & E. A. Schwarz (Asclepiadaceae) (endemic to Atlantic Forest). Refs.: Maia 2001a, Gagné & Jaschhof 2017. 31. Asphondylia rochae Tavares, 1918a (gall-inducer). Distribution: Brazil: Ceará: Fortaleza (Atlantic Forest). Host plant: Ludwigia sp. (Onagraceae). Refs.: Tavares 1918a, Gagné 1994, Gagné & Jaschhof 2017.

32. Asphondylia sanctipetri Urso-Guimarães & Amorim, 2002 (gallinducer). Distribution: Brazil: São Paulo state: Ribeirão Preto (Cerrado); Minas Gerais: Delfinópolis (Cerrado). Host plant: *Didymopanax morototoni* (Aubl.) Decne. & Planch (Araliaceae) (native to Brazil) (all Brazilian biomes). Refs.: Urso-Guimarães & Amorim 2002, Gagné & Jaschhof 2017., Proença & Maia in print.

33. Asphondylia sennae Maia & Couri, 1992 (gall-inducer). Distribution: Brazil: Rio de Janeiro state, Maricá (Atlantic Forest). Host plant: Senna bicapsularis (L.) Roxb. (Fabaceae) (exotic). Refs.: Maia & Couri 1992, Maia 2001b, Gagné & Jaschhof 2017.

34. Asphondylia serrata Maia, 2004a (gall-inducer). Distribution: Brazil: Minas Gerais: Tiradentes (Cerrado), Serra do Ibitipoca (Atlantic Forest), Serra Azul de Minas (Cerrado), Serra do Cabral (Cerrado), São Tomé das Letras (Atlantic Forest and Cerrado), Serra do Caparaó (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest); Rio de Janeiro state: Petrópolis, Nova Friburgo (Atlantic Forest). Host plant: Eremanthus erythropappus (DC.) MacLeish (= Vanillosmopsis erythropappa (DC.) Sch. Bip.) (Asteraceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia 2004a, Maia 2011, 2013, Coelho et al. 2013, Gagné & Jaschhof 2017, Maia & Flor 2020.

35. Asphondylia stachytarpheta Barnes, 1932 (gall-inducer). Distribution: Trinidad and Brazil: Rio de Janeiro state: Mangaratiba (Atlantic Forest). Host plants: *Stachytarpheta cayennensis* (Rich.) Vahl. (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal) and *Stachytarpheta* sp. (Verbenaceae). Refs.: Barnes 1932, Gagné 1994, Rodrigues et al. 2014, Gagné & Jaschhof 2017.

36. *Asphondylia struthanthi* Rübsaamen, 1915 (gall-inducer). Distribution: Brazil: Ceará: Serra do Baturité (Atlantic Forest). Host plant: *Struthanthus* sp. (Loranthaceae). Refs.: Rübsaamen 1915, Möhn 1973, Gagné 1994, Gagné & Jaschhof 2017.

37. Asphondylia sulphurea Tavares, 1909 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: Poss on *Smilax* sp. (Smilacaceae). Refs.: Tavares 1909, Gagné 1994, Gagné & Jaschhof 2017.

38. Asphondylia tournefortiae Rübsaamen, 1915 (gall-inducer). Distribution: El Salvador; Brazil: Amazonas: Auristela and São Francisco on Acre River (Amazon Forest). Host plants: *Heliotropium* angustiflorum (Ruiz & Pav.) Govaerts (=Tournefortia angustiflora Ruiz & Pav.) (native to Brazil) (Amazon Forest) and Myriopus volubilis Small (=Tournefortia volubilis L.) (Boraginaceae) (native) (Atlantic Forest). Refs.: Rübsaamen 1915, Gagné 1994, Gagné & Jaschhof 2017.

39. Asphondylia ulei Rübsaamen, 1908a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Palmeiras (Atlantic Forest). Host plant: Mikania sp. (Asteraceae). Refs.: Rübsaamen 1908a, Möhn 1973, Gagné 1994, Gagné & Jaschhof 2017.

40. Asteromyia modesta (Felt, 1907a) (gall-inducer). Distribution: Widespread eastern Nearctic, Argentina, and Brazil: Minas Gerais (unstated municipality), Rio de Janeiro state (Atlantic Forest). Host plants: *Conyza canadensis* (L.) Cronquist (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal) and *Erigeron strigosus* Muhl. ex Willd. (Asteraceae) (exotic). Refs.: Felt 1907a, Gagné 1968, 1994, Gagné & Jaschhof 2017. 41. *Autodiplosis parva* (Tavares, 1916) (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: undetermined Fabaceae. Refs.: Tavares 1916, Gagné 1994, Gagné & Jaschhof 2017.

42. Baccharomyia magna Maia, 2012 (gall-inducer). Distribution: Brazil: Minas Gerais: Parque Estadual do Itacolomi (Atlantic Forest). Host plant: Baccharis pseudomyriocephala Malag. (Asteraceae) (endemic to Atlantic Forest); HT; ♂; MNRJ. Distr.: Brazil (Minas Gerais). Refs.: Maia 2012, Gagné & Jaschhof 2017.

43. *Baccharomyia ramosina* Tavares, 1917a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Baccharis crispa* Spreng. (= *B. trimera* (Less.) DC. (Asteraceae) (native to Brazil) (Caatinga, Cerrado, Atlantic Forest, and Pampa). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

44. *Brethesiamyia retorta* Maia, 2009 (gall-inducer). Distribution: Brazil: Minas Gerais: Três Marias (Cerrado). Host plant: *Myrcia retorta* Cambess (Myrtaceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia et al. 2009, 2010a, Gagné & Jaschhof 2017.

45. *Bruggmannia acaudata* Maia, 2004b (gall-inducer). Distribution: Brazil: Espírito Santo: Santa Teresa (Atlantic Forest); Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Maricá, Carapebus, Arraial do Cabo; São Francisco de Itabapoana (Atlantic Forest); Bahia: Porto Seguro-Trancoso (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001b, 2004b, 2014, Maia & Oliveira 2010, Maia et al. 2014, Rodrigues & Maia 2014, Maia & Carvalho-Fernandes 2016, Gagné & Jaschhof 2017.

46. *Bruggmannia braziliensis* Tavares, 1906 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: *Myrsine* sp. (Myrsinaceae). Refs.: Tavares 1906, Möhn 1962, Gagné & Jaschhof 2017.

47. Bruggmannia chapadensis Proença & Maia, 2018 (gall-inducer). Distribution: Brazil: Mato Grosso: Parque Nacional da Chapada dos Guimarães (Cerrado). Host plant: *Guapira pernambucensis* (Casar.) Lundell (Nyctaginaceae) (endemic to Atlantic Forest). Refs.: Proença & Maia 2018

48. Brugmannia depressa (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Pará: Belém; Acre: Juruá Mirim (Amazon Forest); Rio de Janeiro state: Teresópolis, Rio de Janeiro (Floresta da Tijuca) (Atlantic Forest); Santa Catarina (unstated municipality); Minas Gerais (unstated municipality). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

49. Bruggmannia elongata Maia & Couri, 1993 (gall-inducer). Distribution: Brazil: Bahia: Porto Seguro-Trancoso (Atlantic Forest); Espírito Santo: Conceição da Barra, Guarapari (Atlantic Forest); Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Rio de Janeiro (Marambaia), Maricá, Saquarema, Araruama, Carapebus, Arraial do Cabo (Ilha do Cabo Frio), Cabo Frio, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest); Rio Grande do Sul: Canela (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia & Couri 1993, Monteiro et al. 1994. Maia 2001b, 2014, Maia et al. 2008, Maia & Oliveira 2010, Maia & Souza 2013, Rodrigues & Maia 2014, Arriola et al. 2015, Carvalho-Fernandes et al. 2016, Maia & Carvalho-Fernandes 2016, Maia & Silva 2016, Goetz et al. 2018, Melo-Júnior et al. 2018, Maia 2020a. 50. Bruggmannia globulifex (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Serra dos Órgãos (Atlantic Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

51. *Bruggmannia lignicola* (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Serra do Macaé (Atlantic Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

52. *Bruggmannia longicauda* (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Atlantic Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

53. Brugmannia longiseta (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Amazonas: Barcelos (Marari), Juruá (Amazon Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

54. *Bruggmannia micrura* (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Santa Catarina (unstated municipality) (Atlantic Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

55. Bruggmannia monteiroi Maia & Couri, 1993 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia & Couri 1993, Gagné & Jaschhof 2017.

57. Bruggmannia robusta Maia & Couri, 1993 (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga (Atlantic Forest); Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Reserva Biológica União, Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Carapebus, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); Espírito Santo: Santa Tereza, Conceição da Barra (Atlantic Forest); Bahia: Porto Seguro-Trancoso (Atlantic Forest); Rio Grande do Sul: Canela (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia & Couri 1993, Monteiro et al. 1994, Maia 2001b, 2014, Maia et al. 2008, Maia & Oliveira 2010, Maia et al. 2014, Rodrigues & Maia 2014, Maia & Carvalho-Fernandes 2016, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Goetz et al. 2018, Maia & Siqueira 2020, Maia 2020a.

58. *Bruggmannia ruebsaameni* (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Santa Catarina: Pedras Grandes (Atlantic Forest). Host plant: *Neea* sp. (Nyctaginaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

59. Bruggmanniella braziliensis Tavares, 1909 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: Sorocea bonplandii (Baill.) W. C. Burger et al. (= Sorocea ilicifolia Miq.) (Moraceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pantanal). Refs.: Tavares 1909, Möhn 1963, Gagné 1994, Gagné & Jaschhof 2017.

60. Bruggmanniella byrsonimae (Maia & Couri, 1992) (gallinducer). Distribution: Brazil: Bahia: Viçosa (Atlantic Forest); Espírito Santo: Linhares (Atlantic Forest); Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Marambaia), Maricá, Araruama, Arraial do Cabo, Carapebus, São João da Barra (Atlantic Forest). Host plant: Byrsonima sericea DC. (Malpighiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia & Couri 1992, Maia 1999a, Maia 2001b, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017, Maia & Flor 2020.

61. Bruggmanniella doliocarpi Maia, 2010 (gall-inducer). Distribution: Venezuela: Santa Lucia; Brazil: Pernambuco: Recife (Atlantic Forest); D.F.: Planaltina (Cerrado); Minas Gerais: Dores do Indaiá (Cerrado). Host plant: Doliocarpus dentatus (Aubl.) Standl. (Dilleniaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Maia et al. 2010a, Gagné & Jaschhof 2017, Maia & Flor 2020.

62. Bruggmanniella duguetiae Urso-Guimarães & Amorim, 2005 (gall-inducer). Distribution: Brazil: São Paulo state: São Carlos, Luiz Antônio (Cerrado). Host plant: Duguetia furfuraceae (A.St.-Hil.) Saff. (Annonaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Urso-Guimarães & Amorim 2005, Saito & Urso-Guimarães 2012, Gagné & Jaschhof 2017.

63. Bruggmanniella ingae Urso-Guimarães & Amorim, 2005 (gallinducer). Distribution: Brazil: São Paulo state: between São José do Rio Preto (Atlantic Forest and Cerrado) and Tapiratiba (Atlantic Forest). Host plant: *Inga edulis* Mart. (Fabaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Urso-Guimarães & Amorim 2005, Gagné & Jaschhof 2017.

64. Bruggmanniella maytenuse (Maia & Couri, 1992) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, São João da Barra, São Francisco de Itabapoana (Atlantic Forest). Host plant: Monteverdia obtusifolia (Mart.) Biral (=Maytenus obtusifolia Mart.) (Celastraceae) (endemic to Brazil) (Amazon Forest and Atlantic Forest). Refs.: Maia & Couri 1992, Maia 1999a, Maia 2001b, Carvalho-Fernandes et al. 2016, Maia & Carvalho-Fernandes 2016, Gagné & Jaschhof 2017.

65. *Bruggmanniella miconiae* Carvalho-Fernandes, Maia & Rodrigues, 2020 (gall-inducer). Distribution: Brazil: Minas Gerais: Dores do Indaiá (Cerrado). Host plant: *Miconia theaezans* (Bonpl.) Cogn. (Melastomataceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Rodrigues et al. 2020

66. Bruggmanniella miconia Garcia, Lamas and Urso-Guimarães, 2020 (gall-inducer). Distribution: Brazil: São Paulo state: Sorocaba (Atlantic Forest). Host plant: *Miconia* cf. *cinnamomifolia* (Melastomataceae). Refs.: Garcia et al. 2020.

67. *Bruggmanniella notatae* Rodrigues & Maia, 2020 (gallinducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba (Ilha da Marambaia) (Atlantic Forest). Host plant: *Ocotea notata* (Nees and Mart.) Mez (Lauraceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Rodrigues et al. 2020.

68. *Bruggmanniella oblita* Tavares, 1920d (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Schinus* sp. (Anacardiaceae). Refs.: Tavares 1920d, Gagné 1994, Gagné & Jaschhof 2017.

69. *Bruggmanniella sideroxyli* Rodrigues & Maia, 2020 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba (Ilha da Marambaia) (Atlantic Forest). Host plant: *Sideroxylon obtusifolium* (Roem. and Schult.) T. D. Penn. (Sapotaceae) (native to Brazil) (Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Rodrigues & Maia 2020.

70. *Burseramyia braziliensis* Maia & Fonseca, 2012 (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest). Host plant: *Swartzia langsdorffii* Raddi (Fabaceae) (endemic to Atlantic Forest). Refs.: Maia et al. 2008, Maia & Fonseca 2012, Maia 2014, Gagné & Jaschhof 2017. 71. *Cerciplanus cipo* Garcia & Urso-Guimarães, 2020 (gallinducer). Distribution: Brazil: Minas Gerais: Serra do Cipó (Cerrado). Host plant: *Heteropterys* sp. (Malpighiaceae). Ref: Garcia et al. 2020.

72. Cerciplanus tocantinensis Garcia & Urso-Guimarães, 2020 (gall-inducer). Distribution: Brazil: Tocantins: Araguaína (Cerrado). Host plant: *Ouratea spectabilis* (Mart.) Engl. (Ochnaceae) (endemic to Cerrado). Ref.: Garcia et al. 2020.

73. *Cleitodiplosis graminis* (Tavares, 1916) (gall-inducer). Distribution: Brazil: Bahia (unstated municipality); Rio de Janeiro state (unstated municipality) (Atlantic Forest). Host plant: *Paspalum conjugatum* P. J. Bergius (Poaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal). Refs.: Tavares 1916, 1921, Gagné 1994, Gagné & Jaschhof 2017.

74. *Clinodiplosis agerati* Maia, 2016 (gall-inducer). Distribution: Brazil: Minas Gerais: Dores de Indaiá (Cerrado). Host plant: *Ageratum conyzoides* L. (Asteraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal). Refs.: Maia & Oliveira 2016, Gagné & Jaschhof 2017.

75. *Clinodiplosis alternantherae* Gagné, 2004 (gall-inducer). Distribution: Uruguay, Argentina and Brazil (unstated locality), Host plant: *Alternanthera philoxeroides* (Mart.) Griseb. (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal) and *A. aquatica* (D. Parodi) Chodat (Amaranthaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Gagné et al. 2004, Gagné & Jaschhof 2017.

76. *Clinodiplosis bahiensis* (Tavares, 1917a) (gall-inducer). Distribution: Brazil: Bahia: Salvador, Madre de Deus (Atlantic Forest). Host plant: undetermined Asteraceae. Refs.: Tavares 1917a, Gagné 2004, Gagné & Jaschhof 2017.

77. *Clinodiplosis bellum* Urso-Guimarães & Carmo-Neto, 2015 (gall-inducer). Distribution: Brazil: São Paulo state: Altinopólis (Cerrado). Host plant: *Diplopteryx pubipetala* (A. Juss.) W. R. Anderson & C. C. Davis (Malpighiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Urso-Guimarães & Carmo-Neto 2015, Gagné & Jaschhof 2017, Ribeiro et al. 2019.

78. *Clinodiplosis cattleyae* Felt, 1908 (gall-inducer). Distribution: Western Europe, immigr: Hawaii, USA, Mexico, Jamaica, Ecuador, and Brazil (unstated locality). Host plants: *Cattleya* spp., *Epidendrum* spp., and *Laelia* spp. (Orchidaceae). Refs.: Felt 1908, Gagné 1994, Gagné & Jaschhof 2017.

79. *Clinodiplosis cearensis* (Tavares, 1917a) (gall-inducer). Distribution: Brazil: Ceará: Fortaleza (Atlantic Forest). Host plant: undetermined Asteraceae. Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

80. *Clinodiplosis cecropiae* Proença & Maia, 2020 (gall-inducer). Distribution: Brazil: Rondônia: Monte Negro (Amazon Forest). Host plant: *Cecropiae* sp. (Urticaceae). Refs.: Proença & Maia 2020.

81. *Clinodiplosis chlorophorae* Rübsaamen, 1905a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Fábrica (Atlantic Forest). Host plant: *Maclura tinctoria* (L.) D. Don ex Steud. (*= Chlorophora tinctoria* (L.) Gaudich. ex B.D. Jackson|) (Moraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal). Refs.: Rübsaamen 1905a, Gagné 1994, Gagné & Jaschhof 2017.

82. *Clinodiplosis conica* Oliveira & Maia, 2008 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus, Arraial do Cabo (Atlantic Forest). Host plant: *Microstachys corniculata* (Vahl) Griseb. (*=Sebastiania glandulosa* (Mart.) Pax.) (Euphorbiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2001b, Oliveira & Maia 2008, Gagné & Jaschhof 2017.

83. *Clinodiplosis costai* Maia, 2005 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus, Arraial do Cabo (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plant: *Paullinia weinmanniifolia* Mart. (Sapindaceae) (endemic to Atlantic Forest). Refs.: Maia 2001b, 2005, Maia et al. 2008, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

84. *Clinodiplosis diodiae* Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Carapebus, Arraial do Cabo (Atlantic Forest). Host plant: *Borreria palustris* (Cham. & Schltdl.) Bacigalupo & E. L. Cabral (= *Diodia gymnocephala* (DC.) K.Schum.) (Rubiaceae) (native to Brazil) (Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2001a,b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

85. *Clinodiplosis eupatorii* (Felt, 1911a) (gall-inducer). Distribution: St. Vincent, Trinidad, Costa Rica, Brazil: Pará (unstated locality) (Amazon Forest). Host plant: *Chromolaena odorata* (L.) R. M. King & H. Rob. (native to Brazil) (all phytogeographic domains) and *Eupatorium* spp. (Asteraceae). Refs.: Felt 1911a, Gagné 1994, Gagné & Jaschhof 2017.

86. *Clinodiplosis floricola* Novo-Guedes & Maia, 2008 (free living phytophagous). Distribution: Brazil: Rio de Janeiro state: Maricá, Rio de Janeiro (Marambaia) (Atlantic Forest). Host plant: *Heteropterys nitida* (Lam.) DC. (Malpighiaceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia 2001b, Novo-Guedes & Maia 2008, Maia & Silva 2016, Gagné & Jaschhof 2017.

87. *Clinodiplosis iheringi* (Tavares, 1925) (gall-inducer). Distribution: Brazil: Santa Catarina: Joinville (Atlantic Forest). Host plant: *Aegiphila integrifolia* (Jacq.) Moldenke (= *Aegiphila arborescens* (Aubl.) J. F. Gmel.) (Verbenaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1925, Gagné 1994, Gagné & Jaschhof 2017.

88. *Clinodiplosis marcetiae* (Tavares, 1917b) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plan t: *Marcetia* sp. (Melastomataceae). Refs.: Tavares 1917b, Gagné 1994, Gagné & Jaschhof 2017.

89. *Clinodiplosis maricaensis* Fernandes & Maia, 2011 (inquiline). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus (Atlantic Forest). Host plant: *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) (endemic to Atlantic Forest). Refs.: Maia 2001b, Maia & Fernandes 2011, Gagné & Jaschhof 2017.

90. *Clinodiplosis melissae* Maia, 1993a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Melissa officinalis* L. (Lamiaceae) (exotic) (cultived plant). Refs.: Maia 1993a, Gagné & Jaschhof 2017.

91. *Clinodiplosis profusa* Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Marambaia, Grumari), Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo (Ilha do Cabo Frio), São João da Barra (Atlantic Forest); Rio Grande do Sul: Santa Tereza (Atlantic Forest). Host plant: *Eugenia uniflora* L. (Myrtaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pampa). Refs.: Monteiro et al. 1994, Maia 2001a, b, Oliveira & Maia 2005, Maia 2008, Silva & Rodrigues 2011, Rodrigues & Maia 2014, Carvalho-Fernandes et al. 2016, Maia & Silva, 2016, Gagné & Jaschhof 2017, Goetz et al. 2018.

92. *Clinodiplosis pulchra* (Tavares, 1917a) (gall-inducer). Distribution: Brazil: Bahia: Salvador, Madre de Deus (Atlantic Forest).

Host plant: *Lantana* sp. (Verbenaceae). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

93. Clinodiplosis quartelensis Maia & Oliveira 2019 (gall-inducer). Distribution: Brazil: Minas Gerais: Quartel São João (Cerrado). Host plant: Banisteriopsis membranifolia (A. Juss.) B. Gates (Malpighiaceae) (endemic to Brazil) (Amazon and Atlantic Forests). Ref.: Maia & Oliveira 2019

94. *Clinodiplosis rubiae* (Tavares, 1918a) (gall-inducer). Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Rubia* sp. (Rubiaceae). Refs.: Tavares 1918a, Gagné 1994, Gagné & Jaschhof 2017.

95. *Clusiamyia granulosa* Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Casimiro de Abreu, Carapebus, Arraial do Cabo (Atlantic Forest); Espírito Santo: Guarapari (Atlantic Forest). Host plant: *Clusia hilariana* Schltdl. (Clusiaceae) (endemic to Atlantic Forest). Refs.: Maia 2001a, b, Bregonci et al. 2010, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

96. *Clusiamyia nitida* Maia, 1997 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Paraty, Rio de Janeiro, Maricá, Silva Jardim, Quissamã, Macaé, Arraial do Cabo (Atlantic Forest). Host plant: *Clusia lanceolata* Cambess. (Clusiaceae) (endemic to Atlantic Forest). Refs.: Maia 1997, 2001b, Gagné & Jaschhof 2017.

97. Compsodiplosis itaparicana Tavares, 1922 (gall-inducer). Distribution: Brazil: Bahia: Salvador (Itaparica) (Atlantic Forest). Host plant: unknown. Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017.

98. Compsodiplosis luteoalbida (Tavares, 1909) (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: ?Smilax sp. (Smilacaceae). Refs.: Tavares 1909, Gagné 1994, Gagné & Jaschhof 2017.

99. Contarinia gemmae Maia, 2003 (gall-inducer). Distribution: Brazil: Amazonas: Amanã (Amazon Forest); Bahia: Sebastião Laranjeiras (Cerrado); Goiás: Pirenópolis, Teresina de Goiás, Cavalcante (Cerrado); Minas Gerais: Januária (Cerrado), São Tomé das Letras (Atlantic Forest, Cerrado); Rio de Janeiro state: Carapebus (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plant: Calophyllum brasiliense Cambess. (Calophyllaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Madeira et al. 2003, Maia et al. 2008, Arriola et al. 2015, Proença & Maia 2015, Gagné & Jaschhof 2017, Maia 2019a.

100. *Contarinia ubiquita* Gagné, 2001 (inquiline). Distribution: Brazil: Rio de Janeiro state: Reserva Biológica de Poço das Antas (Atlantic Forest). Host plant: *Mikania glomerata* Spreng. (Asteraceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Gagné et al. 2001, Gagné & Jaschhof 2017.

101. Contodiplosis friburgensis (Tavares, 1915) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Styrax* sp. (Styracaceae). Refs.: Tavares 1915, Gagné 1994, Gagné & Jaschhof 2017.

102. *Contodiplosis humilis* (Tavares, 1915) (gall-inducer). Distribution: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Styrax* sp. (Styracaceae). Refs.: Tavares 1915, Gagné 1994, Gagné & Jaschhof 2017.

103. Contodiplosis tristis (Tavares, 1915) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Styrax* sp. (Styracaceae). Refs.: Tavares 1915, Gagné 1994, Gagné & Jaschhof 2017.

104. Cordiamyia globosa Maia, 1996a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Reserva Biológica União, Saquarema, Cabo Frio, Arraial do Cabo, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); Espírito Santo: Guarapari, Conceição da Barra, Itaúnas (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest). Host plant: *Varronia curassavica* Jacq. (*= Cordia verbenacea* DC.) (*= Cordia curassavica* (Jacq.) Roem. & Schult.) (Boraginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pampa). Refs.: Monteiro et al. 1994, Maia 1996a, 2001b, Maia et al. 2008, Bregonci et al. 2010, Arriola et al. 2015, Carvalho-Fernandes et al. 2016, Maia & Carvalho-Fernandes 2016, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018, Maia & Siqueira 2020, Maia 2020a.

105. Costadiplosis maricaensis Viceconte & Maia, 2009 (gallinducer). Distribution: Brazil: Bahia*: Porto Seguro (RB557173) (Atlantic Forest); Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Psittacanthus dichroos* (Mart.) Mart. (Loranthaceae) (endemic to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2001b, Viceconte & Maia 2009, Gagné & Jaschhof 2017.

106. Couridiplosis vena Maia, 2004a (gall-inducer). Distribution: Bahia*: Ilhéus (RB732206) (Atlantic Forest); Espírito Santo*: Santa Leopoldina (RB440351) (Atlantic Forest); Minas Gerais: Tiradentes (Cerrado); São Paulo state*: Bananal (RB511509) (Atlantic Forest); Paraná*: Diamante do Norte (RB460265) (Atlantic Forest). Host plant: *Croton floribundus* Spreng (Euphorbiaceae) (native) (Atlantic Forest). Refs.: Maia 2004a, Maia & Fernandes 2004, Gagné & Jaschhof 2017.

107. Dactylodiplosis heisteriae Rübsaamen, 1915a (gall-inducer). Distribution: Brazil: Acre: Auristela, São Francisco (Amazon Forest). Host plant: *Heisteria acuminata* (Humb. & Bonpl.) Engl. (*=Heisteria cyanocarpa* Poepp.) (Olacaceae) (native) (Amazon Forest). Refs.: Rübsaamen 1915a, Gagné 1994, Gagné & Jaschhof 2017.

108. Dactylodiplosis heptaphylli Maia, 2004 (gall-inducer). Distribution: Brazil: Bahia*: Conde (RB37968), Caravelas (RB507605) (Atlantic Forest); Espírito Santo*: Linhares (RB252596, RB34113), Guarapari (RB535217) (Atlantic Forest); Rio de Janeiro state, Carapebus, Macaé* (RB393518), São João da Barra, São Francisco de Itabapoana (Atlantic Forest); Minas Gerais: São Tomé das Letras (Atlantic Forest). Host plant: Protium heptaphyllum (Aubl.) Marchand (Burseraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Narahara et al. 2004, Maia 2001b, 2013, Carvalho-Fernandes et al. 2016, Maia & Carvalho-Fernandes 2016, Gagné & Jaschhof 2017.

109. Dactylodiplosis icicaribae Maia, 2002 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Carapebus (Atlantic Forest). Host plant: *Protium icicariba* (DC.) Marchand (Burseraceae) (endemic to Atlantic Forest). Refs.: Maia et al. 2002, Rodrigues et al. 2014, Gagné & Jaschhof 2017.

110. Dactylodiplosis petibaurum Maia, 2021 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Parque Nacional da Restinga de Jurubatiba (Atlantic Forest). Host plant: Ocotea pulchella (Nees & Mart) Mez (Lauraceae) (native) (Atlantic Forest, Cerrado, and Pampa).

111. Dasineura braziliensis (Tavares, 1922) (gall-inducer). Distribution: Brazil: Mato Grosso*: Fazenda Palmeiras (RB 314383) (Cerrado); Piauí: Piracuruca (Parque Nacional das Sete Cidades) (RB181551) (Cerrado); Bahia (unstated); MG*: Carrancas (RB560635) (Cerrado). Host plant: *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017. 112. Dasineura byrsonimae Maia, 2010b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Marambaia), Reserva Biológica União, Maricá, Saquarema, Carapebus, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); Espírito Santo: Conceição da Barra Atlantic Forest). Host plant: *Byrsonima sericea* DC. (Malpighiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2001b, Maia 2008, 2010b, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Carvalho-Fernandes 2016, Maia & Silva 2016, Gagné & Jaschhof 2017, Maia & Siqueira 2020, Maia 2020a.

113. Dasineura copacabanensis Maia, 1993b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Saquarema, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra (Atlantic Forest). Host plant: *Eugenia copacabanensis* Kiaersk. (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1993b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

114. Dasineura couepiae Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro, Maricá, Araruama, Arraial do Cabo, Cabo Frio (Atlantic Forest); Espírito Santo: Guarapari, São Mateus (Atlantic Forest); Bahia: Caravelas, Conde, Porto Seguro (Atlantic Forest). Host plant: *Couepia ovalifolia* (Schott) Benth. ex Hook.f. (Chrysobalanaceae) (endemic to Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001a, b, Bregonci et al. 2010, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Maia & Cruz, 2020.

115. *Dasineura gigantea* Angelo & Maia, 1999 (gall-inducer). Distribution: Brazil: Paraná: Piraquara, Pontal do Paraná (Atlantic Forest); Santa Catarina: Babitonga, Itapoá (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Host plant: *Psidium cattleyanum* Sabine (Myrtaceae) (endemic to Brazil) (Caatinga, Cerrado, and Atlantic Forest). Refs.: Angelo & Maia 1999, Maia et al. 2008, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018.

116. Dasineura globosa Maia, 1996b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Grumari, Marambaia), Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra (Atlantic Forest). Host plant: *Eugenia astringens* Cambess. (= *Eugenia rotundifolia* Casar) (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1996b, 2001b, Oliveira & Maia 2005, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017.

117. Dasineura marginalis Maia, 2005 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Saquarema, Macaé, Araruama, Arraial do Cabo, Cabo Frio (Atlantic Forest). Host plant: *Eugenia astringens* Cambess (= *Eugenia umbelliflora* O. Berg., *E. rotundifolia* Casar) (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 2001b, Maia et al. 2005, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

118. *Dasineura myrciariae* Maia, 1996b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Marambaia), Maricá, Carapebus, São Francisco de Itabapoana (Atlantic Forest); Espírito Santo: Guarapari, Santa Teresa (Atlantic Forest). Host plant: *Myrciaria floribunda* (H. West ex Willd.) O.Berg (Myrtaceae (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 1996b, Bregonci et al. 2010, Maia 2001b, Maia et al. 2014, Maia & Carvalho-Fernandes 2016, Maia & Silva 2016, Gagné & Jaschhof 2017.

119. Dasineura occulta Pereira-Colavite & Urso-Guimarães,
 2013 (gall-inducer). Distribution: Brazil: São Paulo state: São Carlos

(Atlantic Forest). Host plant: *Hypochaeris chillensis* (Kunth) Britton (Asteraceae) (native to Brazil) (Atlantic Forest and Pampa). Refs.: Pereira-Colavite & Urso-Guimarães 2013, Gagné & Jaschhof 2017.

120. Dasineura ovalifoliae Fernandes & Maia, 2011 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Grumari, Marambaia), Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Carapebus (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest). Host plant: *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) (endemic to Atlantic Forest). Refs.: Maia 2001b, Maia & Fernandes 2011, Maia et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017.

121. Dasineura tavaresi Maia, 1996b (gall-inducer). Distribution: Brazil: State of de Janeiro: Maricá, Araruama, Carapebus, Arraial do Cabo, Cabo Frio, São João da Barra (Atlantic Forest). Host plant: *Neomitranthes obscura* (DC.) N. Silveira (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1996b, 2001b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

122. *Diadiplosis abacaxii* Culik & Ventura, 2013a (predator of *Dysmicoccus brevipes* Cockerell, 1893 Hemiptera: Pseudococcidae). Distribution: Brazil: Espírito Santo: Cachoeiro de Itapemirim (Atlantic Forest). Refs.: Culik & Ventura 2013a, Gagné & Jaschhof 2017.

123. *Diadiplosis bellingeri* Culik & Ventura, 2012 (predator of Pseudococcidae and Coccidae: Hemiptera). Distribution: Brazil: Espírito Santo: Domingos Martins (Atlantic Forest). Refs.: Culik & Ventura 2012, Gagné & Jaschhof 2017.

124. Diadiplosis coccidivora (Felt, 1911b) (predator of *Pulvinaria* urbicola (Cockrell, 1893) (Hemiptera: Coccidae), *Alichtensia* sp., *Coccus* sp., *Pulvinaria* spp., *Saissetia* spp. (Hemiptera: Coccidae), and *Eriococcus* sp. (Hemiptera: Eriococcidae). Distribution: USA (Florida); Bermuda; Jamaica; Guadeloupe, Panama, Guyana, Argentina, Brazil: São Paulo state (unstated municipality). Refs.: Felt 1911b, Borgmeier 1931, Gagné & Jaschhof 2017.

125. *Diadiplosis floridana* (Felt, 1915a) (predator of Pseudococcidae). Distribution: USA (Florida), Cuba, Paraguay, Brazil: Espírito Santo*: Domingos Martins, Sooretama, Cachoeiro de Itapemirim (material of MNRJ) (Atlantic Forest). Refs.: Felt 1915a, Gagné & Jaschhof 2017.

126. *Diadiplosis jamboi* Culik & Ventura, 2013b (predator of *Planococcus halli* Ezzat & McConnell, 1956 (Hemiptera: Pseudococcidae). Distribution: Brazil: Espírito Santo: Vitória (Atlantic Forest). Refs.: Culik & Ventura 2013b, Gagné & Jaschhof 2017.

127. Diadiplosis martinsensis Culik & Ventura, 2013b (predator of *Pseudococcus* cf. *jackbeardsleyi* (Hemiptera: Pseudococcidae). Distribution: Brazil: Espírito Santo: Domingos Martins (Atlantic Forest). Refs.: Culik & Ventura 2013b, Gagné & Jaschhof 2017.

128. Diadiplosis multifila (Felt, 1907b) (predator of scale insects; Ferrisia sp., Planococcus citri Risso, 1813, Phenacoccus solani Ferris, 1918 (Hemiptera: Pseudococcidae) and Icerya montserratensis Riley & Howard, 1890 (Hemiptera: Margarodidae). Distribution: West Indies (Dominican Republic to Trinidad), Brazil: Espírito Santo*: Vitória (material of MNRJ) (Atlantic Forest), Fiji, Israel. Refs.: Felt 1907b, Gagné & Jaschhof 2017.

129. Diadiplosis pseudococci Felt, 1921b (predator of *Pseudococcus bromeliae* Hempel 1912; *Dysmicoccus brevipes* Cockrell 1893 and *D. neobrevipes* Beardsley 1959 (Hemiptera: Pseudococcidae). Distribution: Hawaii, Mexico, Guatemala, Honduras, Jamaica, Guyana, and Brazil (unstated locality). Refs.: Felt 1921b, Gagné & Jaschhof 2017.

130. *Diadiplosis saccharum* Urso-Guimarães, 2020 (predador of *Saccharicoccus sacchari* (Cockrell, 1895) (Hemiptera, Pseudococcidae). Distribution: Brazil: Jaboticabal, São Carlos (Cerrado). Ref.: Urso-Guimarães et al. 2020.

131. *Diadiplosis vaupedis* (Harris, 1968) (predator of undetermined coccoid; *Planococcus* sp. (Hemiptera: Pseudococcidae). Distribution: Colombia, Guadeloupe, Brazil: Espírito Santo*: Domingos Martins (material of MNRJ) (Atlantic Forest). Refs.: Harris 1968, Gagné & Jaschhof 2017.

132. Dialeria styracis Tavares, 1918a (inquiline). Distribution: Brazil: Bahia: Caetité (Atlantic Forest, Caatinga). Host plant: Styrax sp. (Styracaceae). Refs.: Tavares 1918a, Gagné 1994, Gagné & Jaschhof 2017.

133. *Dichodiplosis triangularis* (Felt, 1908) (fungivorous). Distribution: USA (widespread), Costa Rica, and Brazil: Bahia: Salvador (Atlantic Forest). Refs.: Felt 1908, Gagné & Jaschhof 2017.

134. *Didactylomyia longimana* (Felt, 1908) (kleptoparasite in spider web). Distribution: Sri Lanka, USA, Colombia, Dominican Republic, Mexico, and Brazil: Mato Grosso do Sul: Aquidauana (Cerrado). Refs.: Felt, 1908, Gagné & Jaschhof 2017, Carmo-Neto et al. 2019.

135. *Elachypalpus psidii* Maia & Nava, 2011 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: Pelotas (Pampa) (cultived area). Host plant: *Psidium cattleyanum* Sabine (Myrtaceae) (endemic to Brazil) (Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia & Nava 2011, Gagné & Jaschhof 2017.

136. Epihormomyia miconiae Maia, 2001a (gall-inducer). Distribution: Brazil: Espírito Santo*: Santa Teresa (RB493591) (Atlantic Forest); Rio de Janeiro state: Carapebus (Atlantic Forest). Host plant: *Miconia cinnamomifolia* (DC.) Naudin (Melastomataceae) (endemic to Atlantic Forest). Refs.: Maia 2001a, b, Gagné & Jaschhof 2017.

137. Eugeniamyia dispar Maia, Mendonça & Romanowski, 1997 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: Pelotas (Pampa), Porto Alegre (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plant: Eugenia uniflora L. (Myrtaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pampa). Refs.: Maia, Mendonça & Romanowski 1997, Maia et al. 1997, 2008, Bierhals et al. 2012, Mendonça & Romanowski 2012, Gagné & Jaschhof 2017.

138. *Eugeniamyia triangularis* Maia & Nava, 2011 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Eugenia uniflora* L. (Myrtaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pampa). Refs.: Maia & Nava 2011, Gagné & Jaschhof 2017.

139. Feltiella curtistylus Gagné, 1984 (predator of Tetranychus evansi Baker & Pritchard, 1960 (Acarina: Tetranychidae). Distribution: Brazil: Pernambuco: Petrolina (Caatinga); USA: Florida. Refs.: Gagné 1984, 1994, Gagné & Jaschhof 2017.

140. *Fernandesia meridionalis* Rodrigues & Maia, 2013 (gallinducer). Distribution: Brazil: Rio Grande do Sul: São Francisco de Paula (Atlantic Forest). Host plant: *Myrciaria delicatula* (DC.) O.Berg (Myrtaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pampa). Refs.: Rodrigues et al. 2013, Gagné & Jaschhof 2017.

141. *Frauenfeldiella coussapoae* Rübsaamen, 1905b (gall-inducer). Distribution: Brazil: Acre: Juruá Mirim (Amazon Forest); Rio de Janeiro state: Rio de Janeiro (Gávea) (Atlantic Forest). Host plant: *Coussapoa* sp. (Moraceae). Refs.: Rübsaamen 1905b, Gagné 1994, Gagné & Jaschhof 2017.

142. Geraldesia eupatorii Tavares, 1917a (gall-inducer). Distribution: Brazil: Rio de Janeiro state (Atlantic Forest). Host plant: *Eupatorium* sp. (Asteraceae). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

143. Gnesiodiplosis itaparicae Tavares, 1917a (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: undetermined Rubiaceae. Refs.: Tavares, 1917a, Gagné 1994, Gagné & Jaschhof 2017.

144. *Guareamyia purpura* Maia, 2007a (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga (Atlantic Forest). Host plant: *Guarea macrophylla* Vahl (Meliaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2007a, Maia et al. 2008, Gagné & Jaschhof 2017.

145. *Guarephila albida* Tavares, 1909 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: *Guarea* sp. poss. *guidonia* (L.) Sleumer (Meliaceae) (*Guarea guidonia* = *G. trichilioides* L.) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1909, Gagné 1994, Gagné & Jaschhof 2017.

146. *Haplopalpus serjaneae* Rübsaamen, 1915a (gall-inducer). Distribution: Brazil: Acre, Auristela (Amazon Forest). Host plant: *Serjania* sp. (Sapindaceae). Refs.: Rübsaamen 1915a, Gagné 1994, Gagné & Jaschhof 2017.

147. *Haplusia braziliensis* (Felt, 1915b) (Fungivorous species). Distribution: Brazil: Pará, Igarapé Açu (Amazon Forest). Refs.: Felt 1915b, Gagné 1994, Gagné & Jaschhof 2017.

148. *Haplusia plumipes* Karsch, 1877 (Fungivorous species). Distribution: Brazil: Bahia (unstated locality). Refs.: Karsch 1877, Gagné 1994, Gagné & Jaschhof 2017.

149. Houardodiplosis rochae Tavares, 1925 (gall-inducer). Distribution: Brazil: Ceará: Fortaleza (Atlantic Forest, Caatinga), Aracati (Caatinga). Host plant: Combretum leprosum Mart. (Combretaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1925, Gagné 1994, Maia 2002, Gagné & Jaschhof 2017.

150. *Iatrophobia brasiliensis* (Rübsaamen, 1908a) (gall-inducer). Distribution: Costa Rica, Guadeloupe, St. Vincent, Trinidad and Tobago, Guyana, Surinam, and Brazil: Amazonas: Fortaleza, Juruá Mirim (Amazon Forest); Rio de Janeiro state: Mauá, Palmeiras, São Francisco do Itabapoana (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Tubarão (Atlantic Forest). Host plants: *Manihot esculenta* Crantz (= *Manihot utilissima* Pohl.) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest) and *Manihot* sp. (Euphorbiaceae). Refs.: Rübsaamen 1908a, Gagné 1994, Maia et al. 2008, Maia & Carvalho-Fernandes 2016, Gagné & Jaschhof 2017.

151. Jorgenseniella eugeniae Maia, 2005 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Arraial do Cabo (Atlantic Forest). Host plant: Eugenia astringens Cambess. (= E. umbelliflora O. Berg. = E. rotundifolia Casar) (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia et al. 2005, Gagné & Jaschhof 2017.

152. *Lestodiplosis braziliensis* (Tavares, 1920b) (predator). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Refs.: Tavares 1920b, Gagné 1994, Gagné & Jaschhof 2017.

153. Lestodiplosis floricola (Rodrigues & Maia, 2010a) (predator). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). On Jacquemonita holosericea (Weinm) O'Donell (Convolvulaceae). Refs.: Rodrigues & Maia 2010a, Gagné & Jaschhof 2017. 154. Lestodiplosis maricaensis Santos & Maia, 2009 (predator). Distribution: Rio de Janeiro state: Mangaratiba, Maricá, Carapebus (Atlantic Forest). On *Stylosanthes guianensis* (Aubl.) Sw. (Fabaceae) (native to Brazil) (all phytogeographic domains). Refs.: Maia 2001b, Santos & Maia 2009, Rodrigues et al. 2014, Gagné & Jaschhof 2017.

155. *Liodiplosis conica* Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Silva Jardim, Reserva Biológica União, Rio de Janeiro (Parque Nacional da Tijuca, Grumari), Valença, Angra dos Reis (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Rio Grande do Sul: Canela (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* (Asteraceae). Refs.: Gagné et al. 2001, Oliveira & Maia 2005, Maia et al. 2008, Maia & Oliveira 2010, Proença & Maia 2012, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017, Goetz et al. 2018, Maia & Siqueira 2020.

156. *Liodiplosis cylindrica* Gagné, 2001 (gall-inducer). Distribution: Distr: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Paraty, Mangaratiba, Silva Jardim, Valença, Parque Nacional do Itatiaia (Atlantic Forest); Minas Gerais: Viçosa (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest); Rio Grande do Sul: Canela (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest), *Mikania cf biformis, Mikania trinervis* Hook. & Arn. (endemic to Atlantic Forest) (Asteraceae). Refs.: Gagné et al. 2001, Maia et al. 2008, Maia & Oliveira 2010, Carvalho-Fernandes & Maia 2011, Proença & Maia 2012, Rodrigues et al. 2014, Maia & Proença 2016, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017, Melo-Júnior et al. 2018, Goetz et al. 2018.

157. Liodiplosis spherica Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Paraty, Mangaratiba, Valença, Parque Nacional do Itatiaia, Poço das Antas (Atlantic Forest); Minas Gerais: Viçosa (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Rio Grande do Sul: Canela (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* (Asteraceae). Refs.: Gagné et al. 2001, Maia et al. 2008, Maia & Oliveira 2010, Carvalho-Fernandes & Maia 2011, Proença & Maia 2012, Rodrigues et al. 2014, Maia & Proença 2016, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017, Goetz et al. 2018.

158. Lopesia aldinae Fernandes & Maia, 2010 (gall-inducer). Distribution: Brazil: Amazonas: Manaus (Amazon Forest). Host plant: Aldina heterophylla Spruce ex Benth. (Fabaceae) (endemic to Amazon Forest). Refs.: Fernandes et al. 2010, Gagné & Jaschhof 2017.

159. Lopesia andirae Garcia, Lima, Calado & Urso-Guimarães, 2017 (gall-inducer). Distribution: Mato Grosso: Chapada dos Guimarães (Cerrado); Bahia: Barreiras (Cerrado); São Paulo state: Luiz Antônio (Cerrado). Host plant: Andira humilis Mart. ex Benth. (Fabaceae) (endemic to Brazil) (Amazon Forest, Caatinga, and Cerrado). Refs.: Garcia et al. 2017, Lima & Calado 2018.

160. *Lopesia bilobata* Maia, 2004a (gall-inducer). Distribution: Brazil: Minas Gerais: Tiradentes (Cerrado). Host plant: *Guapira* sp. (Nyctaginaceae). Refs.: Maia 2004a, Maia & Fernandes 2004, Gagné & Jaschhof 2017.

161. *Lopesia brasiliensis* Rübsaamen, 1908b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Fábrica (Atlantic Forest); Santa Catarina: Tubarão (Atlantic Forest). Host plant: *Ossaea* sp. (Melastomataceae). Refs.: Rübsaamen 1908b, Gagné 1994, Maia 2007b, Gagné & Jaschhof 2017.

162. Lopesia caulinaris Maia, 2003 (gall-inducer). Distribution: Brazil: Amazonas: Amanã; Amapá: Oiapoque (Amazon Forest); Bahia: São Sebastião (Atlantic Forest); Rio de Janeiro state: Angra dos Reis, Carapebus (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Minas Gerais: São Tomé das Letras (Atlantic Forest) Januária (Atlantic Forest, Cerrado); Goiás: Pirenópolis, Cavalcante (Cerrado); Santa Catarina: Babitonga (Atlantic Forest). Host plant: *Calophyllum brasiliense* Cambess. (Calophyllaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Madeira et al. 2003, Maia 2013, 2008, Arriola et al. 2015, Proença & Maia 2015, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018, Maia 2019a.

163. Lopesia chapadensis Garcia & Urso-Guimarães, 2018 (gallinducer). Distribution: Brazil: Mato Grosso: Chapada dos Guimarães (Cerrado). Host plant: Andira vermifuga (Mart.) Benth. (Fabaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Ref.: Garcia & Urso-Guimarães 2018.

164. Lopesia conspicua Maia, 2003 (gall-inducer). Distribution: Brazil: Amazonas: Amaná (Amazon Forest); Amapá: Oiapoque (Amazon Forest); Goiás: Pirenópolis (Cerrado); Rio Grande do Norte: Canguaretama (Atlantic Forest); Bahia: Sebastião Laranjeiras (Caatinga, Cerrado); Minas Gerais: São Tomé das Letras (Atlantic Forest, Cerrado); Januária (Atlantic Forest, Cerrado); Rio de Janeiro state: Carapebus (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: São Francisco do Sul (Atlantic Forest); Paraná: Paranaguá (Atlantic Forest). Host plant: *Calophyllum brasiliense* Cambess. (Calophyllaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Madeira et al. 2003, Maia 2013, Arriola et al. 2015, Proença & Maia 2015, Gagné & Jaschhof 2017.

165. Lopesia davillae Maia & Monteiro, 2017 (free living phytophagous). Distribution: Brazil: Rio de Janeiro state: Teresópolis (Atlantic Forest). Host plant: Davilla rugosa Poir (Dilleniaceae) (native to Brazil) (Amazon Forest and Atlantic Forest). Ref.: Maia & Monteiro 2017.

166. Lopesia eichhorniae Urso-Guimarães, 2015 (gall-inducer). Distribution: Brazil: São Paulo state: Luiz Antônio (Cerrado). Host plant: *Eichhornia azurea* (Sw.) Kunth (Pontederiaceae) (native to Brazil) (all Brazilian phytogeographic domains). Refs.: Urso-Guimarães et al. 2015, Gagné & Jaschhof 2017.

167. Lopesia elliptica Maia, 2003 (gall-inducer). Distribution: Mexico, Guatemala, Costa Rica, Dominican Republic, Cuba, Bolivia, Guyana, Peru, and Brazil: Amazonas: Amanã (Amazon Forest); Amapá: Oiapoque (Amazon Forest); Pará: Moju (Amazon Forest); Tocantis: Formoso do Araguaia (Cerrado); Rondônia: Chupinguaia (Amazon Forest); Goiás: Pirenópolis (Cerrado); Mato Grosso: Santa Terezinha (Cerrado); Rio Grande do Norte: Canguaretama (Atlantic Forest); Maranhão: São Luís (Amazon Forest); Pernambuco: Rio Preto (Cerrado); Bahia: Sebastião Laranjeiras (Caatinga, Cerrado); Rio de Janeiro state: Carapebus (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Minas Gerais: São Tomé das Letras (Atlantic Forest, Cerrado); Santa Catarina: Babitonga (Atlantic Forest); Paraná: Paranaguá (Atlantic Forest). Host plant: Calophyllum brasiliense Cambess. (Calophyllaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Madeira et al. 2003, Maia et al. 2008, Maia 2013, Arriola et al. 2015, Proença & Maia 2015, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018.

168. Lopesia erythroxyli Rodrigues & Maia, 2010b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Rio de Janeiro (Grumari, Marambaia), Maricá, Carapebus, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest); São Paulo state: Itanhaém (Atlantic Forest). Host plant: *Erythroxylum ovalifolium* Peyr. (Erythroxylaceae) (endemic to Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001b, Rodrigues & Maia 2010b, Fernandes & Maia 2011, Maia et al. 2014, Maia & Silva 2016, Gagné & Jaschhof 2017, Maia 2021.

169. Lopesia grandis Maia, 2001a (gall-inducer). Distribution: Brazil: Paraíba: Mataraca (Atlantic Forest); Bahia: Camamu, Porto Seguro-Trancoso, Santa Cruz de Cabrália, Itacaré, Nova Viçosa, Uma, Ilhéus, Valença, Belmonte (Atlantic Forest); Espírito Santo: Aracruz, Conceição da Barra, Guarapari; Presidente Kennedy, São Mateus (Atlantic Forest); Rio de Janeiro state: Angra dos Reis (Ilha Grande), Paraty, Mangaratiba, Rio de Janeiro (Marambaia), Carapebus, Maricá, Araruama, Arraial do Cabo, São Francisco de Itabapoana (Atlantic Forest); São Paulo state: Bertioga, Ubatuba, Cananeia (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest). Host plants: *Dalbergia ecastophyllum* (L.) Taub. (native to Brazil) (Amazon Forest and Atlantic Forest) and *Dalbergia frutescens* (Vell.) Britton (native to Brazil) (Amazon Forest, Atlantic Forest, Caatinga, and Cerrado) (Fabaceae). Refs.: Maia 2001a, b, 2015, Maia et al. 2008, Maia & Oliveira 2010, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018, Maia 2020^a, Maia 2021.

170. Lopesia indaiensis Maia & Oliveira, 2018 (gall-inducer). Distribution: Brazil: Pernambuco: Tamandaré; Rio de Janeiro state: Mangaratiba (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Minas Gerais: Dores do Indaiá (Cerrado). Host plant: Andira fraxinifolia Benth (Fabaceae) (endemic to Brazil) (Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia et al. 2008, Santos et al. 2012, Rodrigues et al. 2014, Maia & Oliveira 2018.

171. Lopesia leandrae Maia, 2019b (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga (Atlantic Forest). Host plant: Leandra ionopogon (Mart.) Cogn. (Melastomataceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia et al. 2008, Maia, 2019b.

172. Lopesia linearis Maia 2003 (gall-inducer). Distribution: Mexico, Costa Rica, Trinidad and Tobago, Cuba, Colombia, Guyana, Peru, Bolivia, Paraguay, Brazil: Amazonas: Amanã (Amazon Forest); Pará: Moju (Amazon Forest); Rondônia: Chipinguaia (Amazon Forest); Roraima: Caracaraí (Amazon Forest); Rio Grande do Norte: Canguaretama (Atlantic Forest); Mato Grosso: Santa Terezinha (Amazon Forest and Cerrado), Corumbá (Cerrado); Minas Gerais: São Tomé das Letras (Atlantic Forest); Espírito Santo: Linhares (Atlantic Forest); Rio de Janeiro state: Carapebus (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest); Paraná: Paranaguá (Atlantic Forest). Host plant: *Calophyllum brasiliense* Cambess. (Calophyllaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Madeira et al. 2003, Maia et al. 2008, Maia 2013, Arriola et al. 2015, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018.

173. Lopesia marginalis Maia, 2001a (gall-inducer). Distribution: Brazil: Bahia: Caravelas, Conde (Atlantic Forest); Espírito Santo: Alto Limoeiro, São Mateus, Linhares, Itaguaçu, Itarana (Atlantic Forest); Rio de Janeiro state: Rio de Janeiro, Niterói, Casimiro de Abreu, Araruama, Carapebus, Cabo Frio, Arraial do Cabo (Atlantic Forest). Host plant: *Couepia ovalifolia* (Schott) Benth. ex Hook.f. (Chrysobalanaceae) (endemic to Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001a, b, Gagné & Jaschhof 2017, Maia & Cruz 2020, Maia 2021.

174. Lopesia maricaensis Rodrigues & Maia, 2010b (gallinducer). Distribution: Peru: Iquitos (Amazon Forest); Brazil: Amazonas (Amazon Forest), Paraíba: Caaporã (Atlantic Forest); Minas Gerais: Diamantina, Lagoa Santa, Serra do Cipó, Santa Rita do Riacho, Jaboticatubas (Cerrado); Rio de Janeiro state: Rio de Janeiro, Mangaratiba, Niterói, Maricá, Casimiro de Abreu, Cabo Frio; Carapebus (Atlantic Forest). Host plant: *Protium brasiliense* (Spreng.) Engl. (Burseraceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia 2001b, Rodrigues & Maia 2010b, Rodrigues et al. 2014, Gagné & Jaschhof 2017, Maia 2021.

175. Lopesia mataybae Garcia & Urso-Guimarães, 2018 (gallinducer). Distribution: Brazil: Mato Grosso: Chapada dos Guimarães (Cerrado). Host plant: *Matayba guianensis* Aubl. (Sapindaceae) (native to Brazil) (Amazon Forest, Cerrado, Atlantic Forest, and Pantanal). Ref.: Garcia & Urso-Guimarães2018.

176. Lopesia mimosae Maia, 2010 (gall-inducer). Distribution: Brazil: Pernambuco, Parnamirim (Caatinga). Host plant: Mimosa tenuiflora (Willd.) Poir. (= Mimosa hostilis Benth. (Mimosaceae) (native to Brazil) (Caatinga and Cerrado). Refs.: Maia et al. 2010b, Gagné & Jaschhof 2017.

177. Lopesia pernambucensis Maia, 2010 (gall-inducer). Distribution: Brazil: Pernambuco, Parnamirim (Caatinga), Garanhuns* (RB376469) (Caatinga); Bahia: Ibiassucê (Caatinga). Host plant: *Mimosa tenuiflora* (Willd.) Poir. (= *Mimosa hostilis* (Fabaceae) (native to Brazil) (Caatinga and Cerrado). Refs.: Maia et al. 2010b, Gagné & Jaschhof 2017, Brito et al. 2018.

178. Lopesia similis Maia, 2004 (gall-inducer). Distribution: Brazil: Pará: Serra do Cachimbo, Ponta da Pedra (Amazon Forest); Paraíba: João Pessoa (Atlantic Forest); Distrito Federal: Brasília (Cerrado); Alagoas: Maceió (Atlantic Forest); Sergipe: Itaporanga d'Ajuda (Atlantic Forest); Bahia: Conde, Porto Seguro (Atlantic Forest); Espírito Santo: Conceição da Barra (Atlantic Forest); Rio de Janeiro state: Quissamã, Macaé, Arraial do Cabo (Ilha do Cabo Frio), Macaé, São João da Barra (Atlantic Forest); Minas Gerais: Perdizes (Cerrado), Itamonte (Atlantic Forest), São Tomé das Letras (Atlantic Forest, Cerrado); Mato Grosso: Cocalino (Cerrado); Mato Grosso do Sul: Aquidauana (Cerrado). Host plant: *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Narahara et al. 2004, Maia 2013, Maia & Souza 2013, Maia 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Maia 2020, Maia 2021.

179. Lopesia simplex Maia, 2002 (gall-inducer). Distribution: Brazil: Minas Gerais: Belo Horizonte (Cerrado), Ouro Preto (Cerrado-Atlantic Forest transition); Rio de Janeiro state: Mangaratiba, Rio de Janeiro, Maricá, Carapebus, Macaé, Quissamã, Arraial do Cabo, São João da Barra (Atlantic Forest); Espírito Santo: Guarapari, Vila Velha (Atlantic Forest). Host plant: *Protium icicariba* (DC.) Marchand (Burseraceae) (endemic to Atlantic Forest). Refs.: Maia 2001b, Maia et al. 2002, Bregonci et al. 2010, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

180. Lopesia singularis Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Maricá, Rio de Janeiro (Grumari, Marambaia), Arraial do Cabo (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plant: *Pouteria venosa* (Mart.) Baehni (Sapotaceae) (native to Brazil) (Amazon and Atlantic Forest). Refs:. Monteiro et al. 1994, Maia 2001a, b, Oliveira & Maia 2005, Maia et al. 2008, Rodrigues & Maia 2014, Maia & Silva 2016, Gagné & Jaschhof 2017.

181. Lopesia spinosa Maia, 2004a (gall-inducer). Distribution: Brazil: Minas Gerais: Tiradentes and Delfinópolis (Cerrado), São Paulo state: Altinópolis and Jundiaí (Serra do Japi) (Atlantic Forest). Host plant: *Croton floribundus* Spreng (Euphorbiaceae) (native to Brazil) (Atlantic Forest). Refs.: Maia 2004a, Maia & Fernandes 2004, Gagné & Jaschhof 2017, Ribeiro et al. 2019, Urso-Guimarães 2019a.

182. Lopesia tibouchinae Maia, 2004a (gall-inducer). Distribution: Brazil: Minas Gerais: Tiradentes, Patrocínio (Cerrado); Rio de Janeiro state: Santa Maria Madalena (Atlantic Forest). Host plant: *Pleroma candolleanum* (Mart. ex DC.) Triana (= *Tibouchina candolleana* (Mart. ex DC.) Cogn.) (Melastomataceae) (endemic to Cerrado). Refs.: Maia 2004a, Gagné & Jaschhof 2017, Maia 2021.

183. Lopesia ubatubensis Garcia & Urso-Guimarães, 2018 (gallinducer). Distribution: Brazil: São Paulo state: Ubatuba (Atlantic Forest). Host plant: *Clidemia* sp. (Melastomataceae). Ref.: Garcia & Urso-Guimarães, 2018.

184. *Machaeriobia gemmae* Maia, 2016 (gall-inducer). Distribution: Rio de Janeiro state: Parque Nacional da Serra de Órgãos (Atlantic Forest). Host plant: *Machaerium macaense* C. V. Mendonça, A. M. G. Azevedo & H. C. Lima (Fabaceae) (endemic to Atlantic Forest). Refs.: Maia et al. 2016, Gagné & Jaschhof 2017.

185. *Machaeriobia machaerii* (Kieffer, 1913) (gall-inducer). Distribution: Brazil: São Paulo state: Ribeirão Preto (Cerrado); Santa Catarina: Tubarão (Atlantic Forest). Host plants: *Machaerium* sp. and *Machaerium hirtum* (Vell.) Stellfeld (Fabaceae) (native to Brazil) (Amazon, Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

186. *Macroporpa peruviana* Rübsaamen, 1915a (gall-inducer). Distribution: Brazil: Acre: Auristela (Amazon Forest). Host plant: undetermined Malpighiaceae. Refs.: Rübsaamen 1915, Möhn 1962, Gagné 1994, Gagné & Jaschhof 2017.

187. *Macroporpa ulei* Rübsaamen, 1915a (gall-inducer). Distribution: Brazil: Acre: São Francisco (Amazon Forest). Host plant: undetermined Lauraceae. Refs.: Rübsaamen 1915a, Möhn 1962, Gagné 1994, Gagné & Jaschhof 2017.

188. *Manilkaramyia notabilis* Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Carapebus, Maricá (Atlantic Forest); Espírito Santo: Guarapari (Atlantic Forest). Host plant: *Manilkara subsericea* (Mart.) Dubard (Sapotaceae) (endemic to Atlantic Forest). Refs.: Maia 2001a, b, Bregonci et al. 2010, Maia & Silva 2016, Gagné & Jaschhof 2017.

189. *Mayteniella distincta* Maia, 2001a (gall-inducer). Distribution: Brazil: Espírito Santo: Guarapari, Presidente Kennedy* (RB311268, RB311270) (Atlantic Forest); Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Marambaia, Grumari, Ilha das Folhas* R145329, Copacabana* R74076, Jacarepaguá* RB159930, Cagarras), Maricá, Carapebus, Macaé* (R209511, RB565802), Saquarema, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest). Host plant: *Monteverdia obtusifolia* (Mart.) Biral (= *Maytenus obtusifolia* Mart.) (Celastraceae) (endemic to Brazil) (Amazon and Atlantic Forests). Refs.: Monteiro et al. 1994, Maia 2001a, b, Oliveira & Maia 2005, Rodrigues et al. 2014, Maia & Carvalho-Fernandes 2016, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018, Maia 2020a.

190. *Megaulus sterculiae* Rübsaamen, 1915a (gall-inducer). Distribution: Bolívia and Brazil: Acre: São Francisco (Amazon Forest). Host plant: *Sterculia* sp. (Sterculiaceae). Refs.: Rübsaamen 1915a, Gagné 1994, Gagné & Jaschhof 2017. 191. Metasphondylia squamosa Tavares, 1918b (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: undetermined Malvaceae. Refs.: Tavares 1918b, Gagné 1994, Gagné & Jaschhof 2017.

192. *Meunieriella dalechampiae* Rübsaamen, 1905b (inquiline). Distribution: Brazil: Rio de Janeiro state: Palmeiras (Atlantic Forest). Host plant: *Dalechampia ficifolia* Lam. (Euphorbiaceae) (endemic to Brazil) (Cerrado and Atlantic Forest). Refs.: Rübsaamen 1905b, Gagné 1994, Gagné & Jaschhof 2017.

193. *Meunieriella insignis* (Tavares, 1922) (inquiline). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: *Protium heptaphyllum* (Aubl.) Marchand (Burseraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017.

194. *Meunieriella lantanae* (Tavares, 1918a) (inquiline). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Lantana* sp. (Verbenaceae). Refs.: Tavares 1918a, Gagné 1994, Gagné & Jaschhof 2017.

195. *Meunieriella spinosa* Urso-Guimarães, 2018b (inquiline). Distribution: Brazil: Minas Gerais: Delfinópolis (Cerrado). Host plant: *Inga edulis* Mart. (Fabaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Urso-Guimarães 2018b.

196. *Mikaniadiplosis annulipes* Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Rio de Janeiro (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Rio Grande do Sul: Santa Tereza (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (Asteraceae) (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* Refs.: Gagné et al. 2001, Maia et al. 2008, Maia & Oliveira 2010, Gagné & Jaschhof 2017, Goetz et al. 2018.

197. Mycodiplosis rubida (Felt, 1911c) (fungivorous). Distribution: Jamaica, St. Vincent, Hawaii, and Brazil (unstated locality). Host plants: Uromyces pisi (DC.) G.H. Otth (native to Brazil) (Rio Grade do Sul) and Puccinia sp. (Pucciniales). Refs.: Felt 1911c, Gagné 1994, Gagné & Jaschhof 2017.

198. Myrciamyia maricaensis Maia, 1996c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus, Cabo Frio, Arraial do Cabo (Atlantic Forest). Host plant: Myrcia ovata Cambess. (Myrtaceae) (endemic to Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 1996c, 2001b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

199. Myrciamyia pterandrae Maia & Flor, 2018 (gall-inducer). Distribution: Brazil: Minas Gerais: Quartel São João (Cerrado). Host plant: *Pterandra pyroidea* A. Juss. (Malpighiaceae) (endemic to Cerrado). Refs.: Maia et al. 2018.

200. Myrciariamyia admirabilis Maia, 2007 (gall-inducer). Distribution: Brazil: Minas Gerais: Tiradentes, São Tomé das Letras (Cerrado); São Paulo state: Ingaí (Cerrado); Goiás: Floresta Nacional de Silvânia (Cerrado), Hidrolândia (Cerrado). Host plant: *Erythroxylum suberosum* A. St.-Hil. (Erythroxylaceae) (native to Brazil) (Amazon Forest and Cerrado). Refs.: Maia & Fernandes 2007, Malves & Frieiro-Costa 2012, Maia 2013, Bergamini et al. 2017, Gagné & Jaschhof 2017, Silva et al. 2018.

201 *Myrciariamyia bivalva* Maia, 1995 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus (Atlantic Forest). Host plant: *Myrciaria floribunda* (H.West ex Willd.) (Myrtaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 1995, 2001b, Gagné & Jaschhof 2017. 202. Myrciariamyia fernandesi Maia, 2004a (gall-inducer). Distribution: Minas Gerais: Tiradentes (Cerrado). Host plant: Myrciaria tenella (DC.) O.Berg (Myrtaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2004a, Maia & Fernandes 2004, Gagné & Jaschhof 2017.

203. Neolasioptera cerei (Rübsaamen, 1905a) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Maricá, Cabo Frio, Arraial do Cabo (Ilha do Cabo Frio), São João da Barra (Atlantic Forest); Espírito Santo: Conceição da Barra (Atlantic Forest). Host plant: *Hylocereus setaceus* (Salm-Dyck) R. Bauer (= *Selenicereus setaceus* (Salm-Dyck) Berg (Cactaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Rübsaamen 1905a, Gagné 1994, Maia 1999b, Maia 2001b, Maia & Oliveira 2010, Maia & Souza 2013, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Maia 2020a.

204. Neolasioptera cupheae Gagné, 1998 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Silva Jardim (Atlantic Forest). Host plant: *Cuphea carthagenensis* (Jacq.) J.F.Macbr. (Lythraceae) (native to Brazil) (all Brazilian phytogeographic domains). Refs.: Gagné et al. 1998, Gagné & Jaschhof 2017.

205. Neolasioptera eugeniae Maia, 1993b (gall-inducer). Distribution: Brazil: Espírito Santo: Conceição da Barra, Guarapari (Atlantic Forest); Rio de Janeiro state: Angra dos Reis (Ilha Grande), Paraty, Mangaratiba, Parque Nacional do Itatiaia, Rio de Janeiro (Marambaia, Grumari), Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo (Ilha do Cabo Frio), São João da Barra (Atlantic Forest); Minas Gerais: Itamonte (Atlantic Forest). Host plant: *Eugenia uniflora* L. (Myrtaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pampa). Refs.: Maia 1993b, Monteiro et al. 1994, Oliveira & Maia 2005, Maia & Oliveira 2010, Silva & Rodrigues 2011, Carvalho-Fernandes & Maia 2011, Maia & Souza 2013, Maia 2014, Rodrigues & Maia 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Maia & Mascarenhas 2017, Gagné & Jaschhof 2017, Maia 2020a.

206. *Neolasioptera fariae* (Tavares, 1922) (inquiline). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: unknown. Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017.

207. Neolasioptera ingae Möhn, 1964b (gall-inducer). Distribution: El Salvador and Brazil: Rio de Janeiro state: Rio de Janeiro (Marambaia) (Atlantic Forest). Host plants: *Inga vera* Will. (*= Inga spuria* Humb. & Bonpl. ex Willd.) (native to Brazil) (Amazon Forest, Cerrado, Atlantic Forest, and Pantanal) and *Inga punctata* Will. (*= Inga leptoloba* Schltdl. (Fabaceae) (native to Brazil) (Amazon Forest). Refs.: Möhn 1964b, Gagné 1994, Gagné & Jaschhof 2017, Maia & Silva 2016.

208. Neolasioptera lantanae (Tavares, 1922) (gall-inducer). Distribution: Brazil: Bahia: Salvador (Atlantic Forest). Host plant: *Lantana* sp. (Verbenaceae). Refs.: Tavares 1922, Gagné 1994, Gagné & Jaschhof 2017.

209. *Neolasioptera pantaneira* Maia, 2017 (gall-inducer). Distribution: Brazil: Mato Grosso do Sul: Corumbá (Pantanal). Host plant: *Aeschynomene denticulata* Rudd. (Fabaceae) (native to Brazil) (all Brazilian phytogeografic domains). Ref.: Maia et al. 2017.

210. *Neolasioptera ramicola* Maia, 2009 (gall-inducer). Distribution: Brazil: Rio Grande do Sul: Bento Gonçalves (Atlantic Forest). Host plant: *Physalis angulata* L. (Solanaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pantanal). Refs.: Maia Maia et al. 2009, Gagné & Jaschhof 2017.

211. Neolasioptera urvilleae (Tavares, 1909) (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: Urvillea uniloba Radlk. (Sapindaceae) (native to Brazil) (Atlantic Forest). Refs.: Tavares 1909, Gagné 1994, Gagné & Jaschhof 2017.

212. Neomitranthella robusta Maia, 1996c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Carapebus (Atlantic Forest). Host plant: *Neomitranthes obscura* (DC.) N. Silveira (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1996c, 2001b, Gagné & Jaschhof 2017.

213. *Novocalmonia fici* Ozdikmen, 2009 (gall-inducer). Distribution: Brazil: Bahia: Itaparica and Santo Antonio da Barra (Atlantic Forest). Host plant: *Ficus* sp. (Moraceae). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

214. Novocalmonia urostigmatis (Tavares, 1917a) (gallinducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plant: *Ficus* sp. (Moraceae). Refs.: Tavares 1917a, Gagné & Jaschhof 2017.

215. *Ouradiplosis aurata* Felt, 1915b (gall-inducer). Distribution: Brazil: Pará: Igarapé-Açú (Amazon Forest). Host plant: unknown. Refs.: Felt 1915b, Gagné & Jaschhof 2017.

216. Parametasphondylia piperis Maia & Santos 2007 (gallinducer). Distribution: Brazil: Minas Gerais: Tiradentes (Cerrado). Host plant: *Piper* sp. (Piperaceae). Refs.: Maia & Fernandes 2004, Maia & Santos 2007, Gagné & Jaschhof 2017.

217. Parazalepidota clusiae Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Grumari), São Gonçalo, Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Quissamã, Macaé, Petrópolis, Mauá (Atlantic Forest). Host plant: *Clusia fluminensis* Planch. & Triana (Clusiaceae) (endemic to Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001a, b, Oliveira & Maia 2005, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Maia 2020b.

218. Parkiamyia paraensis Maia, 2006 (gall-inducer). Distribution: Brazil: Pará: Oriximiná (Amazon Forest). Host plant: Parkia pendula (Willd.) Benth. ex Walp. (Fabaceae) (native to Brazil) (Amazon and Atlantic Forests). Refs.: Maia & Fernandes 2006, Gagné & Jaschhof 2017.

219. Paulliniamyia ampla Maia, 2001a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Carapebus, São João da Barra, São Francisco de Itabapoana (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plants: *Paullinia weinmanniifolia* Mart. (endemic to Atlantic Forest) and *Matayba guianensis* Aubl. (Sapindaceae) (native to Brazil) (Amazon Forest, Cerrado, Atlantic Forest, and Pantanal). Refs.: Maia 2001a, b, Maia et al. 2008, Maia & Carvalho-Fernandes 2016, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

220. Perasphondylia mikaniae Gagné, 2001 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Paraty, Parque Nacional do Itatiaia, Silva Jardim (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest). Host plants: *Mikania glomerata* Spreng. (native to Brazil) (Cerrado and Atlantic Forest) and *Mikania cf biformis* (Asteraceae). Refs.: Gagné et al. 2001, Maia et al. 2008, Carvalho-Fernandes & Maia 2011, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017.

221. Perasphondylia reticulata Möhn, 1960 (gall-inducer). Distribution: Mexico, El Salvador, Trinidad, Bolivia, and Brazil: Pará (Amazon Forest). Host plants: Chromolaena odorata (L.) R. M. King & H. Rob. (native to Brazil) (all phytogeographic domains) and Eupatorium sp. (Asteraceae). Refs.: Möhn 1960, Gagné 1994, Gagné & Jaschhof 2017.

222. *Pisphondylia brasiliensis* Couri & Maia, 1992 (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga; Rio de Janeiro state: Maricá, Arraial do Cabo (Ilha do Cabo Frio), São João da Barra (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest); Minas Gerais: Brumadinho (Atlantic Forest); Bahia: Porto Seguro-Trancoso (Atlantic Forest); Santa Catarina: Babitonga, São Francisco do Sul (Atlantic Forest); Rio Grande do Sul: Porto Alegre (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz. (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Couri & Maia 1992, Maia 2001b, 2014, Maia et al. 2008, Maia 2010, Maia et al. 2010, Maia & Souza 2013, Arriola et al. 2015, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018.

223. Primadiplosis microgramma Maia, 2011 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest). Host plant: *Microgramma vacciniifolia* (Langsd. & Fisch.) Copel. (Polypodiaceae) (native to Brazil) (Cerrado and Atlantic Forest). Refs.: Maia & Santos 2011, Gagné & Jaschhof 2017.

224. *Proasphondylia brasiliensis* Felt, 1915c (gall-inducer). Distribution: Brazil: Pernambuco: Bonito (Atlantic Forest). Host plant: unknown. Refs.: Felt 1915c, Gagné & Jaschhof 2017.

225. Proasphondylia formosa Maia, 1994 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz. (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 1994, Gagné & Jaschhof 2017, Melo-Júnior et al. 2018.

226. Proasphondylia guapirae Maia, 1994 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Angra dos Reis (Ilha Grande), Mangaratiba, Rio de Janeiro (Marambaia), Maricá, Carapebus, Arraial do Cabo (Ilha do Cabo Frio) (Atlantic Forest); Espírito Santo: Santa Teresa (Atlantic Forest); São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: Babitonga (Atlantic Forest). Host plant: *Guapira opposita* (Vell.) Reitz. (Nyctaginaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 1994, 1999, 2001b, Maia et al. 2008, Maia & Oliveira 2010, Maia & Souza 2013, Maia 2014, Rodrigues & Maia 2014, Arriola et al. 2015, Maia & Silva 2016, Melo-Júnior et al. 2018.

227. Procontarinia mangiferae (Felt, 1911d) (gall-inducer). Distribution: India, China, Reunion, Iran, Guadeloupe, St. Vincent, Trinidad, and Brazil: Bahia: Salvador (Atlantic Forest). Host plant: *Mangifera indica* L. (Anacardiaceae) (exotic) (cultivated plant in all Brazilian domains). Refs.: Felt 1911d, Gagné & Jaschhof 2017.

228. Prodiplosis floricola Felt, 1907b (free living phytophagous). Distribution: USA, Colombia, and Brazil: São Paulo state (unstated locality). Host plants: Spiraea salicifolia L. (Rosaceae) (native to the temperate Northern Hemisphere), Clematis sp. (Ranunculaceae), Caryocar brasiliense Cambess. (Caryocaraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest), and Citrus sp. (Rutaceae). Refs.: Felt 1907b, Gagné 1994, Gagné & Jaschhof 2017.

229. *Rhoasphondylia friburgensis* (Tavares, 1917a) (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Nova Friburgo (Atlantic Forest). Host plants: *Baccharis lateralis* Baker (= *B. schultzi* Baker) (endemic to Brazil) (Atlantic Forest and Cerrado), *B. dracunculifolia* DC. (native to Brazil) (Cerrado, Atlantic Forest, and Pampa), and *B. trinervis* Pers. (Asteraceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, Atlantic Forest, and Pampa). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

230. *Rochadiplosis tibouchinae* Tavares, 1917b (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Petrópolis, Tijuca, and Nova Friburgo (Atlantic Forest); São Paulo state: Altinópolis (Cerrado). Host plant: *Tibouchina* sp. (Melastomataceae). Refs.: Tavares 1917b, Gagné 1994, Gagné & Jaschhof 2017, Ribeiro et al. 2019.

231. Schismatodiplosis lantanae (Rübsaamen, 1908a) (gall-inducer). Distribution: Mexico, Veracruz, Tabasco, Quintana Roo, Trinidad, and Brazil: Pará: Oriximiná (Amazon Forest); Rondônia: Campo Novo de Rondônia (Amazon Forest); Minas Gerais: Aimorés (Atlantic Forest), Vale do Rio Doce (Atlantic Forest, Cerrado); Rio de Janeiro state: Parque Nacional do Itatiaia, Valença, Mangaratiba, Casimiro de Abreu, Reserva Biológica União, Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo (Ilha do Cabo Frio), Rio das Ostras, São João da Barra (Atlantic Forest); Santa Catarina: Tubarão (Atlantic Forest). Host plants: Lantana camara L. (naturalized) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest), Lantana hispida (no records in Brazil) and L. urticifolia (Verbenaceae) (no records in Brazil). Refs.: Rübsaamen 1908a, Gagné 1994, Maia 2001b, Maia & Souza 2013, Proença & Maia 2014, Rodrigues & Maia 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017, Maia & Mascarenhas 2017, Maia & Siqueira 2020.

232. Schizomyia barreirensis Santos, Maia & Calado, 2019 (gallinducer). Distribution: Brazil: Bahia: Barreiras (Cerrado). Host plant: *Bauhinia cupulata* Benth. (Fabaceae) (native to Brazil) (Amazon Forest, Caatinga, and Cerrado). Refs.: Santos et al. 2019.

233. Schizomyia macrocapillata Maia, 2005 (gall-inducer). Distribution: Brazil: Goiás*: Hidrolândia (RB308777), Monte Alegre (RB357341), Cavalcante (RB 466730) (Cerrado); Mato Grosso*: Selvíria (RB295665) (Cerrado); Bahia: Barreiras (Cerrado) and Caetité (Caatinga); Minas Gerais: Três Marias (Cerrado). Host plant: *Bauhinia brevipes* Vogel (Fabaceae) (native to Brazil) (Amazon Forest, Cerrado, and Atlantic Forest). Refs.: Maia & Fernandes 2005b, Costa et al. 2014, Gagné & Jaschhof 2017, Lima & Calado 2018.

234. Schizomyia manihoti Tavares, 1925 (gall-inducer). Distribution: Colombia and Brazil: Ceará (unstated locality). Host plant: *Manihot esculenta* Crantz (= *Manihot utilissima* Pohl.) (Euphorbiaceae) (native to Brazil) (Amazon Forest and Cerrado). Refs.: Tavares 1925, Gagné 1994, Gagné & Jaschhof 2017.

235. Schizomyia maricaensis Sousa & Maia, 2007 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest); São Paulo state: Ribeirão Preto (Cerrado). Host plant: *Tetrapterys phlomoides* (Spreng.) Nied. (Malpighiaceae) (native to Brazil) (Cerrado, Atlantic Forest, and Pantanal). Refs.: Sousa & Maia 2007, Gagné & Jaschhof 2017, Urso-Guimarães 2019b.

236. Schizomyia mimosae Tavares, 1925 (gall-inducer). Distribution: Brazil: Ceará (unstated locality). Host plant: *Mimosa caesalpiniifolia* Benth. (Fabaceae) (endemic to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Tavares 1925, Gagné 1994, Gagné & Jaschhof 2017.

237. Schizomyia santosi Maia & Araújo, 2009 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Marambaia) and Maricá (Atlantic Forest). Host plant: Jacquemontia holosericea (Weinm.) O' Donell (Convolvulaceae) (native to Brazil) (all Brazilian phytogeographic domains). Refs.: Maia 2001b, Maia & Araújo 2009, Maia & Silva 2016. Gagné & Jaschhof 2017.

238. Schizomyia spherica Maia & Oliveira, 2007 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Carapebus, Arraial do Cabo (Atlantic Forest). Host plant: *Microstachys corniculata* (Vahl) Griseb. (= *Sebastiania glandulosa* (Mart.) Pax.) (Euphorbiaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001b, Maia & Oliveira 2007, Gagné & Jaschhof 2017.

239. Schizomyia tuiuiu Urso-Guimarães & Amorim, 2002 (gallinducer). Distribution: Mato Grosso: Cuiabá (Cerrado); São Paulo state: Ribeirão Preto (Cerrado). Host plant: *Bauhinia holophylla* (Fabaceae) (as *B. rufa* (Bong.) Steud. in Urso-Guimarães & Amorim, 2002) (native to Brazil) (endemic to Cerrado). Refs.: Urso-Guimarães & Amorim 2002, Gagné & Jaschhof 2017, Urso-Guimarães 2019b.

240. Smilasioptera candelariae Möhn, 1975 (gall-inducer). Distribution: El Salvador, Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Grumari, Marambaia), Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, Carapebus, São João da Barra (Atlantic Forest). Host plants: Smilax spinosa Mill. (= Smilax mexicana Griseb. ex Kunth.) (no records in Brazil) and Smilax rufescens Griseb. (Smilacaceae) (endemic to Brazil) (Amazon Forest, Cerrado, and Atlantic Forest). Refs.: Möhn 1975, Gagné 1994, Maia 2001b, Oliveira & Maia 2005, Rodrigues & Maia 2014, Carvalho-Fernandes et al. 2016, Maia & Silva 2016, Gagné & Jaschhof 2017.

241. Sphaeramyia flava Maia, 2007a (gall-inducer). Distribution: Brazil: São Paulo state: Bertioga (Atlantic Forest); Santa Catarina: São Francisco do Sul (Atlantic Forest). Host plant: *Guarea macrophylla* Vahl (Meliaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Maia 2007a, Maia et al. 2008, Melo-Júnior et al. 2018.

242. *Sphaerodiplosis dubia* Rübsaamen, 1915 (gall-inducer). Distribution: Brazil (locality unstated). Host plant: unknown. Refs.: Rübsaamen 1915, Gagné 1994, Gagné & Jaschhof 2017.

243. *Stephomyia clavata* (Tavares, 1920b) (gall-inducer). Distribution: Brazil: Bahia: Madre de Deus (Atlantic Forest). Host plant: undetermined Myrtaceae. Refs.: Tavares 1920b, Gagné 1994, Gagné & Jaschhof 2017.

Comments: Maia 2001b and Silva & Maia 2014 recorded Stephomyia cf. clavata on Eugenia hiemalis Cambess. (=Eugenia multiflora Cambess.) (native to Brazil) (Cerrado, Atlantic Forest) in the Rio de Janeiro state: Rio de Janeiro (Marambaia) and Carapebus.

244. Stephomyia epeugeniae Gagné, 1994 (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Botanical Garden) (Atlantic Forest); Minas Gerais: Belo Horizonte (Cerrado); Host plants: Eugenia sp. and Eugenia punicifolia (Kunth.) DC. (= Eugenia ovalifolia Cambess.) (Myrtaceae) (endemic to Brazil) (Amazon Forest, Atlantic Forest, Caatinga, and Cerrado). Refs.: Tavares 1916, Möhn 1962, Gagné 1994, Fernandes et al. 1988, Gagné & Jaschhof 2017.

245. *Stephomyia espiralis* Maia, 1993c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Maricá, Araruama, Arraial do Cabo, Cabo Frio (Atlantic Forest). Host plant: *Eugenia copacabanensis* Kiaersk. (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1993c, 1999a, 2001b, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

246. *Stephomyia mina* Maia, 1993c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Araruama, Carapebus, Arraial do Cabo (Atlantic Forest). Host plant: *Neomitranthes obscura* (DC.) N. Silveira (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1993c, 2001b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

247. Stephomyia rotundifoliorum Maia, 1993c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá (Atlantic Forest); Espírito Santo: Conceição da Barra (Atlantic Forest). Host plant: *Eugenia astringens* Cambess. (*= Eugenia rotundifolia* Casar) (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1993c, 2001b, Gagné & Jaschhof 2017, Maia 2020a. 248. *Stephomyia tetralobae* Maia, 1993c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Maricá, Arraial do Cabo (Atlantic Forest). Host plant: *Eugenia capacabanensis* Kiaersk. (Myrtaceae) (endemic to Atlantic Forest). Refs.: Maia 1993c, 2001b, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

249. *Stomatosema camilae* Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Corumbá (Pantanal). Refs.: Carmo-Neto et al. 2019.

250. *Stomatosema paratudo* Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Corumbá (Pantanal). Refs.: Carmo-Neto et al. 2019.

251. Stomatosema pantaneirum Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Corumbá (Pantanal), Bodoquena (Cerrado). Refs.: Carmo-Neto et al. 2019.

252. *Stomatosema sisbiota* Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Corumbá (Pantanal), Bodoquena (Cerrado), Aquidauana (Cerrado). Refs.: Carmo-Neto et al. 2019.

253. *Stomatosema terena* Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Aquidauana (Cerrado). Refs.: Carmo-Neto et al. 2019.

254. *Stomatosema terere* Carmo-Neto, Lamas & Urso-Guimarães, 2019 (fungivorous). Distribution: Brazil: Mato Grosso do Sul: Aquidauana (Cerrado). Refs.: Carmo-Neto et al. 2019.

255. *Styraxdiplosis caetitensis* Tavares, 1915 (gall-inducer). Distribution: Brazil: Bahia: Caetité (Atlantic Forest, Caatinga). Host plant: *Styrax* sp. (Styracaceae). Refs.: Tavares 1915, Gagné 1994, Gagné & Jaschhof 2017.

256. *Styraxdiplosis cearensis* Tavares, 1925 (gall-inducer). Distribution: Brazil: Ceará (unstated locality) (Atlantic Forest). Host plant: *Croton hemiargyreus* Müll. Arg. (Euphorbiaceae) (endemic to Atlantic Forest). Refs.: Tavares 1925, Gagné 1994, Gagné & Jaschhof 2017.

257. Termitomastus leptoproctus Silvestri, 1901 (fungivorous). Distribution: Argentina and Brazil: Mato Grosso: Cuiabá (Cerrado). Refs.: Silvestri 1901, Gagné 1994, Gagné & Jaschhof 2017.

258. Trotteria quadridentata Maia, 2001c (inquiline). Distribution: Brazil: Rio de Janeiro state: Maricá, Arraial do Cabo (Atlantic Forest). Host plant: Pouteria caimito (Ruiz & Pav.) Radlk (=Pouteria caimito var. laurifolia (Gomes) Baehni (Sapotaceae) (native to Brazil) (Amazon Forest, Cerrado, and Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001b, c, Gagné & Jaschhof 2017.

259. Uleella dalbergiae Rübsaamen, 1908a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Jacarepaguá) (Atlantic Forest). Host plant: *Dalbergia* sp. (Fabaceae). Refs.: Rübsaamen, 1908a, Gagné 1994, Gagné & Jaschhof 2017.

260. Uleia clusiae Rübsaamen, 1905a (gall-inducer). Distribution: Brazil: Amazonas: Santa Clara and Bonfim on Juruá River (Amazon Forest). Host plant: *Clusia* sp. (Clusiaceae). Refs.: Rübsaamen 1908a, Gagné 1994, Gagné & Jaschhof 2017.

261. Youngomyia matogrossensis Proença & Maia, 2019 (gallinducer). Distribution: Brazil: Mato Grosso: Chapada dos Guimarães (Cerrado); São Paulo state: Luiz Antônio and Santa Rita do Passa Quatro (Cerrado). Host plant: *Pouteria torta* (Mart.) Radlk. (Sapotaceae) (native to Brazil) (Amazon Forest, Caatinga, Cerrado, and Atlantic Forest). Refs.: Urso-Guimarães & Scareli-Santos 2006, Saito & Urso-Guimarães 2012, Urso-Guimarães et al. 2016, Proença & Maia 2019.

262. Youngomyia pouteriae Maia 2001c (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Mangaratiba, Rio de Janeiro (Grumari), Maricá, Saquarema, Araruama, Arraial do Cabo, Cabo Frio, São João da Barra (Atlantic Forest). Host plant: *Pouteria caimito* (Ruiz & Pav.) Radlk (*=Pouteria caimito* var. *laurifolia*) (Sapotaceae) (native to Brazil) (Amazon Forest, Cerrado, and Atlantic Forest). Refs.: Monteiro et al. 1994, Maia 2001b, c, Rodrigues & Maia 2014, Carvalho-Fernandes et al. 2016, Gagné & Jaschhof 2017.

263. Zalepidota ituensis (Tavares, 1917a) (gall-inducer). Distribution: Brazil: São Paulo state: Salto de Itú (Atlantic Forest). Host plant: *Porophyllum* sp. (Asteraceae). Refs.: Tavares 1917a, Gagné 1994, Gagné & Jaschhof 2017.

264. Zalepidota piperis Rübsaamen, 1908a (gall-inducer). Distribution: Brazil: Rio de Janeiro state: Rio de Janeiro (Tijuca) (Atlantic Forest). Host plant: *Piper* sp. (Piperaceae). Refs.: Rübsaamen 1908a, Gagné 1994, Gagné & Jaschhof 2017.

265. Zalepidota tavaresi (Kieffer, 1913) (gall-inducer). Distribution: Brazil: Rio Grande do Sul: São Leopoldo (Pampa). Host plant: *Piper* sp. (Piperaceae). Refs.: Kieffer 1913, Gagné 1994, Gagné & Jaschhof 2017.

In order to provide the state of the art of the Cecidomyiidae fauna in each Brazilian phytogeographic domain, data are presented separately.

AMAZON FOREST

Twenty-nine species of Cecidomyiidae of 19 genera have been reported in the Amazon Forest, being 28 gall-inducers and one fungivorous species. Among the former, 23 are monophagous, while five are oligophagous species: *Asphondylia tournefortiae* Rübsaamen, 1915, *Clinodiplosis eupatorii* (Felt, 1911a), *Iatrophobia brasiliensis* (Rübsaamen, 1908a), *Perasphondylia reticulata* Möhn, 1960, and *Schismatodiplosis lantanae* (Rübsaamen, 1908a).

Gall-inducers are associated with 17 plant families, 20 genera and 12 determined species. Callophylaceae is the plant families with the greatest richness of gall midge species (five), followed by Asteraceae with three, and Asteraceae and Burseraceae, both with two. All other families (13) shelter a single gall midge species (Table 5). Nine gall midge species are associated with host plants identified only in genus: *Cecropia* sp., *Clusia* sp., *Coussapoa* sp., *Mikania* sp., *Neea* sp., *Serjania* sp., *Solanum* sp., and *Sterculia* sp., and two gall midge species were obtained from host plants identified only in family, one from Malpighiaceae and the other from Lauraceae. Two cecidomyiids, *Lopesia maricaensis* and *Lopesia aldinae* induce galls on Brazilian endemic plants, *Protium brasiliense* (Burseraceae) and *Aldina heterophylla* (Fabaceae), being the last endemic to Amazon Forest (Table 4). The remainder plants are native to Brazil, except *Lantana camara*, a naturalized species.

The Amazonan fauna comprises 19 genera of Cecidomyiidae, 14 represented by one species, three (*Asphondylia*, *Bruggmannia* and *Clinodiplosis*) by two species, and one, *Lopesia*, by five species, being the most speciose genera (Table 3).

Nineteen gall midge species are known only from the Amazon Forest, while eight species occur in other domains. Twenty-two species have been recorded only in Brazil, while seven occur in other countries too: *Clinodiplosis eupatorii*, *Iatrophobia brasiliensis, Perasphondylia reticulata, Schismatodiplosis lantanae, Lopesia conspicua, Lopesia elliptica*, and *Lopesia linearis*.

List of Cecidomyiidae (Diptera) with occurrence in the Amazon Forest (Brazil)

- 1. Alexomyia ciliata Felt, 1921
- 2. Alycaulus mikaniae Rübsaamen, 1915
- 3. Asphondylia fructicola Maia, 2009
- 4. Asphondylia tournefortiae Rübsaamen, 1915

- 5. Brugmannia depressa (Kieffer, 1913)
- 6. Brugmannia longiseta (Kieffer, 1913)
- 7. Clinodiplosis cecropiae Proença & Maia, 2020
- 8. Clinodiplosis eupatorii (Felt, 1911)
- 9. Contarinia gemmae Maia, 2003
- 10. Dactylodiplosis heisteriae Rübsaamen, 1915
- 11. Frauenfeldiella coussapoae Rübsaamen, 1905
- 12. Haplopalpus serjaneae Rübsaamen, 1915
- 13. Haplusia braziliensis (Felt, 1915)
- 14. Iatrophobia brasiliensis (Rübsaamen, 1908)
- 15. Lopesia aldinae Fernandes & Maia, 2010
- 16. Lopesia caulinaris Maia, 2003
- 17. Lopesia conspicua Maia, 2003
- 18. Lopesia elliptica Maia, 2003
- 19. Lopesia linearis Maia, 2003
- 20. Lopesia maricaensis Rodrigues & Maia, 2010
- 21. Lopesia similis Maia, 2004
- 22. Macroporpa peruviana Rübsaamen, 1915
- 23. Macroporpa ulei Rübsaamen, 1915
- 24. Megaulus sterculiae Rübsaamen, 1915
- 25. Ouradiplosis aurata Felt, 1915
- 26. Parkiamyia paraensis Maia, 2006
- 27. Perasphondylia reticulata Möhn, 1960
- 28. Schismatodiplosis lantanae (Rübsaamen, 1908)
- 29. Uleia clusiae Rübsaamen, 1905

ATLANTIC FOREST

A total of 183 species of Cecidomyiidae of 71 genera have been recorded in the Atlantic Forest, 162 of them are gall-inducers, ten are predators (*Diadiplosis abacaxi, D. bellingeri, D. floridana, D. jamboi, D. martinensis, D. multifila. D. vaupedis, Lestodiplosis braziliensis, L. floricola,* and *L. maricaensis*), eight are probably kleptoparasites (*Clinodiplosis maricaensis, Contarinia ubiquita, Dialeria styracis,* Meunieriella dalechampiae, *M. insignis, M. lantanae, N. fariae,* and *Trotteria quadridentata*), two are free-living species (*Clinodiplosis florícola* and *Lopesia davillae*), and a single one has fungivorous larvae (*Dichodiplosis triangularis*).

Sixteen gall-inducing species are oligophagous, while the others are monophagous. Phytophagous species have been recorded on 38 plant families, 73 genera and 82 determined species. Myrtaceae, Asteraceae, Nyctaginaceae, and Fabaceae are the superhost families, which together shelter 69 of the gall midge species (22, 19, 14, and 14, respectively). This value corresponds to about 39% of the gall-inducing species richness (Table 5). Thirty-eight gall midge species, when described, were recorded on 21 host plants identified only in genus. Later, two of them, *Asphondylia stachytarpheta* Barnes, 1932 and *Machaeriobia machaerii* (Kieffer, 1913) were obtained from identified plant species.

Eleven gall midge species have been recorded on five host plants identified only in family (Asteraceae: 2, Fabaceae: 3, Malvaceae: 1, Myrtaceae: 2, and Rubiaceae: 3). Fourty-eight gall midge species induce galls on endemic plants to Brazil, 36 of them on endemic plants to the Atlantic Forest (Table 4). The remainders are associated with native plants, except *Schismatodiplosis lantanae*, *Asphondylia sennae*, and *Clinodiplosis melissae*, inducers of galls on *Lantana camara* (a naturalized plant), *Senna bicapsularis* and *Melissa officinalis*, both exotic. The most speciose genera in the Atlantic Forest are *Lopesia* (with 18 species), *Asphondylia* (with 15), *Clinodiplosis* (with14), and *Bruggmannia* (with 11). They together shelter about 32% of the species richness. The other genera comprise from nine to one species, being the majority (44 genera) represented by a single species (Table 3).

List of Cecidomyiidae (Diptera) with occurrence in the Atlantic Forest (Brazil)

- 1. Alycaulus globulus Gagné, 2001
- 2. Alycaulus trilobatus Möhn, 1964
- 3. Anadiplosis caetetensis Tavares, 1920
- 4. Anadiplosis procera Tavares, 1920
- 5. Anadiplosis pulchra Tavares, 1916
- 6. Anadiplosis venusta Tavares, 1916
- 7. Anasphondylia myrtacea Tavares, 1920
- 8. Andirodiplosis bahiensis Tavares, 1920
- 9. Anisodiplosis waltheriae Maia, 2005
- 10. Apodiplosis praecox Tavares, 1922
- 11. Arrabiadaeamyia serrata Maia, 2001
- 12. Asphondylia bahiensis Tavares, 1917
- 13. Asphondylia borreriae Rübsaamen, 1905
- 14. Asphondylia communis Maia & Couri, 1992
- 15. Asphondylia cordiae Möhn, 1959
- 16. Asphondylia glomeratae Gagné, 2001
- 17. Asphondylia maricensis Maia & Couri, 1992
- 18. Asphondylia moehni Skuhravá, 1989
- 19. Asphondylia parva Tavares, 1917
- 20. Asphondylia peploniae Maia, 2001
- 21. Asphondylia rochae Tavares, 1918
- 22. Asphondylia sennae Maia & Couri, 1992
- 23. Asphondylia serrata Maia, 2004
- 24. Asphondylia stachytarpheta Barnes, 1932
- 25. Asphondylia struthanthi Rübsaamen, 1915
- 26. Asphondylia ulei Rübsaamen, 1908
- 27. Asteromyia modesta (Felt, 1907)
- 28. Autodiplosis parva (Tavares, 1916)
- 29. Baccharomyia magna Maia, 2012
- 30. Baccharomyia ramosina Tavares, 1917
- 31. Bruggmannia acaudata Maia, 2004
- 32. Brugmannia depressa (Kieffer, 1913)
- 33. Bruggmannia elongata Maia & Couri, 1993
- 34. Bruggmannia globulifex (Kieffer, 1913)
- 35. Bruggmannia lignicola (Kieffer, 1913)
- 36. Bruggmannia longicauda (Kieffer, 1913)
- 37. Bruggmannia micrura (Kieffer, 1913)
- 38. Bruggmannia monteiroi Maia & Couri, 1993
- 39. Bruggmannia neeana (Kieffer, 1913)
- 40. Bruggmannia robusta Maia & Couri, 1993
- 41. Bruggmannia ruebsaameni (Kieffer, 1913)
- 42. Bruggmanniella byrsonimae (Maia & Couri, 1992)
- 43. Bruggmanniella doliocarpi Maia, 2010
- 44. Bruggmanniella ingae Urso-Guimarães & Amorim, 2005
- 45. Bruggmanniella maytenuse (Maia & Couri, 1992)
- 46. Bruggmanniella miconia Garcia, Lamas and Urso-Guimarães, 2020
- 47. Bruggmanniella notatae Rodrigues & Maia, 2020

- 48. Bruggmanniella oblita Tavares, 1920
- 49. Bruggmanniella sideroxyli Rodrigues & Maia, 2020
- 50. Burseramyia braziliensis Maia & Fonseca, 2012
- 51. Cleitodiplosis graminis (Tavares, 1916)
- 52. Clinodiplosis bahiensis (Tavares, 1917)
- 53. Clinodiplosis cearensis (Tavares, 1917)
- 54. Clinodiplosis chlorophorae Rübsaamen, 1905
- 55. Clinodiplosis conica Oliveira & Maia, 2008
- 56. Clinodiplosis costai Maia, 2005
- 57. Clinodiplosis diodiae Maia, 2001
- 58. Clinodiplosis floricola Novo-Guedes & Maia, 2008
- 59. Clinodiplosis iheringi (Tavares, 1925)
- 60. Clinodiplosis marcetiae (Tavares, 1917)
- 61. Clinodiplosis maricaensis Fernandes & Maia, 2011
- 62. Clinodiplosis melissae Maia, 1993
- 63. Clinodiplosis profusa Maia, 2001
- 64. Clinodiplosis pulchra (Tavares, 1917)
- 65. Clinodiplosis rubiae (Tavares, 1918)
- 66. Clusiamyia granulosa Maia, 2001
- 67. Clusiamyia nitida Maia, 1997
- 68. Compsodiplosis itaparicana Tavares, 1922
- 69. Contarinia gemmae Maia, 2003
- 70. Contarinia ubiquita Gagné, 2001
- 71. Contodiplosis friburgensis (Tavares, 1915)
- 72. Contodiplosis humilis (Tavares, 1915)
- 73. Contodiplosis tristis (Tavares, 1915)
- 74. Cordiamyia globosa Maia, 1996
- 75. Costadiplosis maricaensis Viceconte & Maia, 2009
- 76. Couridiplosis vena Maia, 2004
- 77. Dactylodiplosis heptaphylli Maia, 2004
- 78. Dactylodiplosis icicaribae Maia, 2002
- 79. Dactylodiplosis petibaurum Maia, 2021
- 80. Dasineura byrsonimae Maia, 2010
- 81. Dasineura copacabanensis Maia, 1993
- 82. Dasineura couepiae Maia, 2001
- 83. Dasineura gigantea Angelo & Maia, 1999
- 84. Dasineura globosa Maia, 1996
- 85. Dasineura marginalis Maia, 2005
- 86. Dasineura myrciariae Maia, 1996
- 87. Dasineura ovalifoliae Fernandes & Maia, 2011
- 88. Dasineura tavaresi Maia, 1996
- 89. Diadiplosis abacaxii Culik & Ventura, 2013
- 90. Diadiplosis bellingeri Culik & Ventura, 2012
- 91. Diadiplosis floridana (Felt, 1915)
- 92. Diadiplosis jamboi Culik & Ventura, 2013
- 93. Diadiplosis martinsensis Culik & Ventura, 2013
- 94. Diadiplosis multifila (Felt, 1907)
- 95. Diadiplosis vaupedis (Harris, 1968)
- 96. Dialeria styracis Tavares, 1918
- 97. Dichodiplosis triangularis (Felt, 1908)
- 98. Epihormomyia miconiae Maia, 2001
- 99. Eugeniamyia dispar Maia, Mendonça & Romanowski, 1997
- 100. Eugeniamyia triangularis Maia & Nava, 2011
- 101. Fernandesia meridionalis Rodrigues & Maia, 2013
- 102. Frauenfeldiella coussapoae Rübsaamen, 1905
- 103. Geraldesia eupatorii Tavares, 1917

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- 104. Gnesiodiplosis itaparicae Tavares, 1917
- 105. Guareamyia purpura Maia, 2007
- 106. Houardodiplosis rochae Tavares, 1925
- 107. Iatrophobia brasiliensis (Rübsaamen, 1908)
- 108. Jorgenseniella eugeniae Maia, 2005
- 109. Lestodiplosis braziliensis (Tavares, 1920)
- 110. Lestodiplosis floricola (Rodrigues & Maia, 2010)
- 111. Lestodiplosis maricaensis Santos & Maia, 2009
- 112. Liodiplosis conica Gagné, 2001
- 113. Liodiplosis cylindrica Gagné, 2001
- 114. Liodiplosis spherica Gagné, 2001
- 115. Lopesia brasiliensis Rübsaamen, 1908
- 116. Lopesia caulinaris Maia, 2003
- 117. Lopesia conspicua Maia, 2003
- 118. Lopesia davillae Maia & Monteiro, 2017
- 119. Lopesia elliptica Maia, 2003
- 120. Lopesia erythroxyli Rodrigues & Maia, 2010
- 121. Lopesia grandis Maia, 2001
- 122. Lopesia indaiensis Maia & Araújo, 2018
- 123. Lopesia leandrae Maia, 2019
- 124. Lopesia linearis Maia, 2003
- 125. Lopesia marginalis Maia, 2001
- 126. Lopesia maricaensis Rodrigues & Maia, 2010
- 127. Lopesia similis Maia, 2004
- 128. Lopesia simplex Maia, 2002
- 129. Lopesia singularis Maia, 2001
- 130. Lopesia spinosa Maia, 2004
- 131. Lopesia tibouchinae Maia, 2004
- 132. Lopesia ubatubensis Garcia & Urso-Guimarães, 2018
- 133. Machaeriobia gemmae Maia, 2016
- 134. Machaeriobia machaerii (Kieffer, 1913)
- 135. Manilkaramyia notabilis Maia, 2001
- 136. Mayteniella distincta Maia, 2001
- 137. Metasphondylia squamosa Tavares, 1918
- 138. Meunieriella dalechampiae Rübsaamen, 1905
- 139. Meunieriella insignis (Tavares, 1922)
- 140. Meunieriella lantanae (Tavares, 1918)
- 141. Mikaniadiplosis annulipes Gagné, 2001
- 142. Myrciamyia maricaensis Maia, 1996
- 143. Myrciariamyia bivalva Maia, 1995

146. Neolasioptera eugeniae Maia, 1993

147. Neolasioptera fariae (Tavares, 1922)

150. Neolasioptera ramicola Maia, 2009

151. Neomitranthella robusta Maia, 1996

152. Novocalmonia fici Ozdikmen, 2009

154. Parazalepidota clusiae Maia, 2001

155. Paulliniamyia ampla Maia, 2001 156. Perasphondylia mikaniae Gagné, 2001

149. Neolasioptera lantanae (Tavares, 1922)

153. Novocalmonia urostigmatis (Tavares, 1917)

157. Pisphondylia brasiliensis Couri & Maia, 1992

http://www.scielo.br/bn

158. Primadiplosis microgramma Maia, 2011

159. Proasphondylia brasiliensis Felt, 1915

148. Neolasioptera ingae Möhn, 1964

144. Neolasioptera cerei (Rübsaamen, 1905) 145. Neolasioptera cupheae Gagné, 1998

160. Proasphondylia formosa Maia, 1994 161. Proasphondylia guapirae Maia, 1994 162. Procontarinia mangiferae (Felt, 1911) 163. Rhoasphondylia friburgensis (Tavares, 1917) 164. Rochadiplosis tibouchinae Tavares, 1917 165. Schismatodiplosis lantanae (Rübsaamen, 1908) 166. Schizomyia maricaensis Sousa & Maia, 2007 167. Schizomyia santosi Maia & Araújo, 2009 168. Schizomyia spherica Maia & Oliveira, 2007 169. Smilasioptera candelariae Möhn, 1975 170. Sphaeramyia flava Maia, 2007 171. Stephomyia clavata (Tavares, 1920) 172. Stephomyia epeugeniae Gagné, 1994 173. Stephomyia espiralis Maia, 1993 174. Stephomyia mina Maia, 1993 175. Stephomyia rotundifoliorum Maia, 1993 176. Stephomyia tetralobae Maia, 1993 177. Styraxdiplosis caetitensis Tavares, 1915 178. Styraxdiplosis cearensis Tavares, 1925 179. Trotteria quadridentata Maia, 2001 180. Uleella dalbergiae Rübsaamen, 1908 181. Youngomvia pouteriae Maia 2001 182. Zalepidota ituensis (Tavares, 1917)

183. Zalepidota piperis Rübsaamen, 1908

CAATINGA

Only eight gall midg species of seven genera have been reported in the Caatinga, six of them are gall-inducers, one is predator, *Feltiella curtistylus*, and one is probably kleptoparasite, *Dialeria styracis*. All inducers are monophagous. The phytophagous species are associated with three plant families, Combretaceae, Fabaceae, and Styracaceae, four genera, *Bauhinia* L., *Combretum* Loefl, *Mimosa* L., and *Styrax* L., and three determined species, *Combretum* leprosum, *Mimosa tenuiflora*, and *Bauhinia brevipes*. Fabaceae shelter the great richness of gall midge species, four (Table 4). Three gall midge species occur on a host plant identified only in genus, *Styrax* sp., and a single species occur in a plant identified only in family (Fabaceae). All host plant species are native to Brasil. None of them is endemic. All gall midge genera are represented by a single species, except *Lopesia*, with two species (Table 3).

List of Cecidomyiidae (Diptera) with occurrence in the Caatinga (Brazil)

- 1. Anadiplosis caetetensis Tavares, 1920
- 2. Dialeria styracis Tavares, 1918
- 3. Feltiella curtistylus Gagné, 1984
- 4. Houardodiplosis rochae Tavares, 1925
- 5. Lopesia mimosae Maia, 2010
- 6. Lopesia pernambucensis Maia, 2010
- 7. Schizomyia macrocapillata Maia, 2005
- 8. Styraxdiplosis caetitensis Tavares, 1915 (possibly)

CERRADO

A total of 60 gall midge species of 25 genera have been recorded in the Cerrado, 52 of them are gall-inducers, six are fungivorous, terena, S. terere, and Termitomastus leptoproctus, one is inquiline, Meunieriella spinosa, and one is predator, Diadiplosis saccharum.

All phytophagous species are monophagous. Phytophagous gall midges have been recorded on 21 plant families, 37 genera and 37 determined species. Fabaceae, Asteraceae and Calophyllaceae comprise the greatest richness of cecidomyiid species, ten, six and six, respectively (Table 5). Seven gall midge species occur on host plants identified only in genus, totaling seven plant genera. All host species are native. Among them, eleven are endemic to Brazil, two being endemic to Cerrado (Table 4). *Lopesia, Asphondylia*, and *Bruggmanniella* are the best represented genera of Cecidomyiidae, with 14, 7, and 4 species, respectively (Table 3).

List of Cecidomyiidae (Diptera) with occurrence in the Cerrado (Brazil)

- 1. Alycaulus hexadentatus Urso-Guimarães, 2018
- 2. Andirodiplosis bahiensis Tavares, 1920
- 3. Arcivena kielmeyerae Gagné, 1984
- 4. Asphondylia canastrae Urso-Guimarães & Amorim, 2002
- 5. Asphondylia cipo Urso-Guimarães, 2018
- 6. Asphondylia cordiae Möhn, 1959
- 7. Asphondylia gochnatiae Maia, 2008
- 8. Asphondylia microcapillata Maia, 2005
- 9. Asphondylia sanctipetri Urso-Guimarães & Amorim, 2002
- 10. Asphondylia serrata Maia, 2004
- 11. Asphondylia stachytarpheta Barnes, 1932
- 12. Brethesiamyia retorta Maia, 2009
- 13. Bruggmannia chapadensis Proença & Maia, 2018
- 14. Bruggmanniella doliocarpi Maia, 2010
- 15. Bruggmanniella duguetiae Urso-Guimarães & Amorim, 2005
- 16. Bruggmanniella ingae Urso-Guimarães & Amorim, 2005
- 17. Bruggmanniella miconiae Carvalho-Fernandes, Maia & Rodrigues, 2020
- 18. Cerciplanus cipo Garcia & Urso-Guimarães, 2020
- 19. Cerciplanus tocantinensis Garcia & Urso-Guimarães 2020
- 20. Clinodiplosis agerati Maia, 2016
- 21. Clinodiplosis bellum Urso-Guimarães & Carmo-Neto, 2015
- 22. Clinodiplosis quartelensis Maia & Oliveira 2019
- 23. Contarinia gemmae Maia 2003
- 24. Couridiplosis vena Maia, 2004
- 25. Dasineura braziliensis (Tavares, 1922)
- 26. Dasineura occulta Pereira-Colavite & Urso-Guimarães, 2013
- 27. Diadiplosis saccharum Urso-Guimarães, 2020
- 28. Didactylomyia longimana (Felt, 1908)
- Lopesia andirae Garcia, Lima, Calado & Urso-Guimarães, 2017
- 30. Lopesia bilobata Maia, 2004
- 31. Lopesia caulinaris Maia, 2003
- 32. Lopesia chapadensis Garcia & Urso-Guimarães, 2018
- 33. Lopesia conspicua Maia, 2003
- 34. Lopesia eichhorniae Urso-Guimarães, 2015
- 35. Lopesia elliptica Maia, 2003
- *36. Lopesia linearis* Maia, 2003
- 37. Lopesia maricaensis Rodrigues & Maia, 2010
- 38. Lopesia mataybae Garcia & Urso-Guimarães, 2018
- 39. Lopesia similis Maia, 2004
- 40. Lopesia simplex Maia, 2002
- 41. Lopesia spinosa Maia, 2004
- 42. Lopesia tibouchinae Maia, 2004
- 43. Machaeriobia machaerii (Kieffer, 1913)
- 44. Meunieriella spinosa Urso-Guimarães, 2018
- 45. Myrciamyia pterandrae Maia & Flor, 2018
- 46. Myrciariamyia admirabilis Maia, 2007
- 47. Myrciariamyia fernandesi Maia, 2004
- 48. Parametasphondylia piperis Maia & Santos 2007
- 49. Rochadiplosis tibouchinae Tavares, 1917
- 50. Schizomyia barreirensis Santos, Maia & Calado, 2019
- 51. Schizomyia macrocapillata Maia, 2005
- 52. Schizomyia maricaensis Sousa & Maia, 2007
- 53. Schizomyia tuiuiu Urso-Guimarães & Amorim, 2002
- 54. Stephomyia epeugeniae Gagné, 1994
- Stomatosema pantaneirum Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema sisbiota Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema terena Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema terere Carmo-Neto, Lamas & Urso-Guimarães, 2019
- 59. Termitomastus leptoproctus Silvestri, 1901
- 60. Youngomyia matogrossensis Proença & Maia, 2019

PAMPA

Only ten species of gall midges of nine genera have been recorded in the Pampa (List 5), nine of them are gall-inducers and one was caught in flight. One species is oligophagous, *Asphondylia moehni*, the other inducers are monophagous. They are associated with eight plant families distributed in eight genera and six determined species. All families shelter a single gall midge species, and there are two doubtful records on Smilacaceae (Table 4). Three gall midge species occur on host plants identified only in genus, totaling three plant genera. All host plants are native, one of them being endemic to Brazil (Table 5).

List of Cecidomyiidae (Diptera) with occurrence in the Pampa (Brazil)

- 1. Asphondylia moehni Skuhravá, 1989
- 2. Asphondylia sulphurea Tavares, 1909
- 3. Bruggmannia braziliensis Tavares, 1906
- 4. Bruggmanniella braziliensis Tavares, 1909
- 5. Compsodiplosis luteoalbida (Tavares, 1909)
- 6. Elachypalpus psidii Maia & Nava, 2011
- 7. Eugeniamyia dispar Maia, Mendonça & Romanowski, 1997
- 8. *Guarephila albida* Tavares, 1909
- 9. Neolasioptera urvilleae (Tavares, 1909)
- 10. Zalepidota tavaresi (Kieffer, 1913)

PANTANAL

Only five species of Cecidomyiidae of two genera have been reported in the Pantanal, one is gall-inducers and the others are fungivorous. The single gall-inducing species is monophagous and occurs on a native, non endemic plant.

List of Cecidomyiidae (Diptera) with occurrence in the Pantanal (Brazil)

- 1. Neolasioptera pantaneira Maia, 2017
- Stomatosema camilae Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema paratudo Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema pantaneirum Carmo-Neto, Lamas & Urso-Guimarães, 2019
- Stomatosema sisbiota Carmo-Neto, Lamas & Urso-Guimarães, 2019

The phytogeographic domain of occurrence of ten species (*Aphidoletes aphidimyza*, *Clinodiplosis alternantherae*, *C. cattleyae*, *Diadiplosis coccidivora*, *Diadiplosis pseudococci*, *Mycodiplosis rubida*, *Prodiplosis floricola*, *Schizomyia manihoti*, *Schizomyia mimosa*, and *Sphaerodiplosis dubia*) was not determined, since data on its locality was not enough detailed.

Discussion

Brazil shelters 265 gall midge species, about 43% of the diversity of the Neotropical fauna. Brazil is the largest country in the Neotropics, with 8, 515, 767. 049 km² of extension (IBGE, 2012), has a peculiar and diverse flora, with 46, 4922 species (Flora do Brasil, 2020), and also its Cecidomyiidae fauna is the most taxonomically studied. These facts together explained this high percentage.

Most species induce galls, since this is the predominant habit in this family (Gagné 1994). The majority of the host plants are identified in species, but 52 hosts are identified at supraespecific categories, what difficults the establishement of the right number of host species.

Most hosts are native, but few are exotic, being represented by introduced useful plants, as *Melissa officinalis* L. (Lamiaceae), a medicinal herb, *Mangifera indica* L. (Anacardiaceae) with edible fruits, *Senna bicapsularis* (L.) Roxb. (Fabaceae), *Spiraea salicifolia* L. (Rosaceae), and *Erigeron strigosus* Muhl. ex Willd. (Asteraceae), ornamental plants. The gall midges associated with *M. indica*, *S. salicifolia*, and *E. strigosus* are also exotic, but those associated with *M. officinalis* and *S. bicapsularis* were described from Brazil and they have never been reported in other countries, suggesting that these hosts were colonized by Brazilian gall midges.

Lantana camara is naturalized and its gall-inducing species was described from Brazil, but it occurs in several Latin American countries. Probably the midge was introduced together with its host.

Incongruities between the host plant and gall-inducing species distributions were observed, suggesting a plant misidentification. In this case, plant vouchers should be examined, but unfortunately the number of vouchers are rare in the literature.

Twenty-seven botanical names were uptaded, but synonyms were cited in brackets to allow their association with the original references. This procedure is important to gather data on the same species, even if under different names.

Most gall-inducing species are monophagous (about 90%), what was expected, as most gall-inducing insects exhibit a high degree of host-plant specificity (Abrahamson et al. 1998, Carneiro et al. 2009). Fabaceae, Asteraceae, and Myrtaceae are the plant families with the greatest richness of gall midge species, as in several inventories of insect galls in Brazil (e. g. Almada & Fernandes 2011, Maia 2013, Araújo et al. 2015, Maia & Mascarenhas 2017, Ascendino & Maia 2018, Maia & Siqueira 2020). Among them, Myrtaceae exhibit the highest average of gall midge species by host plant species, probably because this family is one of the most diverse in the Atlantic Forest, the most investigated biome, and their gall-inducing species are the most studied (e. g. Oliveira & Maia 2005, Rodrigues et al. 2014, Carvalho-Fernandes et al. 2016). Eugenia (Myrtaceae) and Mikania (Asteraceae) highlight among all other plant genera by sheltering the highest richness of gall midges. Similarly, Guapira opposita, Mikania glomerata, and Calophyllum brasiliense highlight among all other plant species. Their richness has also been pointed out by other authors (Maia 2001b, Maia & Mascarenhas 2017, Melo-Júnior et al. 2018).

The best represented gall midge genera are *Lopesia*, *Asphondylia*, and *Clinodiplosis*. The first is predominantly Neotropical while the others are cosmopolitan, but well represented in the Neotropics (Gagné & Jaschhof 2017). Species of *Clinodiplosis*, *Contarinia*, *Dialeria*, *Meunieriella*, *Neolasioptera* and *Trotteria* have been reported as inquilinous species in galls. But in fact, according to the ecological concept, they should be considered kleptoparasites, since they do not promote the production of new tissues, as inquilines do (Luz & Mendonça-Júnior 2019).

Several gall midge species are associated with endemic hosts. This information is very important, because it shows the peculiarities of the Brazilian fauna, revealing species with exclusive occurrence in the country. The high number of species known only from the type-locality indicates how the geographic distribution of Brazilian fauna is still poor. This scarcity of data is reinforced by the high number of species which have never been collected again since their description.

Concerning phytogeographic domains, Atlantic Forest shelters the highest richness of gall midge species, followed by Cerrado, Amazon Forest, Pampa, Caatinga, and Pampa. Amazon Forest and Cerrado are the largest, with an area of 2,196,943 and 2,036, 448 Km², respectively, followed by Atlantic Forest with 1,110,182 Km² and Caatinga with 844.453 Km². The two others are the smallest, Pampa with 176,469 and Pantanal with 150,355 Km². They also differ in richness of flora. Amazon Forest shelters about 50,000 plant species of Angiospermae, Atlantic Forest 20,000, Cerrado 11,600, Caatinga 3,500, Pampa 3,000 and Pantanal only 900 (MMA, 2020). Considering these data, we should expect the highest richness of gall midge species in the Amazon Forest, according to the plant richness hypothesis (Southwood 1960, 1961), since this domain offers quantitatively and qualitatively the greatest amount of resources for the gall-inducers. Nevertheless, the highest richness is reported in the Atlantic Forest, the most investigated domain. This result is an effect of the strong sampling efforts in Atlantic Forest areas, the poor knowledge of the Cecidomyiidae fauna of other Brazilian biomes and the lack of taxonomists in our country.

Most gall midge species occur in a single domain, differing from their host plants which can be found in more than one domain. This suggests that the distribution of gall-inducing species can be wider than that which we known today. Arriola et al. 2016 argued that the distribution of the galling-insects is similar to that of the host plant. So, collections in uninvestigated ou poorly investigated localities are necessary to fill these geographic distribution gaps.

Nevertheless, several gall midge species are associated with plants endemic of a specific phytogeographic domain, as Amazon Forest, Atlantic Forest, and Cerrado. These data reveal the peculiarities of each one and can be useful for the establishement of environmental preservation areas.

Conclusion

This study is the first dataset of gall midge species with occurrence in Brazil. It totalize 265 species (43% of the Neotropical fauna); 85.6% are gall-inducers. Phytophagous gall midges are associated with at least 128 host plant species. Fabaceae, Asteraceae and Myrtaceae shelter the greatest richness of gall-inducing species. Lopesia, Asphondylia and Clinodiplosis are the best represented cecidomyiid genera. Most species (about 90%) are known only from Brazil. The Atlantic Forest as the most investigated domain comprises the highest species richness. Several gall midge species induce galls on plants endemic to Brazil. Each Brazilian phytogeographic domain has its own species composition. There is a high number of cecidomyiid species which are known only from the type-locality. Data indicate that the Brazilian fauna is the most studied in the Neotropical Region, confirm the greatest richness of gall midges on plant families which are well represented in Brazil, reveal the most diverse genera in the country, show the peculiarities of each domain and highlight how the geographic distribution of most species is still poor.

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Conflicts of Interest

The author declares that she has no conflict of interest related to the publication of this manuscript.

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Brazilian mangroves at risk

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Abstract: Recent statements from the Brazilian federal government indicate that impacting economic activities, particularly commercial shrimp farming, are being encouraged in mangrove areas in the near future. Alterations of the National Action Plan and legal instruments that partially protected mangrove areas have created an even weaker legal framework than previously existed. Such changes are leading Brazil far from the global call to conserve mangroves and from the Aichi targets and United Nations Sustainable Development Goals. Unfortunately, the loss of mangrove ecosystems and their ecosystem services will negatively impact living standards for Brazilians in coastal areas. *Keywords: Biodiversity, Carbon capture, Conservation, Nursery, PAN Manguezal.*

Manguezais brasileiros sob risco

Resumo: As recentes determinações do governo Brasileiro indicam que atividades econômicas impactantes para os manguezais, particularmente a carcinocultura, serão incentivadas num futuro próximo. Alterações no Plano de Ação Nacional e em outros instrumentos legais, que parcialmente protegiam os manguezais, fragilizaram ainda mais os meios legais para proteção dessas áreas. Essas mudanças fazem o Brasil seguir na contra-mão da demanda global pela conservação dos manguezais e dos objetivos de desenvolvimento sustentável traçados pela Organização das Nações Unidas, bem como das metas de Aichi para a biodiversidade. Consequentemente, a iminente perda dos ecossistemas de manguezais impactará negativamente o padrão de vida dos Brasileiros que vivem em áreas costeiras. **Palavras-chave:** Berçário, Biodiversidade, Captura de Carbono, Conservação, PAN Manguezal.

Introduction

Mangroves (Figure 1) represent highly productive ecosystems, once relatively common along tropical and subtropical oceanic coastlines, especially around estuaries, and currently present over an area about 181,000 km² globally (Donato et al. 2011, Giri et al. 2011). Only about 2% of mangrove stands occur along temperate coastlines (Morrisey et al. 2010). Historically, several impacts have affected mangrove extents, such as: pollution, logging, and conversion to non-sustainable aquaculture and agriculture (Friess et al. 2019). Consequently, the global mangrove area, which is already smaller than previously estimated, continues to decline (Giri et al. 2011), making mangrove ecosystems top priorities for implementing conservation actions (Friess et al. 2019).

Mangrove ecosystems are characterized by transitional gradients of physiological and ecological characteristics, strongly influenced by salinity, nutrient concentrations, soil type, tidal amplitudes, and freshwater inputs, all of which create hostile conditions for most plant species (Walsh 1974, Cintrón & Schaeffer-Novelli 1983, Araujo & Pereira 2009, Silva et al. 2016). The vegetation is characterized by a homogeneous phytophysiognomy with low species diversity, but remarkable specializations in morphology and physiology (Figure 1) (Walsh 1974, Cintrón & Schaeffer-Novelli 1983, Araujo & Pereira 2009, Silva et al. 2016).

Brazilian mangrove plant associations are dominated by specialized species. These include some species of the genera *Acrostichum* L. (Pteridaceae), *Avicennia* L. (Acanthaceae), *Cinnamodendron* Endl. (Canellaceae), *Conocarpus* L. (Combretaceae), *Crenea* Aubl. (Lythraceae), *Hibiscus* L. (Malvaceae), *Laguncularia* C.F. Gaertn. (Combretaceae), *Rhizophora* L. (Rhizophoraceae), *Spartina* Schreb. (Poaceae), *Spergularia* (Pers.) J. Presl & C. Presl (Caryophyllaceae), and *Talipariti* Fryxell (Malvaceae). Those species include herbaceous, shrubby, and woody plants (Schaeffer-Novelli & Cintrón 1986, Bernini & Rezende 2004, Martins et al. 2011, Santos et al. 2012, Flora do Brazil 2020) (Figure 1). Mangrove forest losses extirpate this unique and specialized flora.

1. Range of Brazilian Mangroves

In Brazil, mangrove areas occur along an extensive coastline, from the Oiapoque River mouth in the north, to Laguna, in Santa Catarina state, in the south (Saenger et al. 1983, Novelli et al. 2000, Maia et al 2006). Their Brazilian range represents 7% of the total global mangrove land cover (Giri et al. 2011). Considering only the Brazilian north coast, the country has the largest continuous formation of this ecosystem type in the world (Souza-Filho 2005).

2. Ecosystem Services

Many animal phyla and species use mangrove forests, such as molluscs, arthropods, fishes, reptiles, birds, and mammals (Giesen et al. 2006). For example, sixty-seven primate taxa, most of them exhibiting rare behaviors shelter or feed in tropical and subtropical mangroves (Supriatna & Wahono 2000, Santos et al. 2019, Presotto et al. 2020). The high productivity rates of mangrove ecosystems are crucial for the benthic and pelagic components of neritic food webs (Robertson et al. 1992), providing direct and indirect resources (Tomlinson 1986, Luther & Greenberg 2009) for transient fishes and crustaceans as nursery and foraging habitats (El-Regal & Ibrahim 2014, Hutchison et al. 2014, Sheaves 2017, Litvin et al. 2018). In particular, mangrove ecosystems are critical for sustaining economically valuable crustaceans (e.g., pink shrimps and swimming crabs), finfishes (e.g., snooks, mullets, sardines,



some catfish species, snappers, several sciaenids, and some groupers), and their fisheries (Robertson & Duke 1990, Sheridan 1992, Rogers & Mumby 2019, Sheaves et al. 2020). In addition, mangrove forests provide several other ecosystem services, delivered to human society across local, regional and global extents (Barbier et al. 2011). These include such critically important services as erosion control, water purification, coastal protection from storms, raw materials and food, maintenance of fisheries, carbon sequestration and cultural services associated with tourism, recreation, education, and research (Figure 2).



Figure 2. Representation of mangrove ecosystem services (green arrows). Water purification: Rybicki (1997), Lee & Dunton (1999), Gacia et al. (1999), Cornelisen & Thomas (2006), Romero et al. (2006), and Koch et al. (2006). Coastal protection: Fonseca & Cahalan (1992), Koch (1996), Prager & Halley (1999), Hemminga & Duarte (2000), Spalding et al. (2003), and Koch et al. (2009). Erosion: Hemminga & Nieuwenhuize (1990). Maintenance of fisheries: Cesar & van Beukering (2004), and Zeller et al. (2007). Raw materials and food: Hemminga & Duarte (2000), de la Torre-Castro & Rönnbäck (2004), and Lauer & Aswani (2010). Cultural Services associated with tourism: Naylor & Drew (1998). Carbon sequestration: Freeman (2003), Pagiola et al. (2004), NRC (2005), Barbier (2007), U.S. EPA (2009), Mendelsohn & Olmstead (2009), and Hanley & Barbier (2009). Recreation: Mathieu et al. (2003).

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For example, Brazil has some conservation units in which mangrove forests play a major role in creating jobs and income, generating millions of reais per year through tourism, handcrafts, crab extraction, and traditional/commercial fishing. The Parque Nacional dos Lençóis Maranhenses (PNLM) and Delta do Rio Parnaíba (DRP) are both examples of areas that are benefited economically by ecosystem services provided from mangrove ecosystems. The PNLM received 151,786 tourists from all over the world in 2019 (ICMBIO 2020) to visit mainly sand dunes with freshwater lagoons, but also mangrove areas. In the DRP, crab extraction and commercial fishing are the main local economic activities, being the major location of crab harvest in Brazil (Legat et al. 2006, Fogaça et al. 2018).

3. Legal Statute Concerns

As with the global trend, Brazilian mangrove forests have been threatened for years, particularly by deforestation to build ponds for crustacean farming (Ferreira & Lacerda 2016a). However, an astonishing set of recent events has further threatened these ecosystems, derived from an orchestrated sequence of Brazilian political decisions. Since the 2012 alterations in the Brazilian Forest Code (Código Florestal law 12.651/2012), mangrove ecosystems have not been fully protected (Ferreira & Lacerda 2016b). Then, amidst the 2019 oil contamination crisis in coastal ecosystems (Escobar 2019) and government inability to protect the affected areas (Brum et al. 2020), the National Action Plan for Mangroves (Plano de Ação Nacional para a Conservação das Espécies Ameaçadas e de Importância Socioeconômica do Ecossistema Manguezal; PAN Manguezal) was altered. The PAN Manguezal, which identifies priority actions for ecosystem protection, was modified in 2019 to exclude the eradication of shrimp farming and the recovery of mangrove as goals. Later, in September 2020, the number of NGOs, universities and indigenous peoples represented in the National Environment Council (Conselho Nacional do Meio Ambiente; CONAMA) was reduced. The Bolsonaro government subsequently revoked CONAMA resolution 303/2002, which established regulations to protect Brazilian mangrove ecosystems. These two recent changes have created a pathway for increasingly unregulated economic activities and impacts on mangrove ecosystems, leaving them without any protective regulations.

The PAN Manguezal and CONAMA changes imperil mangrove ecosystem services (see mangrove ecosystem services in Barbier et al. 2011) and, as a consequence, economic activities (e.g. tourism and commercial fisheries), that represent the main income and budget in several municipalities along the Brazilian coast. The Bolsonaro's government legal changes ignore global perspectives that consider mangroves as vitally important ecosystems for conservation (Friess et al. 2019). In addition, the new statutes are in opposition to the Aichi targets, which Brazil agreed to follow as part of the Convention on Biological Diversity, and the United Nations Sustainable Development Goals (SDGs). The SDGs are a set of goals aimed at reaching a sustainable planet that started to emerge during the 2012 United Nations Conference on Sustainable Development (Rio + 20), which occurred in Rio de Janeiro and brought together global leaders (Sachs 2015). In particular, the statutes violate four SDGs: SDG 2 (End hunger, achieve food security, improve nutrition, and promote sustainable agriculture); SDG 13 (Take urgent action to combat climate change and reduce its impacts); SDG 14 (Conserve and ensure the sustainable use and development of the oceans, seas, freshwater bodies and marine resources); and SDG 15 (Protect, restore and promote the sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt degradation, and halt biodiversity loss). See Table 1 for a summary of the status of these statutes. The Brazilian government must reinstitute legal protections for mangrove ecosystems to abide by these global objectives. Otherwise, mangrove forests will be increasingly subjected to impacts that will threaten their biodiversity and the living standards of millions of Brazilian citizens living in coastal areas, whose lives depend on activities directly related to mangrove conservation.

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International Agreements	Main Resolves and Guidelines	Regulating Organ/ Event	Implementation Date	January 2021	Access
Aichi Biodiversity Targets	Target 5 - Halving of habitat loss ratings; Target 6 - Sustainable management of fisheries and other seaborn stocks; Target 11 - Conservation of at least 10% of coastal and marine areas; Target 14 - Restoration and safeguarding of ecosystems that provide essential services.	Convention on Biological Diversity (1992 Rio Earth Summit)	January 2011	Active	https://www.cbd.int/sp/ targets/
United Nations Sustainable Development Goals (SDGs)	SDG 2 - End hunger, achieve food security and improved nutrition, and promote sustainable agriculture; SDG 13 - Take urgent action to combat climate change and reduce its impacts; SDG 14 - Conserve and ensure the sustainable use and development of the oceans, seas, freshwater bodies and marine resources; SDG 15 - Protect, restore and promote the sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, halt degradation, and halt biodiversity loss.	United Nations Conference on Sustainable Development (Rio + 20)	September 2015	Active	https://www.un.org/sustaina- bledevelopment/sustainable- development-goals/

Table 1. Summary of international and Brazilian mangrove related policies. Links in "Access" column direct to official government internet pages.

continued ...

...continuation

Brazilian Environmental Policies

Resolution CONAMA No 303/2002	Establishes mangroves as permanent preservation areas.	Conselho Nacional do Meio Ambiente (CONAMA)	May 2002	Judicially Active	http://www2.mma.gov. br/port/conama/legiabre. cfm?codlegi=299
Código Florestal (Law 12.651/2012)	Allows and regulates implementation of shrimp farms and salt pans in mangroves and surrounding environments.	Ministério da Casa Civil; Ministério do Meio Ambiente	May 2012	Active	http://www.planalto.gov. br/ccivil_03/_ato2011- 2014/2012/lei/112651.htm
PAN Manguezal (ordinance ICMBio No 9/2015)	Establishes actions and directions aiming for the conservation and sustainable use of mangroves and associated endangered species; Specifically proposes erradication of shrimp farms and salt pans in mangroves.	Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)	January 2015	Revoked	https://www.icmbio.gov. br/cepsul/images/stories/ legislacao/Portaria/2015/p_ icmbio_09_2015_aprova_ pan_manguezais.pdf
PAN Manguezal Updated (ordinance ICMBio No 647/2019	Revokes and replaces the text of previous PAN Manguezal; Unstring regulations and guidelines for shrimp farms and salt pans.	Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio)	October 2019	Active	https://www.icmbio.gov.br/ portal/images/stories/docs- pan/pan-manguezal/1-ciclo/ pan-manguezal-portaria- aprovacao-e-gat.pdf
Resolution CONAMA No 500/2020	Revokes resolution CONAMA No 303/2002.	Conselho Nacional do Meio Ambiente (CONAMA)	October 2020	Judicially Suspended	https://www.in.gov.br/en/ web/dou/-/resolucao-conama/ mma-n-500-de-19-de- outubro-de-2020-284006009

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Inventory

Amphibians and reptiles from the Parque Nacional da Tijuca, Brazil, one of the world's largest urban forests

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Abstract: The Parque Nacional da Tijuca in Rio de Janeiro, Brazil, is considered to be one of the world's largest urban forests, however no systematic inventory of its herpetofauna is available. In the present study, we surveyed the amphibians and reptiles of this park to assess its species composition (including secondary data) and obtain estimates of species richness and abundance. We conducted active searches (460 hours) between January 2013 and December 2015. We identified the taxa endemic to either the Atlantic Forest or Rio de Janeiro state, and verified the conservation status of each species in the international, Brazilian, and state red lists. We also estimated the species richness and sampling sufficiency by rarefaction curves and Bootstrap richness estimator, and analyzed the distribution of the species abundance in Whittaker plots. We recorded 3,288 individuals over 36 months, representing 24 species of amphibians and 25 reptiles. The cumulative species curves, rarefaction, and the richness estimated indicated that sampling effort was adequate. Species abundance adjusted to the log-series model in both amphibians and reptiles. The four most abundant amphibians represented 70% of the individuals recorded in this group, while the two most abundant reptiles represented 60% of the total individuals. The inclusion of the secondary data raised the number of amphibian species to 38, and the number of reptiles to 36. Approximately 80% of the amphibian species and 28% of the reptile species recorded are endemic to the Atlantic Forest, and six of the amphibian species are endemic to Rio de Janeiro state. Six amphibian species and one reptile species are classified under some threat of extinction, and two reptile species were exotic. The considerable diversity of the herpetofauna of the Parque Nacional da Tijuca, which includes endemic and threatened species, reflects the effectiveness of the reforestation of this protected area and emphasizes the importance of its conservation. Keywords: Atlantic Rainforest; Conservation; Endemism; Herpetofauna; Inventory.

Anfíbios e répteis do Parque Nacional da Tijuca, Brasil, uma das maiores florestas urbanas do mundo

Resumo: O Parque Nacional da Tijuca, no Rio de Janeiro, Brasil, é considerado uma das maiores florestas urbanas do mundo, no entanto nenhum inventário sistemático de sua herpetofauna está disponível. No presente estudo, pesquisamos os anfibios e répteis deste parque para acessar sua composição de espécies (incluindo dados secundários) e obter estimativas da riqueza e da abundância de espécies. Realizamos buscas ativas (460 horas) entre janeiro de 2013 e dezembro de 2015. Identificamos os taxa endêmicos da Mata Atlântica ou do estado do Rio de Janeiro, e verificamos o status de conservação de cada espécie nas listas vermelhas internacional, brasileira e estadual. Também estimamos a riqueza de espécies e a suficiência amostral através de curvas de rarefação e do estimador de riqueza Bootstrap, e analisamos a distribuição de abundância das espécies através de plots de Whittaker. Registramos 3.288 indivíduos ao longo dos 36 meses, representando 24 espécies de anfíbios e 25 de répteis. As curvas cumulativas de espécies, a rarefação, e a riqueza estimada indicaram que o esforço amostral foi adequado. A abundância das espécies se ajustou ao modelo de série logarítmica tanto para os anfibios como para os répteis. As quatro espécies de anfíbios mais abundantes representaram 70% dos indivíduos registrados neste grupo, enquanto as duas espécies de répteis mais abundantes representaram 60% do total de indivíduos. A inclusão dos dados secundários elevou o número de espécies de anfibios para 38 e o de répteis para 36. Aproximadamente 80% dos anfibios e 28% dos répteis registrados são endêmicos da Mata Atlântica e seis espécies de anfíbios são endêmicos do estado do Rio de Janeiro. Seis espécies de anfibios e uma de réptil estão classificadas sob alguma ameaça de extinção, e dois répteis constituem espécies exóticas. A considerável diversidade da herpetofauna do Parque Nacional da Tijuca, que inclui espécies endêmicas e ameaçadas, reflete a efetividade do reflorestamento dessa área protegida e enfatiza a importância de sua conservação. Palavras-chave: Mata Atlântica; Conservação; Endemismo; Herpetofauna; Inventário.

Introduction

The Brazilian Atlantic Forest biome is one of the world's biodiversity hotspots (Myers et al. 2000), although it has now been reduced to only around 12% of its original cover as a result of centuries of intense deforestation (Ribeiro et al. 2011, SOS Mata Atlântica, INPE 2018). Approximately 625 amphibian species (Haddad et al. 2013, Rossa-Feres et al. 2017) and 300 reptiles (Tozetti et al. 2017) are known to occur in this biome, and new species are still being steadily described (e.g., Cruz et al. 2019, Maciel et al. 2019, Prates et al. 2019). In the state of Rio de Janeiro, 201 species of amphibian have been recorded, including 197 anurans and four caecilians (Dorigo et al. 2018), together with 149 reptiles (Oliveira et al. 2020). This state has a high rate of endemism of both amphibians (Dorigo et al. 2018, Rossa-Feres et al. 2017) and reptiles (Tozetti et al. 2017), and its metropolitan region is considered to be an important area for the conservation of Brazilian biodiversity (Oliveira et al. 2019).

The state capital, Rio de Janeiro, which has a population of more than six million inhabitants, includes one of the world's largest urban parks, the Parque Nacional da Tijuca (PNT), which is the only Brazilian national park located within an urban zone. Like other coastal ranges within the Atlantic Forest domain, the Tijuca massif has a rich network of water courses (Coelho Netto 2005), which favors the occurrence of amphibians. During the 18th and 19th centuries, however, the forest of this area was almost totally destroyed for the production of charcoal, and the establishment of sugarcane and coffee plantations (Rocha et al. 2003, Rocha 2017). As a result, many of the water sources that supplied the city began to dry up (Abreu & Rodrigues 2010, Rocha 2017). It seems likely that fragments of forest persisted in many of the more inaccessible areas within the Tijuca massif, in particular, the highest mountain peaks and the steepest valleys (Coimbra-Filho et al. 1973). In addition to providing seeds of native species for the subsequent reforestation of the area (Coimbra-Filho et al. 1973, Abreu & Rodrigues 2010), these forest remnants probably also provided important refuges for many local animal species (e.g., Rocha et al. 2003). The deforested areas once covered by plantations were almost completely reconstituted by a reforestation project - the first major project of its kind anywhere in the world - which involved the planting of 100,000 trees, beginning in the second half of the 19th century during the Brazilian Empire period. This initiative resulted in the establishment of an exuberant forest, which was converted into a national park in 1961 (Brasil 1961).

In addition to the PNT, two other large parks are found within the urban zone of Rio de Janeiro - the Parque Estadual da Pedra Branca and the Parque Natural Municipal da Serra do Mendanha (PNM Serra do Mendanha). The PNT has a long history of scientific research, first being visited by naturalists during the 19th and 20th centuries. These naturalists described a number of amphibian species based on specimens collected in this area, including Aplastodiscus albofrenatus (Lutz, 1924), Dendrophryniscus brevipollicatus Jiménez de la Espada 1870, Ischnocnema guentheri (Steindachner, 1864), Ischnocnema octavioi (Bokermann, 1965), Phasmahyla guttata (Lutz, 1924), and Scinax trapicheiroi (A. Lutz and B. Lutz, 1954). More recently, herpetofaunal researches in the park included studies of the ecology, natural history or bioacoustics of some taxa (e.g., Heyer 1973, Hepp & Carvalho-e-Silva 2011, Dorigo et al. 2014, Machado et al. 2016, Hepp et al. 2017, Silvae-Souza 2019, Guarabyra et al. 2020). Introductory lists of amphibian species (Carvalho-e-Silva et al. 2008) and reptiles (Mocelin 2008) were

compiled for the management plan of this conservation unit (ICMBIO 2008), although, up to the present time, no systematic inventory has been produced. Given this, we inventoried the herpetofauna of the PNT over a three-year period, using intensive sampling to obtain species composition and estimates of the richness and abundance of the park's amphibians and reptiles. We supplemented our inventory with records of occurrence obtained from herpetological collections and species reintroduction programs in PNT.

Material and Methods

1. Study area

The Parque Nacional da Tijuca ($22^{\circ}55'-23^{\circ}00'$ S, $43^{\circ}11'-43^{\circ}19'$ W) is located in the Tijuca massif in the city of Rio de Janeiro, capital of the state of Rio de Janeiro, southeastern Brazil. The park covers a total area of 3,958.38 hectares of dense ombrophilous forest at altitudes between 80 m and 1,021 m above sea level (a.s.l.). The park is divided officially into four sectors (ICMBIO 2008): Sector A – the Tijuca Forest, Sector B – the Carioca Mountains, Sector C – Pedra Bonita/Pedra da Gávea, and Sector D – Pretos-Forros/Covanca (Figure 1). The landscape of the park encompasses a range of land uses and vegetation cover, from forest encompassing distinct stages of succession, to open fields, rocky outcrops, as well as infrastructure, such as buildings and roads (Coelho Netto 2005).

The climate is of the Aw type (humid tropical) in the Köppen classification, with intense rains during the austral summer and drier conditions during the winter. Mean monthly temperatures range from 25°C in February to 19°C in June, with an annual mean of approximately 22°C. Annual precipitation is typically between 2,000 and 2,500 mm, although it may reach 3,300 mm in exceptionally rainy years (Coelho Netto 2005, ICMBIO 2008).

2. Data collection

We searched for amphibians and reptiles in the Sector A of the park over a 36-month period, between January 2013 and December 2015, at altitudes between 400 m and 800 m a.s.l. The searches were based on the Visual Encounter Survey (VES) method of Crump & Scott (1994), and resulted in a total sampling effort of 460 person-hours, with 230 hours of diurnal and 230 hours of nocturnal searches. When conducting an active search, always two researchers moved through the forest searching systematically for amphibians and reptiles in microhabitats such as streams, ponds, bromeliads, the leaf litter, tree trunks, and rocks. The searches were conducted only in a single direction, to avoid the resampling of individuals, thus guaranteeing the independence of the records. All individuals encountered during searches were captured, identified, and most of them released at the capture site. We collected voucher specimens, which we deposited in the herpetological collection of the Museu Nacional (MNRJ), Universidade Federal do Rio de Janeiro (voucher number in Appendix 1). We complemented our inventory with secondary records, which included data from herpetological collections (Appendix 2), and from a faunal reintroduction project for the PNT. We applied the scientific nomenclature proposed by Frost (2020) for the amphibian species. For the reptiles, we used the nomenclature of Costa and Bérnils (2018), which we updated whenever necessary, based on Poe et al. (2017) and Hoogmoed et al. (2019).



Figure 1. Location of Parque Nacional da Tijuca in the municipality of Rio de Janeiro, state of Rio de Janeiro, southeastern Brazil. The area of the park is divided officially into four sectors: A) Tijuca Forest; B) Carioca Mountains; C) Pedra Bonita/Pedra da Gávea; D) Pretos-Forros/Covanca. Map created with QGIS software (www.qgis.org), using a shapefile from ICMBIO (www.icmbio.gov.br)

3. Data analysis

We calculated rarefaction curves and confidence intervals (95%) with the moment-based estimator of species richness (Colwell et al. 2004) to verify the efficiency of the sampling effort in terms of the number of species recorded during the study for both amphibians and reptiles. For this analysis, one hour of VES constituted a sampling unit, with a total of 460 samples being obtained during the study period as a whole. We considered the convergence of the confidence intervals to zero as an indicator of sampling sufficiency (Colwell et al. 2004). We also estimated the number of species and standard deviation with the Bootstrap richness estimator (Smith & van Belle 1984) with 1,000 runs. Both the rarefaction curves and Bootstrap were calculated in the program Estimates 8.2.0 (Colwell 2009). We analyzed the distribution of the species abundance, using rank abundance curves or Whittaker plots (Whittaker 1965), which show the ranked log abundance of each species (Krebs 1999). We analyzed these plots in PAST 2.17 (Hammer et al. 2001).

Species endemism in the Atlantic Forest was defined for the amphibians based on Lingnau et al. (2008), Haddad et al. (2016), and Frost (2020), and for reptiles according to Tozetti et al. (2017). We defined the conservation status of each species based on the IUCN Red List (IUCN 2020), the Red List of Threatened Brazilian Fauna (ICMBIO 2018), and the List of the Threatened Fauna of Rio de Janeiro state (Bergallo et al. 2000). The population trend for each species was obtained from IUCN (2020).

Results

We recorded a total of 74 species of herpetofauna (distributed in four orders), being 38 amphibians (37 anurans and one caecilian; Table 1) and 36 reptiles (three chelonians, one amphisbaenian, nine lizards, and 23 snakes; Table 2) at the PNT. Hylidae was the amphibian family with the highest species richness (N = 10 species), followed by Brachycephalidae (N = 5) and Cycloramphidae (N = 5) (Table 1). With the exception of Gymnophthalmidae, with two species, all lizard families were represented by only a single species. The snake family Dipsadidae was represented by 14 species, and Colubridae by five (Table 2). During our field surveys we recorded a total of 3,288 individuals in Sector A of the Parque Nacional da Tijuca, during the 36 months of the study period, representing a total of 49 species. Most (3,174) of these individuals were amphibians (Table 1), belonging to 24 species of the orders Anura (N = 23 species) and Gymnophiona (N = 1) (Figures 2-4). We recorded only 114 reptiles, although these individuals also represented 25 species (Table 2), distributed in two orders, Chelonia (N = 2) and Squamata (N = 23) (Figures 5-7). Two of these amphibian species and 11 of the reptiles were recorded opportunistically during the study period (i.e., not during standardized surveys; Tables 1 and 2).

The cumulative curve for the amphibian species reached the asymptote at 213 hours of sampling with a total richness of 22 species, while the curve for the reptiles reached the asymptote at 276 hours, **Table 1.** Amphibian species recorded in Parque Nacional da Tijuca in the municipality of Rio de Janeiro, state of Rio de Janeiro, southeastern Brazil, with data on total abundance (Ab; number of records) and relative abundance (RA; percentage of all recorded amphibians) for the species recorded during the Visual Encounter Surveys are given. Other information include: the conservation status of each species based on their category of threat in the international (IUCN 2020), Brazilian (ICMBIO/MMA 2018), and Rio de Janeiro (RJ) state lists (Bergallo et al. 2000); whether they are endemic (End) to the Atlantic Forest (AF) or to Rio de Janeiro state (RJ) or exotic (Ex); their population trend (PT; IUCN 2020); and Sectors of PNT (ICMBIO 2008) where each species was recorded based on primary (P; see Appendix 1) or secondary (S; see Appendix 2) data. * Species recorded in non-standardized encounters. Conservation status: DD = Data Deficient, LC = Least Concern, VU = Vulnerable, EN = Endangered, and PA = Presumably Threatened ("Presumivelmente Ameaçada"). Population trend: D = Decreasing; St = Stable; U = Unknown

ТАХА	Ab	Categ	Category of threat		End	End / Ex PT		Sect		tors	
	(RA)	Global	National	RJ	AF	RJ		Α	В	С	D
ANURA											
Aromobatidae											
Allobates olfersioides (A. Lutz, 1925)	-	VU	VU	PA	End		D	S			S
Brachycephalidae											
Brachycephalus didactylus (Izecksohn, 1971)	9 (0.3)	LC	LC	-	End		D	P, S			
Brachycephalus ephippium (Spix, 1824)	158 (5.0)	LC	LC	-	End		St	P, S	S		
Ischnocnema guentheri (Steindachner, 1864)	544 (17.1)	LC	LC	-	End	End	St	P, S	S		
Ischnocnema octavioi (Bokermann, 1965)	6 (0.2)	LC	LC	-	End		D	Р	S	S	
Ischnocnema parva (Girard, 1853)	617 (19.4)	LC	LC	-	End		D	P, S	S		
Bufonidae											
Dendrophryniscus brevipollicatus Jiménez de la Espada, 1870	15 (0.5)	LC	LC	-	End	End	St	P, S			
Rhinella ornata (Spix, 1824)	15 (0.5)	LC	LC	-	End		U	P, S	S		
Centrolenidae											
Vitreorana eurygnatha (A. Lutz, 1925)	-	LC	LC	PA	End		D	S	S		
Vitreorana uranoscopa (Müller, 1924)	-	LC	LC	PA			D	S	S		
Craugastoridae											
Euparkerella brasiliensis (Parker, 1926)	76 (2.4)	LC	LC	-	End	End	D	P, S	S	S	
Haddadus binotatus (Spix, 1824)	328 (10.3)	LC	LC	-	End		St	P, S	S	S	
Cycloramphidae											
Cycloramphus eleutherodactylus (Miranda-Ribeiro, 1920)	-	DD	LC	PA	End		U		S		
Cycloramphus fuliginosus Tschudi, 1838	-	LC	DD		End		D	S	S		
Thoropa lutzi Cochran, 1938	-	EN	DD	PA	End		D		S		
Thoropa miliaris (Spix, 1824)	8 (0.3)	LC	LC	-	End		St	P, S	S	S	
Zachaenus parvulus (Girard, 1853)	29 (0.9)	LC	LC	-	End		D	P, S	S		
Hemiphractidae											
Fritziana goeldii (Boulenger, 1895)	1 (0.1)	LC	LC	-	End		St	P, S	S		
Hylidae											
Aplastodiscus albofrenatus (A. Lutz, 1924)	46 (1.4)	LC	LC	-	End	End	St	P, S	S	S	
Boana albomarginata (Spix, 1824)*	-	LC	LC	-	End		St	P, S	S		
Boana faber (Wied-Neuwied, 1821)	-	LC	LC	-			St	S	S		
Bokermannohyla circumdata (Cope, 1871)	32 (1.0)	LC	LC	-	End		D	P, S	S		
Dendropsophus elegans (Wied-Neuwied, 1824)	-	LC	LC	-			St		S		
Dendropsophus minutus (Peters, 1872)	-	LC	LC	-			St	S	S		
Scinax cuspidatus (A. Lutz, 1925)	-	LC	LC	-	End		St	S	S		
Scinax fuscovarius (A. Lutz, 1925)*	-	LC	LC	-			St	P, S	S		
Scinax perpusillus (A. Lutz and B. Lutz, 1939)	10 (0.3)	LC	LC	-	End		St	Р	S		
Scinax trapicheiroi (A. Lutz and B. Lutz, 1954)	47 (1.5)	NT	LC	-	End	End	D	P, S	S	S	
Hylodidae											
Crossodactylus gaudichaudii Duméril and Bibron, 1841	500 (15.8)	LC	LC	-	End		D	P, S	S	S	
Hylodes nasus (Lichtenstein, 1823)	601 (18.9)	LC	LC	-	End	End	D	P, S	S	S	S
										Con	tinue

Continuation									
Leptodactylidae									
Adenomera marmorata Steindachner, 1867	13 (0.4)	LC	LC	-		St	P, S	S	S
Leptodactylus latrans (Steffen, 1815)	-	LC	LC	-		St		S	
Physalaemus signifer (Girard, 1853)	6 (0.2)	LC	LC	-	End	St	P, S	S	
Microhylidae									
Myersiella microps (Duméril and Bibron, 1841)	-	LC	LC	-	End	St	S	S	
Odontophrynidae									
Proceratophrys boiei (Wied-Neuwied, 1824)	-	LC	LC	-	End	St	S	S	
Phyllomedusidae									
Phasmahyla guttata (Lutz, 1924)	108 (3.4)	LC	LC	-	End	D	P, S	S	
Phyllomedusa burmeisteri Boulenger, 1882	-	LC	LC	-		St	S	S	S
GYMNOPHIONA									
Siphonopidae									
Siphonops hardyi Boulenger, 1888	5 (0.2)	LC	LC	-	End	St	Р		

with a total of 14 species, based on a total sampling effort of 460 hours (Figure 8). The confidence intervals of the rarefaction curves converged to zero for the amphibians, but not for the reptiles, for which the error was estimated as 2.3 species (Figure 8). The species richness estimated by the Bootstrap procedure was similar to that recorded by the VES method for both amphibians (estimated richness = 22.4 species) and reptiles (estimated richness = 15.6 species).

The Whittaker plots (Figure 9) revealed considerable variation in the abundance of the amphibian and reptilian species, which was best adjusted using the log-series model. The most abundant amphibian species (Table 1) were *Ischnocnema parva* (N = 617 records; 19.4% of all amphibians), *Hylodes nasus* (N=601; 18.9%), *Ischnocnema guentheri* (N=544; 17.1%), and *Crossodactylus gaudichaudii* (N = 500; 15.8%). The most abundant reptiles were the lizards *Enyalius brasiliensis* (N=55; 48.2% of all reptiles) and *Ecpleopus gaudichaudii* (N = 15; 13.2%), representing 61.4% of all the reptiles recorded during the surveys (Table 2). The most abundant snakes were *Bothrops jararacussu* (N = 8; 7.0% of all reptiles), *Thamnodynastes* cf. *nattereri* (N = 7; 6.1%), and *Bothrops jararaca* (N = 6; 5.3%).

Overall, 30 of the amphibian (ca. 80% of the total) and ten of the reptile species (28%) recorded in the PNT are endemic to the Atlantic Forest biome (Tables 1 and 2). Six of these amphibians – *Aplastodiscus albofrenatus*, *Dendrophryniscus brevipollicatus*, *Euparkerella brasiliensis*, *Hylodes nasus*, *Ischnocnema guentheri*, and *Scinax trapicheiroi* – are considered endemic to the state of Rio de Janeiro. Most of the amphibian and reptile species are listed as Least Concern or Not Threatened in the three lists of conservation status (international, Brazilian, and state). Six amphibian and one reptile species recorded in the PNT are listed under some category of extinction threat. The populations of 15 amphibian species are thought to be declining (Table 1). Two of the reptile species, the lizard *Hemidactylus mabouia* and the chelonian *Trachemys scripta* are exotic (Table 2).

Discussion

1. Biodiversity: species richness, composition, and abundance

The results of the present study indicate that the forests of the PNT harbor for a large proportion of the herpetofaunal diversity of the Atlantic Forest of the state of Rio de Janeiro. Considering both the records obtained during our fieldwork and from secondary data, the park's amphibian species richness would amount to at least 38 species, which represent 19% of the amphibians known to occur in the state (N = 201 species; Dorigo et al. 2018), 6% of the amphibian species from Atlantic Rainforest (N = 600; Rossa-Feres et al. 2017), and 3% of the amphibian fauna of Brazil (N = 1,136; Segalla et al. 2019). The combination of primary and secondary data also indicates the occurrence of 36 reptile species in the PNT, which represent 24% of the reptiles known to occur in the whole Rio de Janeiro state (N = 149; Oliveira et al. 2020), 12% of the species recorded in the Atlantic Rainforest (N = 300; Tozetti et al. 2017), and 4% of the country's reptilian diversity (N = 795 species; Costa & Bérnils 2018). When considering the sampled area and the effort employed, the number of amphibian and reptile species recorded during surveys in PNT is consistent with the expected. In fact, the cumulative species curves for both groups reached the asymptote by around the middle of the study period, indicating that sampling effort was adequate in both cases. Similarly, while the confidence intervals of the rarefaction curves for the amphibians converged to zero, indicating sampling sufficiency, those calculated for the reptiles approached the convergence, with reduced estimated error.

In comparison with the PNM Serra do Mendanha – other substantial remnant of dense rainforest in the urban zone of Rio de Janeiro for which systematic medium-term data are available – the results of the present study are broadly consistent. Pontes et al. (2015) recorded 45 amphibians and 39 reptiles at PNM Serra do Mendanha, values closely comparable with the 38 amphibians and 36 reptiles we recorded at PNT. The amphibian species richness recorded in the PNT is also relatively high in comparison with other forested areas in the state of Rio de Janeiro for which data are available (Table 3). While the species richness recorded at the different sites fluctuates considerably, it seems likely that much of this variation is related to differences in survey methods, sampling effort, and the study period (Table 3).

The reptilian species richness recorded in the PNT was also relatively high in comparison with the data from other Atlantic Forest remnants in the state of Rio de Janeiro, as recorded in both short-term (Morro São João, Casimiro de Abreu, N = 9 species; Almeida-Gomes et al. 2008) and medium-term studies (Reserva Ecológica de Guapiaçu,

Dorigo, T.A. et al.

Table 2. Reptile species recorded in Parque Nacional da Tijuca in the municipality of Rio de Janeiro, state of Rio de Janeiro, southeastern Brazil, with data on total abundance (Ab; number of records) and relative abundance (RA; percentage of all recorded amphibians) for the species recorded during the Visual Encounter Surveys are given. Other information include: the conservation status of each species based on their category of threat in the international (IUCN 2020), Brazilian (ICMBIO/MMA 2018), and Rio de Janeiro (RJ) state lists (Bergallo et al. 2000); whether they are endemic (End) to the Atlantic Forest (AF) or to Rio de Janeiro state (RJ), or exotic (Ex); their population trend (PT; IUCN 2020); and Sectors of PNT (ICMBIO 2008) where each species was recorded based on primary (P; see Appendix 1) or secondary (S; see Appendix 2) data. * Species recorded in non-standardized encounters. **Visual record only (no specimens collected). *** Refauna Project (Refauna 2019). Conservation status: NE = Not Evaluated, DD = Data Deficient, LC = Least Concern, VU = Vulnerable, EN = Endangered, and PA = Presumably Threatened ("Presumivelmente Ameaçada"). Population trend: D = Decreasing, I = Increasing, St = Stable, U = Unknown or Unspecified

ТАХА	Ab	Category of threat			End / Ex		РТ		Sect	ors	
	(RA)	Global	National	RJ	AF	RJ		А	В	С	D
CHELONIA											
Emydidae											
Trachemys scripta (Thunberg in Schoepff, 1792)*	-	LC	-	-	Ex	Ex	St	P**			
Testudinidae											
Chelonoidis carbonarius (Spix, 1824)*	-	-	LC	-			-	P**			
Chelonoidis denticulatus (Linnaeus, 1766)	-	VU	LC	-			Unsp	S***			
SQUAMATA											
AMPHISBAENIA											
Amphisbaenidae											
Leposternon microcephalum Wagler, 1824*	-	-	LC	-			Unk	Р			
LACERTILIA											
Dactyloidae											
Anolis punctatus Daudin, 1802*	-	LC	-	-			Unk	P**	S		
Gekkonidae											
Hemidactylus mabouia (Moreau de Jonnès, 1818)*	-	-	-	-	Ex	Ex	-	P**, S	S		
Gymnophthalmidae											
Ecpleopus gaudichaudii Duméril and Bibron, 1839	15 (13.2)	LC	LC	-	End		Unk	P, S	S		
Placosoma glabellum (Peters, 1870)*	-	LC	LC	-	End		Unk	Р			
Leiosauridae								-			
	55	LC	LC	_			Unk	P. S	S		
Enyalius brasiliensis (Lesson, 1830)	(48.2)							-,-			
Mabuyidae											
Psychosaura macrorhyncha (Hoge, 1946)*	-	LC	LC	-	End		Unk	Р			
Phyllodactylidae											
Gymnodactylus darwinii (Gray, 1845)	1 (0.9)	LC	LC	-	End		Unk	P, S	S		
Teiidae											
Salvator merianae Duméril and Bibron, 1839	4 (3.5)	LC	LC	-			St	P**, S			
Tropiduridae											
Tropidurus torquatus (Wied, 1820)*	-	LC	LC	-			Unk	P**			
SERPENTES											
Boidae											
Boa constrictor Linnaeus, 1758*	-	-	LC	-			-	P**			
Colubridae											
Chironius bicarinatus (Wied-Neuwied, 1820)	-	LC	LC	-			D	S	S		
Chironius foveatus Bailey, 1955	5 (4.4)	LC	LC	-	End		Unk	P, S			
Chironius fuscus (Linnaeus, 1758)	-	LC	-	-			St	S	S		
Spilotes pullatus (Linnaeus, 1758)	2 (1.8)	LC	LC	-			St	P**, S			
Spilotes sulphureus (Wagler, 1824)	-	LC	-	-			St		S		
Dinsadidae											

Continue...

Continuation										
Cercophis auratus (Schlegel, 1837)*	-	DD	-	-		Unk	Р			
Dipsas indica Laurenti, 1768	2 (1.8)	LC	LC	-		St	P, S			
Echinanthera cephalostriata Di-Bernardo, 1996	2 (1.8)	LC	LC	-	End	Unk	P, S		S	
Echinanthera melanostigma (Wagler, 1824)	-	LC	LC	-		Unk	S			
Elapomorphus quinquelineatus (Raddi, 1820)	-	LC	LC	-		Unk	S	S	S	
Erythrolamprus miliaris (Linnaeus, 1758)	-	LC	LC	-		St	S	S		S
Erythrolamprus poecilogyrus (Wied-Neuwied, 1825)	-	LC	-	-		Ι	S	S		S
Philodryas olfersii (Lichtenstein, 1823)*	-	LC	LC	-		St	P**			
Siphlophis compressus (Daudin, 1803)	-	LC	-	PA		St	S			
Siphlophis pulcher (Raddi, 1820)	-	LC	LC	-		Unk	S	S		
Taeniophallus affinis (Günther, 1858)	5 (4.4)	LC	LC	-	End	Unk	P, S	S		
Taeniophallus persimilis (Cope, 1869)	-	LC	LC	-		Unk	S			
Thamnodynastes cf. nattereri (Mikan, 1828)	7 (6.1)	-	LC	-		-	P, S			
Xenodon neuwiedii Günther, 1863	1 (0.9)	LC	LC	-		Unk	P, S			
Elapidae										
Micrurus corallinus (Merrem, 1820)	1 (0.9)	LC	LC	-	End	Unk	P, S	S		
Viperidae										
Bothrops jararaca (Wied, 1824)	6 (5.3)	LC	LC	-	End	Unk	P, S	S		
Bothrops jararacussu Lacerda, 1884	8 (7.0)	LC	LC	-	End	Unk	P, S			



Figure 2. Examples of the anurans recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Brachycephalus didactylus*; B) *Brachycephalus ephippium*; C) *Ischnocnema guentheri*; D) *Ischnocnema octavioi*; E) *Ischnocnema parva*; F) *Dendrophryniscus brevipollicatus*; G) *Rhinella ornata*; H) *Euparkerella brasiliensis*. Photographs by T. A. Dorigo

Figure 3. Examples of the anurans recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Haddadus binotatus*; B) *Thoropa miliaris;* C) *Zachaenus parvulus;* D) *Fritziana goeldii;* E) *Aplastodiscus albofrenatus;* F) *Bokermannohyla circumdata;* G) *Boana albomarginata;* H) *Scinax perpusillus.* Photographs by T. A. Dorigo



Figure 4. Examples of the amphibians recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Scinax trapicheiroi;* B) *Phasmahyla guttata;* C) *Crossodactylus gaudichaudii;* D) *Hylodes nasus;* E) *Adenomera marmorata;* F) *Physalaemus signifer;* G) *Siphonops hardyi* (Gymnophiona). Photographs by T. A. Dorigo

N = 37 species; Almeida-Gomes et al. 2014a), and even the short-term study of Vrcibradic et al. (2011) at the Estação Ecológica Estadual do Paraíso, which reached a total of 29 species with the inclusion of secondary data.

Hylidae (N = 10) was the most diverse amphibian family in the PNT, followed by Brachycephalidae and Cycloramphidae, with five species. A similar predominance of hylids in the amphibian assemblage has been recorded not only at other Atlantic Forest sites (e.g., Heyer et al. 1990, Almeida-Gomes et al. 2014a, Silva et al. 2017), but also at other localities in the Neotropical region (e.g., Toft & Duellman 1979, Duellman 1988, Menin et al. 2017). The high diversity of hylids in South America has been explained by the fact that this region is considered to be the center of origin and diversification of this clade (Wiens et al. 2006).

With 14 species, Dipsadidae was the most diverse reptilian family in the PNT, which is consistent with the pattern typically found for the Squamata in Atlantic Forest remnants in the state of Rio de Janeiro (e.g., Pontes et al. 2009, Rocha et al. 2018, Martins et al. 2019). The family Dipsadidae, which is widely distributed in the Americas and West Indies (Hedges et al. 2009, Zaher et al. 2009, Grazziotin et al. 2012), includes the vast majority of the snakes of the Neotropical region, with more than 700 species (see Uetz et al. 2019).

Figure 5. Examples of the reptiles recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Trachemys scripta*; B) *Chelonoidis carbonarius;* C) *Hemidactylus mabouia;* D) *Ecpleopus gaudichaudii;* E) *Placosoma glabellum;* F) *Enyalius brasiliensis;* G) *Psychosaura macrorhyncha;* H) *Gymnodactylus darwinii.* Photograph by T. A. Dorigo

The PNT and the PNM Serra do Mendanha (Pontes et al. 2015) shared 25 amphibian species (Sørensen index of similarity = 0.6; Magurran & McGill 2011) and 29 reptiles (= 0.8). Despite these similarities, 13 amphibian species were exclusive to the PNT, while 27 were exclusive to the Serra do Mendanha. Similarly, seven species of reptiles were exclusive to PNT and ten to the Serra do Mendanha. Similarities of the herpetofauna between these two areas would be accounted at least partly because they would have originally been part of a single continuous forest in the past, and can also be indicative of the effectiveness of the reforestation of the Tijuca massif, carried out during the 19th century (Rocha et al. 2003, Rocha 2017). However, the existence of a unique fauna in both areas also reinforces the importance of the preservation of both forests for the protection of the herpetofauna of the Atlantic Forest in general.

The relative abundance of both amphibian and reptilian species in the PNT was adjusted to a log-series model, a pattern typical of communities of medium diversity, showing certain degree of equilibrium between the rare and dominant species (Magurran & McGill 2011). In this model, most species are relatively rare in comparison with the more abundant taxa (Magurran 2004). The four most abundant amphibian species (*I. parva, H. nasus, I. guentheri*, and *C. gaudichaudii*) contributed to around 70% of the total records of amphibians obtained in the park. The genus *Ischnocnema* belongs to the superfamily Brachycephaloidea (*sensu* Padial et al. 2014), which spawn on the moist forest floor and have direct development, so they do not depend on bodies of water for



Figure 6. Examples of the reptiles recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Tropidurus torquatus*; B) *Chironius foveatus*; C) *Cercophis auratus*; D) *Dipsas indica*; E) *Echinanthera cephalostriata*; F) *Taeniophallus affinis*; G) *Thamnodynastes* cf. *nattereri*; H) *Xenodon neuwiedii.* Photograph by T. A. Dorigo

their breeding and development (Haddad & Prado 2005, Pombal & Haddad 2007). Species that have direct development tend to be dominant in leaf litter-dwelling anuran communities in Neotropical rainforests (e.g., Crump 1971, Allmon 1991, Donnelly 1994, Rocha et al. 2001, Siqueira et al. 2014). Two stream-dwelling frog species, *Hylodes nasus* and *C. gaudichaudii*, were the most abundant species recorded at the PNT. Hylodidae species are generally abundant in small streams in preserved Atlantic Rainforest areas (e.g., Weygoldt 1989, Patto & Pie 2001, Almeida-Gomes et al. 2008, Siqueira et al. 2011b).

The most abundant reptiles in the PNT were *Enyalius brasiliensis* and *Ecpleopus gaudichaudii*, and accounted for approximately 60% of the total reptilian abundance recorded in the present study. *Enyalius brasiliensis* is typical of forested environments, and was relatively abundant in the area in comparison with other forest remnants in the state of Rio de Janeiro, based on short-term (Morro São João; Almeida-Gomes et al. 2008) or mid-term studies (Guapiaçu Ecological Reserve; Almeida-Gomes & Rocha 2014a). *Ecpleopus gaudichaudii* is a Gymnophtalmidae endemic to the Atlantic Forest, which is typically a major component of local leaf-litter reptile communities (e.g., Dixo & Verdade 2006, Almeida-Gomes & Rocha 2014a, Cruz et al. 2014).

Two of the three most abundant snakes recorded during our surveys were members of the family Viperidae (*B. jararaca* and *B. jararacussu*),



Figure 7. Examples of the reptiles recorded during the present study in the Parque Nacional da Tijuca, southeastern Brazil: A) *Micrurus corallinus;* B) *Bothrops jararaca;* C) *Bothrops jararacussu.* Photograph by T. A. Dorigo

whose representatives are common in areas of mainland Atlantic Forest (Marques & Sazima 2004, Pontes et al. 2009). The Dipsadidae *Thamnodynastes* cf. *nattereri* was the second most abundant snake in the PNT, and was also one of the most abundant in the PNM Serra do Mendanha (Pontes et al. 2009).

2. Conservation: endemism, threat status, and exotic species

Most (ca. 80%) of the amphibian species recorded in the PNT are endemic to the Atlantic Forest (Rossa-Feres et al. 2017). This was somewhat expected, as that biome has a high degree of endemism for amphibians (Haddad et al. 2013). By contrast, only 28% of the reptilian



Figure 8. Cumulative (black line) and rarefaction (gray line, with 95 % confidence intervals shown by the dotted lines) curves for the (A) amphibians and (B) reptiles recorded in the Parque Nacional da Tijuca, southeastern Brazil, according to the VES sampling effort (number of hours)



Figure 9. Rank-abundance curves (black dots) with the trend line of the species of (A) amphibians and (B) reptiles recorded by the VES method in the Parque Nacional da Tijuca, southeastern Brazil

species recorded are endemic to the Atlantic Forest, although this is also typical for this group (Tozetti et al. 2017). Six of the amphibians are also considered to be endemic to the state of Rio de Janeiro, including two (*H. nasus* and *I. guentheri*) that are endemic to the municipality of Rio de Janeiro. *Hylodes nasus* is known to be restricted to the forests of this municipality, and has, in fact, only been recorded, up to now, in the PNT (Nascimento et al. 2001, Lingnau et al. 2008) and the PNM Serra do Mendanha (Pontes et al. 2015). However, Canedo (2008) has analyzed specimens that may be associated with *H. nasus*, which were collected in the municipality of Volta Redonda, in the state of Rio de Janeiro, and also in Cachoeiro de Itapemirim, in the state of Espírito Santo, although further research is required to confirm the identity of the taxon. *Ischnocnema guentheri* is currently considered to be a species complex (Kwet & Solé 2005, Gehara et al. 2013). Based on mitochondrial and nuclear sequences, combined with bioacoustic data, Gehara et al. (2013) identified *I. guentheri sensu stricto* as a lineage restricted to the PNT, although it seems likely that this form is also present in adjacent areas.

Overall, only six of the species recorded in the PNT (*Allobates* olfersioides, *Cycloramphus eleutherodactylus, Scinax trapicheiroi, Thoropa lutzi, Vitreorana eurygnatha*, and *V. uranoscopa*) are listed under some category of extinction threat (Bergallo et al. 2000, ICMBIO 2018, IUCN 2020), although the populations of 15 species are thought to be declining (IUCN 2020). Of these six species, we recorded only

Table 3. The species richness (S) of amphibians recorded in forest remnants in the state of Rio de Janeiro, Brazil. The information provided is: location, municipality, sampling period, total effort employed in the visual survey (VES, in hours), quadrat (m^2), and pitfall trap methods (with the bucket volume in liters), other methods or sources used (OMS), and the respective reference. * Information on sampling period or effort was not available. PNM = Parque Natural Municipal, RPPN = Reserva Particular do Patrimônio Natural. In the pitfall trap column, BD = bucket-days, BH = bucket-hours. Other methods/sources included zoological collections [CC1 = Centro de Primatologia do Rio de Janeiro (CPRJ), CC2 = Coleção de Anfíbios da Universidade Federal do Estado do Rio de Janeiro (UNIRIO), CC3 = Coleção Eugenio Izecksohn da Universidade Federal do Rio de Janeiro (ZUFRJ)], acoustic searches (AS), funnel traps for tadpoles (FT), tube traps (TT), and Management Plan (MP)

Local	Municipality	Date	VES (h)	Quadrats (m ²)	Pitfall traps (liters)	OMS	S	Reference
Reserva Ecológica de Guapiaçu	Cachoeiras de Macacu	Sep/2004, Jul/2007- Feb/2014	2631	4750	6600 BD (30 and 60)		73	Almeida-Gomes et al. 2014a
Centro Marista São José das Paineiras	Mendes	Apr/2009- Dec/2013	*			CC2,3,4,5 AS, FT	51	Folly et al. 2014
PNM Taquara	Duque de Caxias	Sep/2006- Oct/2008, monthly	*				50	Salles et al. 2009
PNM Serra do Mendanha	Rio de Janeiro	2002-2014	*	*	*	AS, TT	45	Pontes et al. 2015
Espraiado, Serra do Mato Grosso	Maricá, Saquarema, Tanguá	Jan/2011- Dec/2012, monthly	411	180			44	Martins et al. 2014
Serra da Tiririca	Niterói e Maricá	Jul/2008- Jun/2010	435			CC4	42	Pontes & Pontes 2016
Parque Nacional da Tijuca	Rio de Janeiro	Jan/2013- Dec/2015	460			CC4	38	This study
Reserva Rio das Pedras	Mangaratiba	May/1997- Sep/2006	*				41	Carvalho-e- Silva et al. 2008
RPPN Campo Escoteiro Geraldo Hugo Nunes	Guapimirim	Nov/1983- 2010	*			CC2,3,4	40	Silva-Soares et al. 2010
Caledônia, Parque Estadual dos Três Picos	Nova Friburgo	Mar/2008- Mar/2010	400				35	Siqueira et al. 2011b
Estação Ecológica Estadual do Paraíso	Guapimirim	Sep/2004	107	700	600 BD -30	CC1	30	Vrcibradic et al. 2011
Parque Estadual do Cunhambebe	Itaguaí	Jan- Nov/2010	128				28	Ouvernay et al. 2012
Pedra do Sino, Parque Nacional da Serra dos Órgãos	Teresópolis	2009- 2013, 15 days	*	*	*	AS	28	Folly et al. 2016
Floresta Nacional Mário Xavier	Seropédica	Feb/2010- Jan/2011, Apr/2012	416				26	Caram et al. 2016
Parque Estadual do Cunhambebe	Angra dos Reis	*	82		1440 BH -2		21	Lima et al. 2014
Monte Verde	Cambuci	Sep/2006	75	750	450 BD		20	Almeida-Gomes et al. 2010
Morro São João	Casimiro de Abreu	May- Jun/2005	60	750	450 BD -30		16	Almeida-Gomes et al. 2008
Parque Estadual do Desengano	Santa Maria Madalena	May- Jun/2006, 11 days	75	750	330 -30		13	Siqueira et al. 2011a

one, S. trapicheiroi, during our field surveys. However, although S. trapicheiroi is listed as Near Threatened by the IUCN due to its greatly reduced area of occurrence of less than 20,000 km² (Rodrigues & Carvalho-e-Silva 2004), it is assigned to the Least Concern category in the Brazilian red list (ICMBIO 2018). Verdade and Rodrigues (2007) allocated three other specific names for geographically widespread populations into synonymy of Allobates olfersioides (Lutz, 1925): A. alagoanus (Bokermann, 1967), A. capixaba (Bokermann, 1967), and A. carioca (Bokermann, 1967). According to this arrangement, A. olfersioides is widely distributed along much of the eastern coast of Brazil, between Rio de Janeiro and the state of Alagoas, 2,000 km to the north. However, Haddad et al. (2013) still considered these disconnected populations as different species: A. olfersioides in the state of Rio de Janeiro, A. capixaba in the states of Espírito Santo, and A. alagoanus in the state of Alagoas. The clarification of whether these populations are distinct species or lineages of the same species is essential (Forti et al. 2017). In their assessment of the conservation status of this amphibian, Haddad et al. (2016) considered only A. carioca to be a synonym of A. olfersioides, which restricted the species distribution to the metropolitan region of Rio de Janeiro. In this case, the reduction of the area of occurrence of the taxon to approximately 7,730 km², the lack of records from some localities, including the PNT, over the past 30 years, and the loss of habitat quality over this period has led to the classification of this species as Vulnerable in both the Brazilian red list (ICMBIO 2018) and that of the IUCN (2020).

Thoropa lutzi is classified as Endangered by the IUCN (2020), but as Data Deficient in Brazil (ICMBIO 2018). This species is known to occur only in the states of Rio de Janeiro and Espírito Santo. Populations have not been observed in Rio de Janeiro over the past 30 years, however, including PNT (Sabbag et al. 2018). The other three species, Cycloramphus eleutherodactylus, Vitreorana eurygnatha, and V. uranoscopa, are listed only at the state level (Bergallo et al. 2000), as Presumably Threatened, and only C. eleutherodactylus is listed by the IUCN (2020), as Data Deficient. Cycloramphus eleutherodactylus is known to occur in the Brazilian states of Paraná, São Paulo, Rio de Janeiro, and Minas Gerais (Rossa-Feres et al. 2017, Santos-Pereira et al. 2018), although some studies have reported its disappearance by the beginning of the 20th century (Izecksohn & Carvalho-e-Silva 2001, Eterovick et al. 2005). We did not collect C. eleutherodactylus during fieldwork and it has not been found at PNT since 1972 (Matos 2011). Nevertheless, Lima et al. (2012) concluded that this species may be relatively abundant in some cave environments, and emphasized the importance of including these habitats in amphibian inventories.

Hylodidae species of three genera, *Crossodactylus*, *Hylodes* and *Megaelosia*, are rheophilic and endemic to the Atlantic Forest (Laia & Rocha 2012), where they are typically found in well-preserved forest fragments (e.g., Santos-Pereira et al. 2016, Malagoli et al. 2017). These species are strongly associated with this type of environment, and depend on streams throughout their life cycle (eggs, tadpoles, and adults), being thus vulnerable to the loss of riparian forest (Almeida-Gomes et al. 2014b). These anurans are particularly relevant to conservation initiatives, given that their effective area of occupation is normally restricted to water courses, a minor fraction of the total area of most forests, which makes them especially vulnerable to extinction (Almeida-Gomes et al. 2014b).

Only one of the reptiles recorded in the present study, the tortoise *Chelonoidis denticulatus*, is considered to be under some threat of extinction, being classified as Vulnerable by the IUCN (2020), although it is considered to be Least Concern in Brazil (ICMBIO 2018). The occurrence of *Chelonoidis denticulatus* was recorded in the Atlantic Forest of Rio de Janeiro in 1820 by Prince Maximilian zu Wied-Neuwied, who found carapaces of this species on the sandy plains in the north of the municipality of Cabo Frio (Vanzolini 1994, Vargas-Ramírez et al. 2010, Vanzolini & Myers 2015). While *C. denticulatus* was originally recorded in the Atlantic Forest, it appears to have been eradicated subsequently from the forests of eastern Brazil (Vanzolini & Myers 2015). This species is being reintroduced into the PNT by the Refauna Project (Refauna 2019).

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One important question that has yet to be answered regards the original occurrence of the tortoise *Chelonoidis carbonarius* in the state of Rio de Janeiro. This species was originally described by Spix (1824) from specimens obtained from the region of the Amazon River. Pritchard and Trebbau (1984), Vanzolini (1994), Ernst and Leuteritz (1999) and Vargas-Ramírez et al. (2010) described the geographic distribution of the species in Brazil, and in particular, indicated its absence in the state of Rio de Janeiro. While a number of studies have indicated the presence (e.g., Rocha et al. 2004, Köhler 2008) or possible occurrence (Tozetti et al. 2017) of *C. carbonarius* in Rio de Janeiro, it is difficult to establish whether this region was part of the original distribution of the species, or whether its occurrence in the region (if confirmed) is due to the deliberate release into the wild of animals raised as pets or for food.

Two of the species of the herpetofauna recorded in the PNT – *Hemidactylus mabouia* and *Trachemys scripta* – are exotic invaders. The house gecko, *H. mabouia*, was originally introduced into Brazil from Africa during the colonial period, and has since expanded its area of distribution considerably in the country's natural environments (Rocha & Bergallo 2011, Telles et al. 2015, Oliveira et al. 2016, Winck et al. 2017). *Hemidactylus mabouia* was observed in the built-up areas of the PNT, which is typical of its occurrence at other localities (Rocha et al. 2011, Oliveira et al. 2016). The presence of this lizard in the vicinity of the park's natural habitats demands special attention due to the potential for the species to become invasive (Rocha et al. 2011).

The pond slider, *Trachemys scripta*, was originally found in the southern United States and northern Mexico, but since the 1970s, large numbers have been raised in the United States for the pet trade, and this resulted in the introduction of the species in many places around the world (e.g., Ramsay et al. 2007, van Dijk et al. 2011). The presence of *T. scripta* in natural environments has negative impacts on the native freshwater turtles (e.g., Cadi & Joly 2004, Hidalgo-Vila et al. 2009). *Trachemys scripta* has been recorded in disturbed areas within the PNT, probably as the result of intentional release of animals raised as pets. It is still unclear whether this exotic species has established a viable population in the state of Rio de Janeiro (Oliveira et al. 2020).

A number of amphibian and reptile species of interest occur in the PNT, including those endemics to either the Atlantic Forest or to Rio de Janeiro state and/or municipality, those under some threat of extinction, and those with declining populations. The presence of all those taxa reinforces the importance of the park for the conservation of the region's herpetofauna. The considerable diversity of reptiles and amphibians found in the PNT also testifies to the success of the reforestation program of this protected area, which now provides an important refuge for species of both groups. The results of our inventory, which recorded a total of 74 species, constitute the first comprehensive published overview of the composition and abundance of the herpetofauna of the Parque Nacional da Tijuca.

Supplementary Material

The following online material is available for this article:

Appendix 1 - Voucher specimens of amphibians and reptiles from the Parque Nacional da Tijuca deposited at the Museu Nacional, Rio de Janeiro (MNRJ).

Appendix 2- List of species of amphibians and reptiles recorded from herpetological collections, and respective voucher numbers, from Parque Nacional da Tijuca, Rio de Janeiro, Brazil.

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Author Contributions

Thiago Arnt Dorigo: 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.

Carla Costa Siqueira: 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.

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Carlos Frederico Duarte Rocha: 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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Species composition of small non-volant mammals in the Parque Estadual das Fontes do Ipiranga, São Paulo, Brasil

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Abstract: This study provides the first inventory of small non-volant mammals in the Parque Estadual das Fontes do Ipiranga (PEFI), a protected area in the city of São Paulo, Brazil. The data was collected from 2015 to 2017 in 16 research campaigns with a duration of five days each. Four areas with different phytophysiognomies were sampled throughout the dry and rainy seasons. We sampled small mammals using live-capture and pitfall traps. Eleven species were captured, composed of six rodents and five marsupials. The sampling effort involved 5,600 traps/night, there were 527 capture events, and we captured 302 distinct individuals: 174 marsupials and 128 rodents. Recaptures accounted for 42.7% of the total captures. No significant differences were observed in the richness and abundance of small non-volant mammals between the different phytophysiognomies. We also found no significant differences in the richness and abundance of small non-volant mammals between the dry and rainy seasons. The relative abundance (Ar) and constancy index (C) of the species showed that the three most abundant and common species in the PEFI are: Didelphis aurita, Akodon montensis and Oligoryzomys nigripes, which represented 93.7% of the captures. Tomahawk traps accounted for 69% of the total captures, and pitfall traps were responsible for detecting the greatest richness, capturing 81.8% of the species. Comparing the efficiency of the different sampling methods in capturing small mammals in the PEFI, we observed significant differences between both pitfall versus Tomahawk and pitfall versus Sherman in the understory. The results obtained in this study are consistent with the past and current situations of the forest fragment which are in recovery after significant altered by anthropic activity. In light of this scenario of degradation and isolation, a defaunation process affecting the mastofauna is very likely in the PEFI, which favors the establishment and dominance of generalist species. This study could be the basis for further monitoring programs of small non-volant mammals. The data obtained here will also increase knowledge about the diversity of small mammals in urban fragments of the Atlantic Forest and demonstrate the importance of the PEFI for the maintenance of ecologically important species within the largest metropolitan region in Brazil. These species play important biological roles for the maintenance of ecological interactions and for the provision of rare ecosystem services for the anthropic landscape, which is of great value to the city of São Paulo. Keywords: Rodentia; Didelphimorphia; inventory; mastofauna.

Composição de espécies de pequenos mamíferos não voadores do Parque Estadual das Fontes do Ipiranga, São Paulo, Brasil

Resumo: Este é o primeiro inventário para pequenos mamíferos não voadores no Parque Estadual das Fontes do Ipiranga (PEFI), uma Unidade de Conservação da cidade de São Paulo. A coleta de dados ocorreu entre 2015 e 2017, em 16 campanhas de cinco dias cada. Quatro diferentes fitofisionomias foram amostradas entre os períodos seco e chuvoso. Amostramos os pequenos mamíferos não voadores utilizando armadilhas de captura viva. Foram capturadas 11 espécies, sendo seis de roedores e cinco de marsupiais. O esforço amostral foi de 5.600 armadilhas/ noite, com 527 eventos de captura, sendo 174 marsupiais e 128 roedores. As recapturas representaram 42.7% do total de eventos. Não foi observada diferença significativa entre riqueza e abundância de pequenos mamíferos

não voadores entre as diferentes fitofisionomias. Nós também não encontramos diferença significativa na riqueza e abundância de pequenos mamíferos entre o período seco e chuvoso. A abundância relativa (Ar) e o índice de constância (C) das espécies foram calculados, demonstrando que as três espécies mais abundantes e comuns no PEFI são: Didelphis aurita, Akodon montensis e Oligoryzomys nigripes, as quais representaram 93.7% das capturas. As armadilhas Tomahawk foram responsáveis por 69% do total de capturas, e as armadilhas de queda foram responsáveis por detectar a maior riqueza, capturando 81.8% das espécies. Comparando a eficiência dos diferentes métodos de amostragem para capturar os pequenos mamíferos do PEFI, nós observamos diferença significativa entre pitfall versus Tomahawk do sub-bosque e pitfall versus Sherman também do sub-bosque. Os resultados obtidos neste estudo condizem com o histórico e situação atual do fragmento florestal, considerado em recuperação após ser severamente alterado por ações antrópicas. Diante do cenário de degradação e isolamento, é muito provável que no PEFI tenha existido um processo de defaunação que acometeu a mastofauna, favorecendo o estabelecimento e predominância de espécies generalistas. Este primeiro estudo poderá servir como base para futuros programas de monitoramento dos pequenos mamíferos não voadores. Os dados aqui obtidos também agregam conhecimento sobre a diversidade de pequenos mamíferos em fragmentos urbanos de Mata Atlântica e demonstram a importância do PEFI para a manutenção de espécies ecologicamente importantes dentro da maior cidade do Brasil. Essas espécies cumprem papel biológico importante para a manutenção das interações ecológicas e provimento de serviços ecossistêmicos raros para a paisagem antrópica, com grande valor para a cidade de São Paulo.

Palavras-chave: Rodentia; Didelphimorphia; inventário; mastofauna.

Introduction

The Atlantic Forest is one of the most threatened biomes in the world and one of the 25 global biodiversity hotspots (Mittermeier et al. 2004, Ribeiro 2009, Jenkins et al. 2013). It originally covered a total area of 1,315,460 km², spread over 17 Brazilian states (Peres 2010). The Atlantic Forest is also one of the regions with the highest biological richness on the planet (Mittermeier et al. 2006). Its remnants currently comprise only 12.4% of its original coverage and are mostly found in small fragments of less than 250 ha, of which only 9.3% are within protected areas (Hirota 2018). Although extremely degraded, the Atlantic Forest remains remarkably heterogeneous and its wide variety of ecosystems allows the occurrence of diverse plant and animal species (Galindo-Leal & Câmara 2005).

Considered to be the Brazilian biome with the second largest number of species and endemic mammals (MMA 2002, Paglia et al. 2012), the Atlantic Forest is one of the most diverse regions of small mammals in South America (Galindo-Leal & Câmara 2003), comprising 105 species of rodents (Patton et al. 2015) and 30 species of marsupials (Bovendorp et al. 2017). However, the absence of published species inventories of some areas has created a significant knowledge gap concerning the presence and distribution of its taxa (Costa et al. 2005, Brito et al. 2009, Galetti et al. 2009, De Vivo et al. 2011). Bovendorp et al. (2017) compiled information from 136 studies conducted on small non-volant mammals from seven different types of vegetation in the biome and these results enabled the identification of priority areas for future sampling efforts. Despite some advances in research, further studies on the diversity of the Atlantic Forest species are necessary in order to increase both the understanding and direct conservation efforts for biodiversity (Galetti et al. 2009, Ribeiro 2009).

The Parque Estadual das Fontes do Ipiranga - PEFI is among the few, but important, remaining areas of Atlantic Forest effectively protected as *Conservation Units* in the city of São Paulo (Whately et al. 2008). Its boundaries have been set since 1893, when the PEFI area had approximately 697 ha (Barbosa et al. 2002). Since then, this fragment has been affected by the construction of highways and avenues, the urbanization of neighboring districts and by fires, leading to a decline in its vegetation cover (Peccinini & Pivello 2002). The PEFI vegetation is typical of dense tropical rainforest of the Atlantic hillside (São Paulo 2008), where altitude ranges from 770 to 825 m (Barbosa et al. 2002). The area is typically used for recreation, teaching and research. A number of studies were carried out in the PEFI, and its hydrography, topography, plant physiognomy and climate are well known (Fernandes et al. 2002, Santos 2008, Villagra & Romaniuc-Neto 2010). However, there have been very few studies on the local fauna (Bicudo et al. 2002, Malagoli et al. 2008) until 2013, when some studies on vertebrates began to be carried out (Perrella & Guida 2013, Benedicto 2015, Monticelli & Morais 2015, Lisboa et al. in press, Moraes 2017, Rossi 2017, Monticelli & Antunes 2018, Perrella et al. 2018, Monticelli 2019, Rossi et al. 2020). The aim of these studies has been to increase the knowledge about local fauna and create opportunities for new research.

Studies concerning mammals in the PEFI were mainly conducted on larger species. Thus, despite the advances in research on mammalian fauna, knowledge remains deficient regarding the composition of the small non-volant mammal community. Even though the PEFI is situated along the Atlantic Forest phytophysionomy best studied regarding to composition of small non-volant mammals (Bovendorp et al. 2017, Fiqueiredo et al. 2017), it is precisely located in the Paulista plateau, a regional sampling gap for the group (Figueiredo et al. 2017). Therefore, this study has three objectives: 1) to inventory the small non-volant mammals occurring in the PEFI in order to create a database for future monitoring programs; 2) to compare the success of distinct sampling methods; and 3) to compare richness and abundance of small non-volant mammals throughout seasons and phytophysiognomies.

Materials and Methods

1. Study area

The Parque Estadual das Fontes do Ipiranga (PEFI) is located in the municipality of São Paulo (Figure 1) and borders the municipality of Diadema (23°38'08" S and 23°40'18" S and 46°36'48" W and 46°38'00" W) (Fernandes et al. 2002). The PEFI is one of the country's largest



Figure 1. Location of the Parque Estadual das Fontes do Ipiranga (PEFI) in the city of São Paulo, Brasil, and indication of the sampled areas

and most important remnants of Atlantic Forest inside an urban area (Bicudo et al. 2002) and the third largest fragment of the biome in the city of São Paulo (Rancura & Cerati 2020). It currently comprises a total area of 526.4 ha with 340 ha of biological reserve (Bicudo et al. 2002).

The original area of the PEFI was composed of lands owned by farmers and which underwent a vegetation recovery process after their expropriation by the São Paulo State government, making it an area of secondary forest with little more than 100 years of recovery (Barbosa et al. 2002, Barros et al. 2002). The PEFI phanerogamic flora is composed of 1,159 species from 129 families (Barros et al. 2002, Villagra 2008).

The climate in the city of São Paulo is categorized as Cwa (according to Koppen classification), also called humid subtropical, and is marked by a dry winter and a rainy summer. The average temperature and rainfall establishes that the driest and coldest periods are between the months of April and September, whereas the warmest and rainiest periods of the year correspond to the months of October to March (IAG 2017).

The Atlantic Forest fragment closest to the PEFI is the Parque Estadual da Cantareira which is located approximately 20 km away. Such a scenario of isolation associated with the urban pressure generated by the growth of the surrounding cities led to negative impacts on PEFI biodiversity (Gomes et al. 2003, Monticelli & Morais, 2015).

Based on the Unit Management Plan directions, this study sampled four different points of the PEFI, namely: Instituto de Botânica 1 (Ibot.1) - an area of dense forest with homogeneous canopy; Instituto de Botânica 2 (Ibot.2) - forest with high-sized heterogeneous canopy; Parque de Ciência e Tecnologia 1 (Cient.1) - forest with sparse homogeneous canopy; Parque de Ciência e Tecnologia 2 (Cient.2) - forest with discontinuous canopy/degraded forest.

2. Data collection, capture and tagging

Data collection took place from 2015 to 2017, for a total of 16 monthly research campaigns with a duration of five days each. In all campaigns, the four aforementioned areas (Ibot.1, Ibot.2, Cient.1 and Cient.2) were sampled. Every area was sampled four times, for two campaigns in the rainy season and two in the dry season.

In order to capture the small non-volant mammals, we employed two types of live capture traps: a box-trap (Sherman, size 30 x 7.5 x 9 cm) and a cage-trap (Tomahawk, size 45 x 20 x 20 cm). Traps were baited with a mixture of sardines, cornmeal, bananas, peanut butter and pineapple essence. The mixture was replenished daily.

Two parallel 100-meter lines were established in each area, 30 m apart from each other. Each line had 10 capture stations equidistant at every 10 m. In every station, three live traps were placed spread along the ground (named "forest layer 1") and at about two meters high (named "forest layer 2"), attached to understory branches, totaling 60 live traps per sample site.

Pitfall traps were also arranged in the sampled points, but at 100 m apart and parallel to the live trap lines. On each site, 10 buckets

were installed, 10 m apart from each other, buried up to the soil level (named "forest layer 0"), connected by a guide fence made with black 80-centimeter high plastic canvas and supported by wooden poles and metal staples. Inside each bucket, a piece of expanded polystyrene foam was set down to avoid animal drowning in case of flooding.

Upon the animal capture, the following procedures were executed: taxonomic identification, individual tagging with a numbered metallic earring (Ear tags, National Band and Tag Company, USA), collecting of feces and ectoparasites, weighing, body measuring, sex and reproductive condition recording and subsequent release at the capture site.

The species identity was determined following Gardner (2007) and Patton et al. (2015). Taxonomic identifications of representatives of genera with cryptic species-diversity were performed based on cytogenetic analyses carried out in the Special Laboratory of Ecology and Evolution of the Instituto Butantan.

As there are no previous studies concerning small non-volant mammals from the PEFI, testimony specimens of all the species captured were collected. The specimens were deposited in the mammal collection of the Museu de Zoologia da Universidade de São Paulo and prepared according to the guidelines established by the institution.

The capture success was determined by the total number of captures multiplied by 100 and divided by the capture effort (traps per night). All procedures were authorized by the pertaining environmental agencies under license SISBIO no. 45520 and SISGEN no. AE48610.

3. Richness, abundance and seasonality

The species richness was assessed through the non-parametric estimator Jackknife 1 (Burnham & Overton 1979), using the software EstimateS 9.0 (Colwell 2013). The analysis was performed with 100 randomizations and the days as the sample unit.

In order to compare the richness and abundance among the different phytophysiognomies sampled, we employed a Kruskal-Wallis test. The capture data was also used to evaluate putative differences in richness and abundance of small mammals between the dry and rainy seasons. Finally, we applied a paired t-test for parametric data and the Mann-Whitney test for non-parametric data. In all cases, the significance level adopted was 5% (p < 0.05).

The relative abundance (Ar) of species was determined by the number of individuals of each species captured multiplied by 100 and divided by the total number of individuals captured. The constancy index (C), which allows species to be grouped into categories based on its capture frequency, was established as: common species - present in more than 50% of the samples; relatively common species - present in 25 to 50% of the samples; and rare species - present in less than 25% of the samples (Dajoz 1983).

4. Capture method assessment

The different methods implemented for the capture of small mammals in the PEFI were compared to assess their efficacy at the study site, using the Kruskal-Wallis test and the Dunn post-test.

Results

1. Data collection, capture and tagging

During this study, 11 species of small non-volant mammals were captured, six of which were rodents belonging to the Cricetidae and Caviidae families and five were marsupials belonging to the Didelphidae family (Figure 2). From a 5,600 traps/night sampling effort, 527 capture events occurred, represented by 302 individuals (174 marsupials and 128 rodents; Table 1). Recaptures accounted for 42.7% of the total capture events. The success rate of small mammal capture was 9.41%, being 5.55% in the dry season and 3.85% in the rainy season. The vegetation formation with the highest number of captures was Cient.1, followed by Cient.2, Ibot.2 and Ibot.1 (Table 1).



Figure 2. Species of small mammals captured in the Parque Estadual das Fontes do Ipiranga (PEFI). A) *Didelphis aurita*, B) *Caluromys philander*, C) *Monodelphis americana*, D) *Gracilinanus microtarsus*, E) *Monodelphis iheringi*, F) *Akodon montensis*, G) *Thaptomys nigrita*, H) *Oligoryzomys nigripes*, I) *Blarinomys breviceps*, J) *Juliomys pictipes*, K) *Cavia aperea*

2. Richness, abundance and seasonality

The estimated richness of small mammals calculated was close to the empirical results, indicating 12.04 species occurring in the PEFI. (Figure 3). There was no significant difference in the small mammal richness among the different sampled areas (H = 3.779; p = 0.286), nor in the abundance among the different sampled physiognomies (H = 0.602; p = 0.895).

The relative abundance analysis showed the following values for each species: *Didelphis aurita* (52.7%), *Akodon montensis* (32.4%), *Oligoryzomys nigripes* (8.44%), *Mododelphis iheringi* (2.03%), *M. americana* and *Thaptomys nigrita* (1.01%), *Gracilinanus microtarsus* and *Juliomys pictipes* (0.67%), *Blarinomys breviceps*, *Cavia aperea* and *Caluromys phylander* (0.34%). The constancy index determined three common species: *D. aurita* (100%), *A. montensis* (87.5%), *O. nigripes* (81.25%); and eight rare species: *M. iheringi* (18.75%), *M. americana*, *T. nigrita*, *G. microtarsus* and *J. pictipes* (12.5%), *B. breviceps*, *C. aperea* and *C. phylander* (6.25%). In general, the three most abundant species represented 93.7% of the captures and the remaining eight rare species represented only 6.3% (Table 2).

Nine species were captured during the dry period, five rodents and four marsupials, and six species during the rainy season, four rodents **Table 1.** Capture of small mammals during the dry and rainy seasons inside four different phytophysiognomies formations of the ParqueEstadual das Fontes do Ipiranga, São Paulo, Brasil. Ibot.1 - Instituto de Botânica 1; Ibot.2 - Instituto de Botânica 2; Cient.1 - Parqueda Ciência e Tecnologia 1; Cient.2 - Parque da Ciência e Tecnologia 2

	а. :	C		Loc	Location		
	Species	Season	Ibot.1	Ibot. 2	Cient. 1	Cient. 2	
Rodentia							
Cricetida	ae						
	Akodon montensis Thomas, 1913	Dry	1	5	31	17	
		Rainy	5	10	12	15	
	Oligoryzomys nigripes (Olfers, 1818)	Dry	2	2	3	3	
		Rainy	2	5	2	6	
	Blarinomys breviceps (Winge, 1887)	Dry	—	—	—	-	
		Rainy	—	—	1	-	
	Juliomys pictipes (Osgood, 1933)	Dry	1	—	—	-	
		Rainy	—	1	—	-	
	Thaptomys nigrita (Lichtenstein, 1829)	Dry	-	—	-	3	
		Rainy	-	-	-	-	
Caviidae							
	Cavia aperea Erxleben, 1777	Dry	—	—	—	1	
		Rainy	—	—	—	—	
Didelphi	morphia						
	Didelphis aurita (Wied-Neuwied, 1826)	Dry	29	22	23	17	
		Rainy	16	19	19	17	
	Monodelphis americana (Müller, 1776)	Dry	-	-	-	-	
		Rainy	-	-	2	1	
	Monodelphis iheringi (Thomas, 1888)	Dry	-	-	2	4	
		Rainy	-	-	-	-	
	Caluromys philander (Linnaeus, 1758)	Dry	1	-	-	-	
		Rainy	—	—	_	-	
	Gracilinanus microtarsus (Wagner, 1842)	Dry	—	1	_	1	
		Rainy	-	_	-	-	
	T / 1	Dry	34	30	59	46	
	10721	Rainy	23	35	36	39	
	Overall total		57	65	95	85	
				3	02		

and two marsupials. Although more captures occurred during the dry months (n = 167) compared to the rainy months (n = 135), we found no significant difference in the total richness of small mammals (rodents and marsupials) between the two periods (t = -1.275; p = 0.211). Similarly, there was no significant difference in the richness of rodents (t = 2.507; p = 0.120) and marsupials (t = -0.547; p = 0.340) separately between the seasons. The total abundance of small mammals did not vary significantly between the dry and rainy periods (t = 1.999; p = 0.147), nor did it for rodents (u = 0.480; p = 0.315) and marsupials (u = 0.626; p = 0.265) separately.

In addition to the species of small non-volant mammals, field activities led to the record of four other non-target native taxa, namely: brown howler monkey (*Alouatta guariba* Humboldt, 1812), orange dwarf porcupine (*Coendou spinosus* F. Cuvier, 1823), nine-banded armadillo (*Dasypus novemcinctus* Linnaeus, 1758) and three-toed sloth (*Bradypus variegatus* Schinz, 1825). Additionally, there were unintentional captures of alien species, such as the common marmoset (*Callithrix jacchus* Linnaeus, 1758), the black-tufted marmoset (*Callithrix penicillata* É. Geoffroy, 1812) and domestic animals, such as the common household cat (*Felis catus* Linnaeus, 1758). Taxonomic identification of non-target species followed Abreu et al. (2020).

3. Capture method assessment

With a sampling effort of 2,400 traps/night, the Tomahawk traps accounted for 69% of the total captures, detecting four of the 11 species. Despite this expressive value, 71.5% of the captures with this



Figure 3. Species accumulation curve based on sampling effort in the Parque Estadual das Fontes do Ipiranga (PEFI), São Paulo, Brasil. Blue square corresponds to the jackknife 1 estimator for species richness

Species	No. of specimen captured	Ar (%)		C (%)	Capture method
Rodentia					
Akodon montensis	96	32.4	87.5	Common	ptf-Sh-T
Oligoryzomys nigripes	25	8.44	81.25	Common	ptf-Sh-T
Blarinomys breviceps	1	0.34	6.25	Rare	ptf
Juliomys pictipes	2	0.67	12.5	Rare	Sh
Thaptomys nigrita	3	1.01	12.5	Rare	ptf
Cavia aperea	1	0.34	6.25	Rare	ptf
Didelphimorphia					r
Didelphis aurita	162	52.7	100	Common	ptf-Sh-T
Monodelphis americana	3	1.01	12.5	Rare	pti shi i
Monodelphis iheringi	6	2.03	18.75	Rare	ptf
Caluromys philander	1	0.34	6.25	Rare	Sh-T
Gracilinanus microtarsus	2	0.67	12.5	Rare	ptf
Total	302	100		1.000	L, tr

Table 2. Species of small non-volant mammals captured in the Parque Estadual das Fontes do Ipiranga and their respective values of relative abundance (Ar) and constancy index (C). Ptf = pitfall traps; sh = Sherman; t = Tomahawk

method were *D. aurita*, the most abundant species in the PEFI. With a sampling effort of 800 traps/night and accounting for only 16% of the total capture events, the pitfall trap was not the most effective method of capture, but it was responsible for detecting nine of the 11 species of small mammals. Despite its lower sampling effort and the absence of bait, this method proved to be the most efficient and was essential for the sampling of small mammals in the area. Box traps (Shermans) were the least successful in terms of capture events. With a 2,400 traps/night sampling effort, the same as the Tomahawk, they captured five of the 11 species and their capture events represented only 15% of the total.

Some species were captured exclusively with pitfall traps (forest layer 0): *T. nigrita, G. microtarsus, M. americana, M. iheringi, B. breviceps* and *C. aperea.* Others were captured only in the understory (forest layer 2): *C. phylander.* Live traps on the forest substrate (forest layer 1) did not show any exclusive captures. Individuals of *D. aurita, A. montensis, O. nigripes* and *J. pictipes* were captured in all three of the forest strata sampled.

Concerning the captures in the different strata of the forest, pitfall traps (0) presented the most successful capture rate of 13.65%, followed by live traps (Sherman and Tomahawk) located in the forest substrate (1), with a

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rate of 12.7%, and live traps in the understory (2), with a rate of 3.66%. Assessing the efficiency of the methods for capturing different species of the PEFI, we found a significant difference between the methods (H = 30.24; p = 0.0001). Post-test analysis showed a significant difference between pitfall *versus* Tomahawk from forest stratum 2 (p = 0.05) and pitfall *versus* Sherman from forest stratum 2 (p = 0.05). We found no significant difference between pitfall, Sherman (1) and Tomahawk (1). There was also no significant difference between Sherman (1), Sherman (2) and Tomahawk (2) methods.

Discussion

Environmental characteristics such as vegetation type, primary production and terrain directly impact the mammalian community present in a given area (Peres 2000, Haugaasen & Peres 2005, Galetti et al. 2009). Anthropic actions, such as habitat suppression and fragmentation, also affect the permanence of mammal populations in different environments (Chiarello 1999, Cullen-Junior et al. 2000, Peres 2000, Galetti et al. 2009, Brocardo & Cândido-Junior 2012). These factors in association with the size of the remaining natural area may determine the richness of mammal species (Chiarello 1999), as the absence of large protected areas has been directly related to the decrease in species, especially those of larger size (Chiarello 2000, Gurd et al. 2001, Ceballos et al. 2005, Cardillo et al. 2005, Jorge et al. 2013).

Large remnants of the Atlantic Forest are related to the viable maintenance capacity of several species of mammals (Chiarello 1999, 2000, Cullen-Junior et al. 2000). Contrarily, the PEFI is an example of the loss of vegetation cover and fragmentation which, due to its isolation as a small area inside the anthropic landscape of São Paulo, the most populous city in Brasil, presents a low richness of small nonvolant mammals when compared with other areas of the Atlantic Forest found nearby and better preserved: 32 species found in the Estação Ecológica do Bananal (Abreu-Junior & Percequillo 2019); 23 in the Reserva Florestal do Morro Grande (Pardini & Umetsu, 2006); and 21 in the Parque Estadual Carlos Botelho (Brocardo et al. 2012). However, the number of species recorded in the PEFI is still compatible with the majority of the studies reported for the Atlantic Forest biome (Figueiredo et al. 2017). These results were expected, considering that a substantial reduction in mammal richness has been reported for small fragments of the Southern Atlantic Forest (Abreu-Junior & Köhler 2009, Brocardo & Cândido-Junior 2012) in Southeastern (Chiarello 1999, Briani et al. 2001, Pardini et al. 2005) and Northeastern Brazil (Silva Junior & Pontes 2008). In light of its loss of vegetation cover and isolation, it is possible that a defaunation process is occurring in the PEFI, affecting not only the small mammals, but mainly other mammals which are larger and/ or have greater habitat requirements. In the PEFI no invasive rodents were captured, which is surprising considering all its anthropogenic modifications. According to Bovendorp et al. (2017), 24% of the Atlantic Forest fragments have at least one species of invasive rodent.

The forest types present in areas Ibot. 1 and Ibot.2 are considered similar to each other according to Bicudo et al. (2002) and they show a relatively larger number of arboreal individuals compared to the other areas, with distinct aggregation patterns and dense understory. Fewer species were found in these areas. The Cient.1 area is comparable to Ibot.1 and 2 (Bicudo et al. 2002) and presented a similar number of species. These three areas are considered to be less degraded than Cient.2 (Bicudo et al. 2002).

Although we did not find significant differences in small mammal richness in the PEFI among four sampled areas, the Cient.2 had the highest number of species, where eight of the 11 species were captured. Of these eight, six were terrestrial or semi-fossorial (*C. aperea*, *T. nigrita*, *A. montensis*, *O. nigripes*, *M. americana* and *M. iheringi*). This area is comprised of continuous forest, degraded by fires that occurred in the late 1990s and early 2000s (Peccinini & Pivello 2002). Pardini & Umetsu (2006) suggest that younger or more altered forests, as found in Cient.2, lead to greater biomass production and, consequently, greater availability of fruits and arthropods which are the main items consumed by small non-volant mammals. In addition, this variety of phytophysiognomy has a relatively more opened canopy with a denser understory which favors the proliferation of terrestrial or understory species and minimizes the chance of occupation by forest canopy species (Pardini & Umetsu 2006).

The Ibot.1 site was the only one in which *C. philander*, an arboreal species commonly found in canopies but also in the understory (Delciellos et al. 2006), was captured. The exclusive capture at this site may be related to the fact that the species is described as arboreal of medium to high canopies (Aragona & Marinho-Filho 2009) and the site presents a large concentration of 4 to 6 m tall trees, due to its absence of recent major impacts.

Communities of small non-volant mammals are long considered to be generally composed of two or three dominant species and other species tend to be in greater rarity (Fleming 1975), a pattern largely considered to be a response to adaptive flexibility of dominant species. The results found in this study are in accordance with this expected pattern for a community of small non-volant mammals in degraded/ recovering areas of the Atlantic Forest (Pardini et al. 2005, Puttker et al. 2008). Other characteristics of the PEFI such as isolation, secondary forest and urban surroundings might also promote this scenario of species occupation. These elements favored the establishment and predominance of three generalist species in the PEFI: *D. aurita, A. montensis* and *O. nigripes*, which accounted for a combined 93.7% of total captures. Seven of the 11 species captured in the PEFI are among the 22 species suggested as hyper-dominant in the Atlantic Forest (Bovendorp et al. 2017).

The process of forest regeneration and the impacts it suffers, such as fires, cause environmental changes that may reflect on the structure of the small mammal community, as observed by Oliveira (1995), creating new species dynamics to be later assessed. Pinotti (2010) suggests that both structural characteristics of the forest (biomass and depth of leaf litter, branch volume and number) as well as food availability (arthropod biomass in the soil, richness of fruiting plants and number of individuals fruiting in the understory) are liable to change as a result of the forest regeneration process and are strongly linked to favoring or disadvantaging species, either specialists or generalists. Generalist species such as D. aurita, A. montensis and O. nigripes also benefit from a greater availability of food resources found in areas at an earlier stage of regeneration. Specialized forest species have a higher occupancy capacity in more mature forests, where these resources are scarcer (Pinotti 2010). However, it is important to note that the absence of connectivity between the PEFI and other forest fragments prevents the recolonization of the area by other species, which could potentially increase the richness of the rodent and marsupial community (Pardini et al. 2005).

As *D. aurita* was the most abundant species in the PEFI, the low richness found may have been influenced by the presence of this species. Fonseca & Robinson (1990) suggested that the increase in the density of *D. aurita*, due to the absence or small abundance of predators, for example, could be related to the low richness of small terrestrial mammals in smaller fragments, either due to competition for resources or even predation (Graipel et al. 2003).

The absence of PEFI predators due to reduced vegetation cover and all the effects of isolation inside the anthropic landscape may also be related to the rates of recapture found in this study. The high recapture value (42.7%) suggests that the areas sampled were not occupied by new individuals during the study period. Such a low turnover of individuals may be related to the absence of medium and large predators, which play an important role in the dynamics and structure of the mammal community (Fonseca 1988). For the PEFI, there are no records of native carnivorous mammals and this absence can decrease the rate of predation, favor the permanence of individuals in the same area for a longer time and, consequently, decrease the turnover of individuals between adjacent territories thus resulting in spatial stability, a fact also observed by Lessa et al. (1999).

Regarding the species found in the different strata, the trapping method alone is not sufficient to estimate the use of vertical space by small mammals (Delciellos et al. 2006, Preveddello et al. 2008). The finding of species described as terrestrial (*A. montensis*) or arboreal (*J. pictipes*) (Paglia et al. 2012) on ground and understory may be related to physical characteristics of the area, such as connectivity due to the presence of lianas and fallen trunks, seasonal variations in food availability and interspecific competition (Begon et al. 2006, Lambert et al. 2006, Hannibal & Caceres 2010). *Didelphis aurita* and *O. nigripes* are considered scansorial and can be found on the ground and understory (Paglia et al. 2012). Although young individuals of *D. aurita* have been previously reported to be more prevalent in the upper strata (Prevedello et al. 2008), in this study, 62.83% (n = 71) of young individuals of *D. aurita* were captured on the ground and 37.17% (n = 42) in understory.

Whereas not significantly different, the capture success rate of this study was greater in the dry season (5.55%) than in the rainy season (3.85%). This can possibly be explained by the variation throughout the year in the availability of food resources. Higher rainfall is related to a greater supply of arthropods (Janzen 1973, Charles-Dominique 1983, Wolda 1993, Santos-Filho et al. 2008) and fruits in the environment (Foster 1982, Charles-Dominique 1983, Julien-Laferrière & Atramentowicz 1990, Bergallo & Magnusson 1999, 2002, Santos-Filho et al. 2008). This makes the animals more likely to find food while moving less (Stallings 1988), thus decreasing the efficiency of the baits and the probability of capturing small mammals (MacClearn et al. 1994). A higher relative rate of capture in the season of lower rainfall has previously been observed in other studies with determined seasonality (Mello 1980, O' Connell 1989, MacClearn et al. 1994, Vieira 2002, Alho 2003, Santos-Filho et al. 2008). Among the five species of marsupials captured, three were captured exclusively in the dry period (M. iheringi, C. philander and G. microtarsus), one was captured in both periods, but more often in the dry period (D. aurita), and one species was captured only in the rainy season (M. americana). For rodents, among the six species captured during the study, one was exclusively captured during the rainy season (B. breviceps), two during the dry season (T. nigita and C. aperea) and three were captured in both periods (A. montensis, O.

nigipes and *J. pictipes*). *A. montensis* was the most frequently captured species in the dry season and *O. nigripes* in the rainy season.

As there is no previous research on small non-volant mammals from the PEFI, this novel study can be a starting point for monitoring programs of this group, aiming to evaluate possible local ecological changes and advances in the defaunation process.

The different capture methods used in this study complemented each other, as they were responsible for the capture of different species, showing the importance of the use of diverse techniques to capture small non-volant mammals (Santos-Filho et al. 2006, Caceres et al. 2011, Bovendorp et al. 2017).

Even though baits are not used in pitfall traps, they have previously been associated with higher capture success rates (Hice & Schmidly 2002, Santos-Filho et al. 2006, Umetsu et al. 2006). In this study, the method with the greatest success in capturing small mammals was the use of cage-type traps (Tomahawk). The difference in the capture success rate of Tomahawks *versus* Shermans was probably due to the size of the traps. Since the most abundant animal species captured in this study reached around 2 kg, this size was probably incompatible with the Shermans culminating in a lower capture rate using this method.

Considering the great influence marsupials and small rodents have on forest dynamics, as well as their role as habitat quality indicators (Pardini & Umetsu 2006), the importance of knowing their community formation cannot be underestimated, as its understanding may direct conservation efforts of the forests and species.

Of the 11 species captured in this study, none were included in the national list of threatened species (ICMBio 2018), but six (54.55%) are endemic to the Atlantic Forest: *B. breviceps*, *J. pictipes*, *T. nigrita*, *D. aurita*, *M. iheringi* and *G. microtarsus* (Paglia et al. 2012). In the state of São Paulo, *M. iheringi* is on the list of threatened species in the "vulnerable" category, and five of the species captured are classified as "almost threatened" (*B. breviceps*, *J. pictipes*, *T. nigrita*, *M. americana* and *G. microtarsus*) (São Paulo 2018).

The results of this study add knowledge about the biodiversity of the Atlantic Forest in an urban fragment in São Paulo. Although species richness is not high and generalist species are predominant, the PEFI is important for the maintenance of different animal and plant species, which in turn, play important biological roles for the maintenance of ecological interactions. This demonstrates the great value of the PEFI to the city of São Paulo, due to its provision of rare ecosystem services for the anthropic landscape of the city.

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Author Contributions

Cauê Monticelli: contributed to the concept and design of the study, to data collection, analysis and interpretation; to manuscript preparation; with critical revision and adding intellectual content. Kauê Souza de Moraes: contributed to data collection, analysis and interpretation; to manuscript preparation and adding intellectual content.

Luan Henrique Morais: contributed to data collection.

Amanda Alves de Moraes: contributed to the concept and design of the study, to data collection, to manuscript preparation; with critical revision and adding intellectual content.

Conflicts of Interest

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

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Terrestrial Gastropoda from the caves of Presidente Olegário, southeastern Brazil

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Abstract: Samples of terrestrial gastropods were collected year-round in seven caves in Presidente Olegário municipality, Minas Gerais state, southeastern Brazil, during several expeditions from 2012 to 2014. Twenty-four taxa (plus a single freshwater species), mainly stylommatophorans, were found in the material. The following species are reported for the first time for Minas Gerais state: *Alcadia iheringi* Wagner, 1910 and *Helicina sordida* King, 1831 (Helicinidae); *Cecilioides consobrina* (d'Orbigny, 1841) (Ferussaciidae); *Entodina gionensis* Morretes, 1940 and *Scolodonta interrupta* (Suter, 1900) (Scolodontidae); *Megalobulimus sanctipauli* (Ihering & Pilsbry, 1900) (Strophocheilidae); *Drymaeus coarctatus* (Pfeiffer, 1845) (Bulimulidae); *Habroconus semenlini* (Moricand, 1846) (Euconulidae); and *Solaropsis* aff. *rosaria* (Pfeiffer, 1849) (Solaropsidae). Furthermore, the species *Drymaeus iracema* (Simone, 2015) and *Drymaeus terreus* (Simone, 2015) are synonymized with *Drymaeus coarctatus* (L. Pfeiffer, 1845).

Keywords: Minas Gerais; Neritimorpha; Stylommatophora; stygofauna; troglofauna..

Gastrópodes terrestres de cavernas da região de Presidente Olegário no sudeste brasileiro

Resumo: Amostras de gastrópodes terrestres foram coletadas em sete cavernas na região do município de Presidente Olegário, Minas Gerais, Brasil, durane múltiplas expedições ao longo dos anos de 2012 a 2014. Vinte-e-quatro táxons (mais uma única espécie dulciaquícola) foram encontrados, em sua maioria Stylommatophora. As seguintes espécies são aqui reportadas pela primeira vez para o estado de Minas Gerais: *Alcadia iheringi* Wagner, 1910 e *Helicina sordida* King, 1831 (Helicinidae); *Cecilioides consobrina* (d'Orbigny, 1841) (Ferussaciidae); *Entodina gionensis* Morretes, 1940 e *Scolodonta interrupta* (Suter, 1900) (Scolodontidae); *Megalobulimus sanctipauli* (Ihering & Pilsbry, 1900) (Strophocheilidae); *Drymaeus coarctatus* (Pfeiffer, 1845) (Bulimulidae); *Habroconus semenlini* (Moricand, 1846) (Euconulidae); e *Solaropsis* aff. *rosaria* (Pfeiffer, 1849) (Solaropsidae). Além disso, as espécies *Drymaeus iracema* (Simone, 2015) e *Drymaeus terreus* (Simone, 2015) são aqui sinonimizadas com *Drymaeus coarctatus* (L. Pfeiffer, 1845).

Palavras-chave: Minas Gerais; Neritimorpha; Stylommatophora; estigofauna; troglofauna.

Introduction

Brazilian cave-dwelling invertebrates have historically received scarce attention from researchers (Simone & Moracchioli 1994; Bichuette & Trajano 1999, 2003; Trajano & Bichuette 2010), a fact that is even more marked for mollusks (Salvador, 2019b). During the past decade, however, Dr. Maria E. Bichuette and her team from the Universidade Federal de São Carlos (UFSCar; São Carlos, Brazil) have been conducting collecting expeditions to cave systems throughout Brazil focusing on several animal taxa. The molluscan material they collected has been deposited in the malacological collection of the Museu de Zoologia da Universidade de São Paulo (MZSP; São Paulo, Brazil) and our team has been steadily studying it over the past years. Those efforts are leading to an increase in the amount of information on cave-dwelling mollusks (e.g.; Simone 2012, 2013; Salvador et al. 2016, 2017; Simone et al. 2020). This increase in studies is very welcome, both from the perspectives of mollusk taxonomy (Salvador 2019b) and of environmental protection, given that cave ecosystems are usually fragile but harbor a reasonable number of endemic species (Trajano 2000; Gallão & Bichuette 2012; Weigand 2013).

Some of those expeditions were undertaken by Dr. Bichuette to cave systems in the municipality of Presidente Olegário, Minas Gerais state, SE Brazil (September/2012 to June/2014). That karst region is famous for its multiple calcareous caves (Secutti & Buchuette 2013) and, amidst a wealth of specimens of varied taxa, Dr. Bichuette's team collected many terrestrial and a few freshwater snails. No surveys of the molluscan fauna have been published from that locality so far, so the material they collected brings new information. We study those specimens herein, reporting twenty-five species, some of which are new records for Minas Gerais state.

Material and Methods

All the material studied here was collected by Maria Elina Bichuette and her team (G.F. Damasceno, J.S. Gallo, L.P.A. Resende, I.A. Ribeiro, and T. Zepon) and is deposited in the MZSP. All the specimens were collected in calcareous caves in the municipality of Presidente Olegário, Minas Gerais state, SE Brazil (Figure 1).



Figure 1. Map showing the municipality of Presidente Olegário in Minas Gerais state, SE Brazil, and the caves in the area: 1, Lapa Arco da Lapa; 2, Lapa da Fazenda São Bernardo; 3, Lapa da Juruva; 4, Lapa do Moacir; 5, Lapa Vereda da Palha; 6, Lapa Zé de Sidinei; 7, Toca do Charco. Abbreviations: DF, Distrito Federal; GO, Goiás state; MG, Minas Gerais state.

Presidente Olegário is located in the southern Alto São Francisco Basin, being inserted into the domains of the Cerrado Biome. The climate is tropical, semi-humid, with four to five months of drought (Nimer 1989). The karst of the region's landscape is rich with calcareous caves (Secutti & Buchuette 2013). Seven different caves were surveyed and collection took place in four instances, covering the whole seasonal range: September/2013, and January, April, and June/2014 (Table 1).

The material comprised both empty shells and a few animals collected live; the latter were preserved in ethanol 70%. For each collection effort, the time of active search used was always the same for each cave, according to the cave's size. Table 1 presents a list with all caves, alongside additional information on each.

Table 1. List of all caves in the municipality of Presidente Olegário, Minas Gerais state, Brazil, where the present material was	collected.
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Locality	Coordinates	Altitude	Cave length	Cave gap	Collection dates
Lapa Arco da Lapa	18°12'31"S 46°08'53"W	798 m	ca. 500 m	15 m	29/Sep/2013, 21/Jan/2014, 14/ Apr/2014, 13/Jun/2014
Lapa da Fazenda São Bernardo	18°16'37"S 46°06'46"W	802 m	ca 2000 m	10 m	30/Sep/2013, 23/Jan/2014, 13/ Apr/2014, 10/Jun/2014
Lapa da Juruva	18°19'19"S 46°04'53"W	808 m	ca. 1100 m	29 m	11/Jun/2014
Lapa do Moacir	18°11'10"S 46°09'34"W	773 m	ca. 200 m	5 m	20/Jan/2014, 17/Apr/2014, 13/ Jun/2014
Lapa Vereda da Palha	18°15'19"S 46°07'34"W	780 m	ca. 2500 m	25 m	27/Sep/2013, 22/Jan/2014, 15/ Apr/2014, 12/Jun/2014
Lapa Zé de Sidinei	18°18'06"S 46°05'41"W	850 m	ca. 650 m	20 m	28/Sep/2013, 24/Jan/2014, 16/ Apr/2014, 14/Jun/2014
Toca do Charco	18°11'06"S 46°09'39"W	764 m	ca. 80 m	3 m	29/Sep/2013, 20/Jan/2014, 17/ Apr/2014, 13/Jun/2014

Identification was conducted based on the original descriptions (and type material whenever possible), the catalog of Simone (2006), further taxonomic literature, and additional comparative material (preferably from Minas Gerais state when possible) housed in the collection of the MZSP. Selected specimens were analyzed under SEM in the Staatliches Museum für Naturkunde Stuttgart (SMNS, Stuttgart, Germany). Some specimens (either juvenile or fragmentary) could not be identified beyond genus or even family level (Table 1).

The following abbreviations are used herein. Shell dimensions: H, shell height (parallel to coiling axis); D, greatest shell width (perpendicular to H); h, aperture height (maximum length parallel to coiling axis); d, greatest width of aperture (maximum width perpendicular to coiling axis). Measurements were made with a digital caliper or, for minute shells, with the aid of computer software (Leica Application Suite [LAS] v.3.8.0 and ImageJ). Material: sh, empty shell(s); spm, specimen(s) collected live, ethanol-preserved. Caves: AdL, Lapa Arco da Lapa; FSB, Lapa da Fazenda São Bernardo; Jur, Lapa da Juruva; Moa, Lapa do Moacir; TdC, Toca do Charco; VdP, Lapa Vereda da Palha; ZdS, Lapa Zé de Sidinei. Cave zones: EP, epigean; EZ, entrance zone; TZ, twilight zone; DZ, dark (aphotic) zone.

Results

The complete list of species, the cave(s) (and the cave zone) where each occurs, and a relation of all the studied material are reported in Table 2. Species that require further discussion (*i.e.*, those with new records for Minas Gerais state) are addressed below and figured (Figure 2), arranged according to the classification of Bouchet et al. (2017). The other species (i.e., not new records for Minas Gerais) are, nevertheless, recorded here for the first time from these caves.

Neritimorpha Family Helicinidae Genus *Alcadia* Gray, 1840

Alcadia iheringi Wagner, 1910 Figure 2A–C

Alcadia iheringi Wagner, 1910: 354, pl. 70, figs. 7–8; Morretes, 1949: 63; Salgado & Coelho, 2003: 151; Simone, 2006: 42, fig. 36; Agudo-Padrón, 2008: 151; Agudo-Padrón, 2012: 40; Agudo-Padrón, 2014: 10; Birckolz et al., 2016: table 1; Salvador et al., 2016: 61, figs. 2–4; Zepon & Bichuette, 2017: 5.

Alcadia (Alcadia) iheringi: Haas, 1959: 365.

Type locality. Nova Teutônia, Santa Catarina state, Brazil.

Previously known distribution. Type locality; caves in Alto Ribeira State Park, São Paulo state.

New records. Presidente Olegário: in caves "Lapa da Fazenda São Bernardo" and "Lapa Zé de Sidinei". The present record extends the species range ca. 700 km to the northeast (ca. 650 km to the north).

Identification. Small conic-globose helicinid shell, with comparatively (among congeners) small and circular aperture.

Genus Helicina Lamarck, 1799 Helicina sordida King, 1832 Figure 2D–F *Helicina sordida* King, 1832: 339; Pfeiffer, 1852: 622; Hupé, 1857: 62; Martens, 1867: 8; Salgado & Coelho, 2003: 152; Simone, 2006: 40, fig. 30.

Type locality. Rio de Janeiro, Brazil.

Previously known distribution. Rio de Janeiro (uncertain if state or city).

New records. Presidente Olegário: in cave "Lapa do Moacir". The present record extends the species range at least 400 km to the northwest.

Identification. Wide helicinid shell with prominent keel on body whorl; aperture D-shaped, laterally elongated, with strongly thickened peristome.

Stylommatophora Superfamily Achatinoidea Family Ferussaciidae Genus Cecilioides Férussac, 1814 Cecilioides consobrina (d'Orbigny, 1841) Figure 2G

Achatina consobrina d'Orbigny 1837: 89, pl. 11 bis, figs. 10–12; d'Orbigny, 1841: 170.

Achatina pygmaea Pfeiffer, 1847: 148.

Cecilioides (*Caecilianopsis*) *consobrina*: Pilsbry, 1909–1910: 39, pl. 5, figs. 81-82; Hylton Scott 1948: 254; Schade, 1965: 2014; Fernandez & Castellanos, 1973: 271.

Cecilioides consobrina: Richards & Hummelinck, 1940: 8; Morretes, 1949: 131; Wurtz, 1950: 107; Parodiz 1957: 131; Altena, 1960: 50; Salgado & Coelho, 2003: 154; Simone, 2006: 183, fig. 666; Aguirre et al. 2007: 10, fig. 4.5; Míquel et al., 2007: 114; Oroño et al., 2007: 21; Míquel & Aguirre 2011: 109, fig. 8; Salvador et al., 2017: 139, figs. 13–14; Salvador et al., 2018: 114, fig. 9H-I.

Cecilioides (Karolus) consobrina: Figueiras, 1963: 87; Quintana, 1982: 80; Míquel & Herrera, 2014: 122.

Cecilioides consobrina consobrina: Ramírez et al, 2003: 276.

Type locality. Cuba, near Matanzas.

Previously known distribution. From Mexico (*C. consobrina veracrucensis* (Crosse & Fischer, 1877)) to Argentina (*e.g.*, Sandoval 1997; Miquel et al. 2007).

New records. Presidente Olegário: in cave "Lapa da Fazenda São Bernardo". Despite this species being widely distributed, this is, up to our knowledge, the first specific record from Minas Gerais state.

Identification. Minute smooth glossy bullet-like shell, with long and narrow aperture.

Superfamily Scolodontoidea Family Scolodontidae Genus Entodina Ancey, 1887 Entodina gionensis Morretes, 1940 Figure 2H–J

Entodina gionensis Morretes, 1940: 257, pl. 1, figs. 1–4; Morretes, 1949: 138; Simone, 2006: 223, fig. 849; Agudo-Padrón, 2008: 164; Agudo-Padrón, 2014: 18; Birckolz et al., 2016: table 1; Salvador et al., 2016: 64, figs. 16–18.

Type locality. Vamiranga Shell Mound, Iguape municipality, São Paulo state, Brazil.

Table 2. List of all species found, with information on locality, cave zone inhabited by the species, whether it is a new occurrence for Minas Gerais state, and catalog number of the MZSP collection. Classification of the genus *Lilloiconcha* Weyrauch, 1965 in the newly-recognized family Cystopeltidae follows Salvador et al. (2020). Abbreviations: Caves: AdL, Lapa Arco da Lapa; FSB, Lapa da Fazenda São Bernardo; Jur, Lapa da Juruva; Moa, Lapa do Moacir; TdC, Toca do Charco; VdP, Lapa Vereda da Palha; ZdS, Lapa Zé de Sidinei. Cave zones: EP, epigean; EZ, entrance zone; TZ, twilight zone; DZ, dark (aphotic) zone.

Species	Family	New record?	Cave	Cave zone	Collection Nr. (MZSP)
Neritimorpha					
Alcadia iheringi Wagner, 1910	Helicinidae	Yes	FSB, ZdS	EP, EZ, TZ, DZ	150032 (1 sh), 150047 (1 sh)
Helicina brasiliensis Gray, 1824	Helicinidae	No	FSB	EZ, TZ	137269 (5 sh), 137482 (2 sh)
Helicina sordida King, 1831	Helicinidae	Yes	Moa	ΤZ	150028 (1 sh)
Helicina lundi Beck in Pfeiffer, 1858	Helicinidae	No	AdL, Moa, TdC	EZ, TZ	137179 (1 sh), 137203 (1 sh), 137272 (1 spm), 150025 (2 sh)
Helicina sp.	Helicinidae	_	AdL, FSB, Jur, TdC, ZdS	EP, EZ, TZ, DZ	137142 (2 sh), 137149 (1 spm), 137185 (2 spm), 137186 (1 sh), 137273 (1 spm), 137564 (2 sh), 137566 (1 sh)
Caenogastropoda					
Habeastrum strangei Simone, Cavallari & Salvador, 2020	Diplommatinidae	No	FSB, Jur, Moa, VdP, ZdS	EP, EZ, TZ, DZ	137054 (5 spm), 137132 (2 spm), 137143 (3 sh), 137145 (5 spm), 137153 (1 spm), 137176 (1 sh), 137181 (6 spm), 137256 (6 sh), 137475 (4 sh), 147862 (2 spm), 147863 (1 spm)
Hygrophila					
<i>Biomphalaria</i> sp.	Planorbidae	_	Moa, TdC	ΤZ	137148 (3 sh), 137178 (1 sh)
Allopeas micra (d'Orbigny, 1835)	Achatinidae	No	AdL, FSB, ZdS	EP, EZ, TZ, DZ	137264 (2 sh), 137265 (1 sh), 137268 (5 sh), 137276 (4 sh), 137284 (5 sh), 137424 (1 sh),
Bulimulus sp	Bulimulidae		FSB	ΕZ	130017 (2 sh) 137479 (1 sh)
Cecilioides consobrina (d'Orbigny, 1841)	Ferussaciidae	Yes	FSB	EP	150049 (1 sh)
Drymaeus coarctatus (Pfeiffer, 1845)	Bulimulidae	Yes	FSB, Moa, VdP	EZ, TZ	137053 (1 sh), 137147 (1 sh), 137259 (2 sh), 137261 (1 sh), 137299 (1 spm), 137473 (2 sh), 150142 (1 sh), 150143 (1 sh)
<i>Dysopeas muibum</i> Marcus & Marcus, 1968	Achatinidae	No	AdL, FSB, Jur, VdP, ZdS	EP, EZ, TZ, DZ	137106 (1 sh), 137138 (1 sh), 137189 (10 sh), 137190 (1 sh), 137192 (2 sh), 137284 (5 sh), 137276 (4 sh), 137422 (1 sh), 137480 (2 sh), 137563 (1 sh)
Entodina gionensis Morretes, 1940	Scolodontidae	Yes	FSB	EZ, TZ	137269 (5 sh), 137474 (5 sh), 150048 (1 sh)
Gastrocopta servilis (Gould, 1843)	Gastrocoptidae	No	FSB	ΤZ	150046 (1 sh)
Habroconus semenlini (Moricand, 1846)	Euconulidae	Yes	AdL, FSB, Moa, VdP	EP, EZ, TZ, DZ	137182 (1 sh), 137270 (5 sh), 137428 (1 sh), 137429 (4 sh), 137472 (3 spm), 150024 (5 sh), 150042 (1 sh), 150053 (2 sh),150137 (2 spm)
Lamellaxis goodalli (Miller, 1822)	Achatinidae	No	FSB	TZ, DZ	137431 (1 sh), 137478 (2 sh)
Leiostracus sp.	Bulimulidae		AdL	EZ	137204 (1 sh)

Continue...

Leptinaria lamellata (Potiez & Michaud, 1838)	Achatinidae	No	FSB, TdC, ZdS	EP, EZ, TZ, DZ	137135 (1 sh), 137136 (1 sh), 137187 (1 sh), 137268 (5 sh), 137294 (1 sh), 137481 (2 sh), 150044 (1 sh)
Lilloiconcha gordurasensis (Thiele, 1927)	Cystopeltidae	No	FSB	EP	150040 (2 sh)
Megalobulimus sanctipauli (Ihering & Pilsbry, 1900)	Strophocheilidae	Yes	ZdS	EP(?)	unnumbered
Odontostomidae indet.	Odontostomidae	—	VdP	EZ, TZ	137101 (1 spm), 137102 (2 sh)
Radiodiscus sp.	Charopidae	—	FSB	TZ, DZ	137260 (2 sh), 137471 (10 spm)
Scolodonta bounobaena (d'Orbigny, 1835)	Scolodontidae	No	AdL, FSB, Jur, TdC, ZdS	EZ, TZ, DZ	137105 (1 sh), 137141 (2 sh), 137152 (1 sh), 137177 (1 sh), 137191 (4 sh), 137208 (3 spm), 137262 (5 sh), 137263 (1 sh),137471 (10 spm), 137564 (2 sh), 137567 (1 sh)
Scolodonta interrupta (Suter, 1900)	Scolodontidae	Yes	FSB, Moa, VdP	EP, EZ, TZ, DZ	137146 (1 sh), 137174 (4 sh), 137258 (1 sh), 137267 (5 sh), 137271 (2 spm), 137293 (10 sh), 137300 (1 spm), 150025 (2 sh)
Solaropsis aff. rosaria (Pfeiffer, 1849)	Solaropsidae	Yes	Moa, TdC	EZ, TZ	137180 (1 sh), 150140 (1 sh), 150141 (1 sh)
Subulinidae indet.	Achatinidae		Moa, VdP, ZdS	EZ, TZ	137144 (2 sh), 137175 (1 sh), 137182 (1 sh)
Scolodontidae indet.	Scolodontidae		FSB, VdP, ZdS	EP, EZ, TZ, DZ	137104 (1 sh), 137266 (10 sh), 137298 (1 spm), 137471 (10 spm)
Tamayoa banghaasi (Thiele, 1927)	Scolodontidae	No	AdL, FSB, Moa, TdC, VdP, ZdS	EP, EZ, TZ, DZ	137056 (2 sh), 137150 (1 spm), 137202 (2 sh), 137274 (1 spm), 137275 (1 spm), 137476 (1 sh), 137568 (3 spm), 150050 (1 sh)

Continuation...

Previously known distribution. Known only from type locality in São Paulo state (Simone 2006). Outside of São Paulo, it has been reported from various municipalities in Santa Catarina state (Agudo-Padrón 2008), but those remain unconfirmed (Salvador et al. 2016).

New records. Presidente Olegário: in cave "Lapa da Fazenda São Bernardo". The present record extends the species distribution ca. 700 to the north.

Identification. Discoid shell with flattened spire, sculptured by strongly sinuous ribs; aperture with strong parietal-columellar barrier and a marked palatal tooth. The present specimens have a slightly less developed parietal-columellar fold than typical *E. gionensis*.

Genus Scolodonta Doering, 1875 Scolodonta interrupta (Suter, 1900) Figure 2K–L

Streptaxis interruptus Suter, 1900: 331, pl. 3, fig. 5.

Scolodonta interrupta: Pilsbry, 1900: 385, pl. 12, figs. 6–8; Gude, 1902: 240; Kobelt, 1906: 69, pl. 51, figs. 21–23; Morretes, 1949: 166; Quintana, 1982: 104; Salgado & Coelho, 2003: 170; Simone, 2006: 225, fig. 856; Salvador et al., 2016: 65, figs. 23–25; Zepon & Bichuette, 2017: 5; Salvador, 2021: 64, figs. 4–6.

Type locality. Brazil, São Paulo state, Perus.

Previously known distribution. Known only from type locality (Salvador 2021). The report of Simone (2006) from Pará state is erroneous.

New records. Presidente Olegário: in caves "Lapa da Fazenda São Bernardo", "Lapa do Moacir" and "Lapa Vereda da Palha". The present record extends the species distribution ca. 500 km to the north.

Identification. Unsculptured scolodontid shell with slightly raised spire and small circular aperture lacking apertural barriers.

Superfamily Rhytidoidea Family Strophocheilidae

Genus Megalobulimus K. Miller, 1878 Megalobulimus sanctipauli (Ihering & Pilsbry, 1900) Figure 2M–N

Strophocheilus oblongus var. sanctaepauli Ihering & Pilsbry in Pilsbry, 1900: 390.

Strophocheilus (Borus) sanctaepauli: Pilsbry, 1901: 123, pl. 20, fig. 63.

Strophocheilus (Borus) sanctipauli: Pilsbry, 1902: v, xciv [emendation].



Figure 2. A–B. *Alcadia iheringi* (MZSP 150047; H = 3.1 mm, D = 3.6 mm). C. *Alcadia iheringi* (MZSP 150032; **D** = 3.5 mm). **D–F**. *Helicina sordida* (MZSP 150028; H = 5.3 mm, D = 7.8 mm). **G**. *Cecilioides consobrina* (MZSP 150049; H = 1.3 mm, D = 0.4 mm). **H–I**. *Entodina gionensis* (MZSP 150048; H = 1.6 mm, D = 4.0 mm). **J**. *Entodina gionensis* (MZSP 137471; D = 3.1 mm). **K–L**. *Scolodonta interrupta* (MZSP unnumbered; H = 4.1 mm, D = 7.0 mm). **M–N**. *Megalobulimus sanctipauli* (MZSP unnumbered; H = 11.5 cm, D = 6.5 cm). **O**. *Drymaeus coarctatus*, adult with thickened lip (MZSP 150142; H = 3.7 cm, D = 2.2 cm). **P**. *Drymaeus coarctatus*, young adult with barely reflected lip (MZSP 150143; H = 3.6 cm, D = 2.0 cm). **Q**. *Habroconus semenlini* (MZSP 150137; H = 3.7 mm, D = 4.7 mm). **R–T**. *Solaropsis* aff. *rosaria* (MZSP 150141; H = 10.0 mm, D = 19.4 mm).

Strophocheilus (Megalobulimus) sanctipauli: Bequaert, 1948: 137, pl. 13, fig. 7; Parodiz, 1957: 132; Fernández & Castellanos, 1973: 276; Quintana, 1982: 85.

Strophocheilus sanctipauli: Morretes, 1949: 141; Cuezzo & Drahg, 1995: 197.

Strophocheilus santipauli: Fernandez, 1978: 149, fig. 1.

Melalobulimus (Megalobulimus) sanctipauli: Morretes, 1953: 68.
Megalobulimus sanctipauli: Abbott, 1989: 76, textfig.; Salgado & Coelho, 2003: 158; Simone, 2006: 219, fig. 831; Beltramino et al., 2012: 19, fig. 1; Beltramino, 2013: 469. Beltramino et al., 2015: 3, fig. 1; Zepon & Bichuette, 2017: 5.

Type locality. Brazil, São Paulo state, Botucatu municipality.

Previously known distribution. Brazil (São Paulo and Paraná states), Paraguay (Canindeyú and Alto Paraná departments), Argentina (Missiones and Corrientes provinces) (Beltramino 2013). Unpublished data also record the species in the two southernmost states of Brazil: Santa Catarina and Rio Grande do Sul (J.H. Fontenelle pers. comm. 2016).

New records. Presidente Olegário: in cave "Lapa Zé de Sidinei". The present record extends the species range ca. 550 km to the northeast (ca. 500 km to the north).

Identification. Relatively tall and non-bulbous spire in comparison with congeners, with suture running almost perpendicular to shell axis.

Superfamily Orthalicoidea Family Bulimulidae Genus Drymaeus Albers, 1850 Drymaeus coarctatus (L. Pfeiffer, 1845) Figure 20–P

Bulimus coarctatus Pfeiffer, 1845: 73; Pfeiffer, 1848: 90; Pfeiffer, 1854: 80, pl. 22, figs. 22–23; Hupé, 1857: 56, pl. 10, fig. 4; Pfeiffer, 1858: 166.

Drymaeus coarctatus: Pilsbry, 1898: 195, pl. 28, figs. 17–20; Morretes, 1943: 117; Breure, 1979: 108; Salgado & Coelho 2003: 161; Simone, 2006: 136, fig. 445: Zepon & Bichuette, 2017: 5.

Drymaeus (Mormus) coarctatus: Morretes, 1949: 150.

Drymaeus (Drymaeus) coarctatus: Breure & Eskens, 1981: 16, pl. 5, fig. 5; Breure & Ablett, 2014: 5; 46, fig. 37F–H.

Kora terrea Simone, 2015: 51, figs. 1–5.

Kora iracema Simone, 2015: 54, figs. 15-20.

Drymaeus iracema: Salvador & Simone, 2016: 3; Birckolz et al., 2016: 136, fig. 14.

Drymaeus terreus: Salvador & Simone, 2016: 3; Birckolz et al., 2016: 138, fig. 15.

Type locality. Brazil (Pilsbry, 1898).

Previously known distribution. Reported only from the municipality of Lussanvira, Pereira Barreto, São Paulo state (Morretes 1943, 1949; Simone 2006).

New records. Presidente Olegário: in caves "Lapa da Fazenda São Bernardo", "Lapa do Moacir", "Lapa Vereda da Palha", as well as in Povoado de Galena, $18^{\circ}25$ 'S $46^{\circ}25$ 'W (type locality of *Kora terrea*). It was also recorded in the Gruta do Morro dos Tapuias, $12^{\circ}30$ 'S $45^{\circ}03$ 'W, municipality of São Desidério, Bahia state (type locality of *K. iracema*). The present record extends the species range ca. 1,000 km to the northeast (ca. 750 km to the north). **Identification.** Bulimoid shell; reticulate protoconch; aperture with reflected and strongly thickened peristome, unique among the congeners in Brazil. The peristome thickens with age, as younger specimens have a "typical" *Drymaeus*-like appearance (Figure 2P).

Remarks. Drymaeus iracema (Simone, 2015) and D. terreus (Simone, 2015) were originally described as troglobiont species classified in the genus Kora Simone, 2012. The former is known from São Desidério municipality in Bahia state and the latter, from Presidente Olegário municipality, in Minas Gerais state. Salvador & Simone (2016) argued that they belonged in Drymaeus Albers, 1850 based on the reticulate protoconch sculpture. Further conchological features such as the strongly expanded peristome, the straight profile of the spire, and the upright columellar region, allow us to propose here that both D. iracema (holotype MZSP 104964) and D. terreus (holotype MZSP 106215), conchologically indistinguishable from one another, are junior synonyms of Drymaeus coarctatus (lectotype NHMUK 1975560; Simone 2006: fig. 445; Breure & Ablett 2014: fig. 37F–H).

Drymaeus coarctatus is thus widely distributed in eastern Brazil: from western Bahia state (São Desidério municipality), through Minas Gerais state (Presidente Olegário municipality, and newly reported specimens MZSP 152050, 152078, and 152091 from the municipalities of Paracatu and Unaí) and Espírito Santo state (newly reported specimen, USNM IZ530536, from the collection of the Smithsonian National Museum of Natural History, Washington, D.C., USA), to northern São Paulo state (Simone 2006: Lussanvira, an old railway station in Pereira Barreto municipality).

The shell of this species can present tooth-like thickenings on the palatal and basal regions of the peristome, as well as a more marked fold on the apical end of the columellar region (e.g., lectotype and specimen USNM IZ530536). All these structures supposedly develop as the individual grows older and deposits more shell material on the peristome. The shell can have a flame-like coloration pattern of the periostracum (MZSP 106215) or a pattern composed of several brown narrow spiral stripes (USNM IZ530536). Such intraspecific variation in shell color is well documented in other species of *Drymaeus* (e.g., Salvador et al. 2018).

Superfamily Trochomorphoidea Family Euconulidae

Genus Habroconus Crosse & P. Fischer, 1872 Habroconus semenlini (Moricand, 1846) Figure 2Q

Helix Semen-lini Moricand, 1846: 149, pl. 5, fig. 17; Pfeiffer, 1846: 457, 1848: 31, 1853: 32; Reeve, 1854: pl. 112, fig. 637; Hupé, 1857: 14.

Helix (Hyalina) semen lini: Albers, 1860: 73.

Hyalina semen lini: Heynemann, 1868: 106; Clessin, 1888: 166. Hyalina (Conulus) semen lini: Martens, 1868: 175.

Conulus semen lini: Ihering, 1894: 38.

Guppya semen-lini: Ancey, 1897: 9.

Guppya seminlini: Pilsbry, 1900: 386.

Vitrea semen lini: Suter, 1900: 331.

Habroconus (Pseudoguppya) semen-lini: Haas, 1953: 205, 1959: 365.

Habroconus (Pseudoguppya) semenlini: Baker, 1928: 12; Morretes, 1949: 137; Figueiras, 1963: 87; Schade, 1965: 214; Fernandez & Castellanos, 1973: 274; Quintana, 1982: 79.

Habroconus semenlini: Klappenbach, 1967: 42; Agudo-Padrón, 2008: 166; Santos et al., 2010: 516; Agudo-Padrón, 2014: 18.

Habroconus semenline: Salgado & Coelho, 2003: 154.

Pseudoguppya semenlini: Salvador et al., 2016: 65; Salvador et al., 2018: 124, figs. 14A–C; Zepon & Bichuette, 2017: 5; Salvador, 2019a: 95; Silva et al., 2019: 184, fig. 4A–C.

Type locality. Bahia state, Brazil.

Previously known distribution. Brazil (Alagoas, Bahia, Rio de Janeiro, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul states), Paraguay, Uruguay, Argentina (Silva et al. 2019).

New records. Presidente Olegário: in caves "Lapa Arco da Lapa" and "Lapa da Fazenda São Bernardo", "Lapa do Moacir" and "Lapa Vereda da Palha". The present record fills a gap in the species' distribution.

Identification. Typical euconulid shell; body whorl with marked angulation and relatively tall compared to congeners; broad D-shaped aperture.

Superfamily Sagdoidea Family Solaropsidae Genus Solaropsis Beck, 1837 Solaropsis aff. rosaria (L. Pfeiffer, 1849) Figure 2R–T

Solaropsis rosarium [sic]: Salvador et al., 2015: 68, figs. 7–9. **Type locality.** Banks of the Amazon River (Pfeiffer, 1853; Pilsbry, 1933).

Previously known distribution. From Amazonas to Mato Grosso do Sul, Brazil (Salvador et al., 2015). Previous reports from Suriname and "New Granada" have been considered spurious (Pilsbry, 1933).

New records. Presidente Olegário: in caves "Lapa do Moacir" and "Toca do Charco". The present record, if confirmed, would mean an increase of ca. 300 km in the species' distribution towards the southeast.

Identification. The present specimens do not exactly match the species *S. rosaria*, typically known from the Amazon biome in South America. However, they are indistinguishable from the specimens from Tocantins state, in central Brazil, that Salvador et al. (2015) called *S. rosarium* [sic]. The present specimens thus belong to the same species as those of Salvador et al. (2015), but their identity remains elusive for now.

The specimens are also reminiscent of *S. derbyi* Ihering, 1900 from São Paulo state, southeastern Brazil. However, that species is poorly defined and its type specimen is badly preserved (Simone 2006: fig. 937).

The taxonomy of *Solaropsis* in Brazil is riddled with poorly-defined species, a few potentially invalid names, several potential synonyms, unrecognized intraspecific variability of shell morphology, and almost no knowledge of anatomical features, DNA, and basic biology (Cuezzo 2002; Calcutt et al. 2020). As such, proper identification of the present specimens will have to wait until a better definition of species boundaries in the genus is in place.

Remarks. This species has been lately classified in the genus *Psadara* Miller, 1878 (e.g., Simone 2006); however, a recent molecular analysis has confirmed that *Psadara* is a synonym of *Solaropsis* (Calcutt et al. 2020).

Discussion

Orthalicoidea is usually the most diverse group of land snails in any sample of mollusks taken in Brazil (Salvador 2019b). However, as already pointed out by Salvador et al. (2016), this does not seem to hold for the troglofauna, in which Subulininae and Scolodontidae are represented by a more diverse assembly of species among the stylommatophorans. In the present material, only four orthalicoid species were found (and only in the epigean and the entrance zone of the caves; Table 2). Meanwhile, several subulinine and scolodontid taxa were recovered from the epigean to the dark aphotic zone of the caves (Table 2). From all species reported herein, only *Habeastrum strangei* is a known cave endemic (Simone et al., 2020); all the others have wider distributions in non-cave environments.

Moreover, a curiously high number (four) of helicinids species was found in the material, including living specimens in the aphotic zones of the caves (Table 2). Our previous studies on cavern snails have always been poor on helicinid specimens (e.g., Salvador et al. 2016, 2017). Neotropical helicinids are typically considered arboreal species in the literature (e.g., Richling 2004), but here it can be seen that they might inhabit other habitats as well.

From the caves studied here, almost all of them have little left of the original Cerrado vegetation in their surroundings; the place was cleared to give way to pasture or agriculture and only a couple of meters of native vegetation remains in the entrance area of each cave (T. Zepon, personal communication 2016). Only a single cave ("Lapa Arco da Lapa") has a larger surrounding area of native vegetation (ca. 100 m) (T. Zepon, personal communication 2016). That does not seem to affect the species composition of that cave, as almost all the species found in Lapa Arco da Lapa were also recorded from other caves; the single exception is *Leiostracus* sp., an arboreal snail found in the epigean area there (Table 2).

Several of the species reported here are first records for Minas Gerais state (Results section; Table 2). This report extends the geographical distribution of some species, while simply filling "distribution gaps" of others. Moreover, the present records are especially important, as they are among the few dealing with molluscan troglofauna in the region and Brazil as a whole. Land snails are considered the most threatened group of animals (Lydeard et al. 2004; Régnier et al. 2008) and many of them inhabit, and could likely be endemic to, caves (Simone & Moracchioli 1994; Simone 2013; Weigand 2013; Salvador 2019b).

The vast majority of caves in Brazil completely lack legal protection. Better known geographical distributions improve arguments for conservation, as the Brazilian legislation states that a cave must be protected if it harbors rare or endemic species (Campos-Filho et al. 2014). Hence the great importance of troglofaunal inventories, as delays in ensuring proper protection to those habitats might result in irreparable biodiversity loss.

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Author Contributions

Rodrigo B. Salvador: conceptualized the study; led the taxonomic study and writing of the manuscript.

Fernanda S. Silva: led the taxonomic study and writing of the manuscript.

Daniel C. Cavallari: contributed to the study and manuscript writing. Luiz Ricardo L. Simone: contributed to the study and manuscript writing.

Conflicts of Interest

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

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Broad and fine-scale threats on threatened Brazilian freshwater fish: variability across hydrographic regions and taxonomic groups

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Abstract: Anthropogenic environmental changes are the main cause of species extinction during the Holocene. Species have been exposed to major source of threats, such as habitat loss and fragmentation, pollution, introduced species, and harvesting, many of which are derived from specific anthropogenic activities, such as urbanization, agriculture, and damming (*i.e.* fine-scale threats). However, the importance of these threats on the species conservation status in a given region depends on the type of impacts they are exposed to and the susceptibility of species to these impacts. In this study, we used a database of threatened Brazilian freshwater fish species to test whether the major source of threats and the specific anthropogenic impacts to species vary across hydrographic regions and taxonomic groups. Our results showed that habitat loss is a ubiquitous major threat jeopardizing the conservation status of the Brazilian fish species. However, different finescale threats mediate this process across hydrographic regions and taxonomic groups. The combination of impacts from agriculture, deforestation, and urbanization affects most of the threatened species in the basins of the Northeast, South, and Southeast, including the species of the most threatened order, the Cyprinodontiformes. Damming is the main human activity affecting threatened species of Siluriformes, Characiformes, Gymnotiformes, and Cichliformes, especially in northern basins (Amazon and Tocantins-Araguaia). Therefore, we found that specific fine-scale threats influencing threatened species vary across hydrographic regions and taxonomic groups, probably due to geographic variability in the incidence of human activities and differential niche requirements and vulnerability of species to these activities. Keywords: Aquatic biodiversity; Conservation; Habitat loss.

Ameaças em ampla e fina escala sobre peixes de água doce ameaçados de extinção do Brasil: variabilidade entre regiões hidrográficas e grupos taxonômicos

Resumo: Alterações ambientais antropogênicas são a principal causa de extinção das espécies no Holoceno. As espécies têm sido expostas à diferentes fontes de ameaças principais, tais como a perda e fragmentação de habitat, poluição, introdução de espécies e coleta de organismos, muitas das quais são decorrentes de atividades antropogênicas específicas, tais como urbanização, agricultura e represamento (i.e. ameaças de escala fina). Entretanto, a importância dessas ameaças no estado de conservação das espécies em uma dada região depende do tipo de ameaça que as espécies são expostas e da susceptibilidade das espécies a esses impactos. Neste estudo, utilizamos a base de dados de espécies de peixes dulcícolas do Brasil ameaçadas de extinção para testar se as principais ameaças e os impactos específicos às espécies variam entre as regiões hidrográficas e grupos taxonômicos. Nossos resultados mostraram que a perda de habitat é uma ameaça principal ubíqua, prejudicando o estado de conservação das espécies de peixes do Brasil. Entretanto, diferentes ameaças de escala mais fina mediam este processo entre a regiões hidrográficas e grupos taxonômicos. A combinação de impactos provenientes da agricultura, desmatamento e urbanização afeta a maior parte das espécies ameaçadas nas bacias do Nordeste, Sul e Sudeste, incluindo as espécies da ordem mais ameaçada, os Cyprinodontiformes. O represamento dos rios é a principal atividade humana afetando as espécies ameaçadas de Siluriformes, Characiformes, Gymnotiformes e Cichliformes, especialmente nas bacias do norte (Amazônica e Tocantins-Araguaia). Portanto, as ameaças em escala fina que afetam as espécies ameaçadas variam entre as regiões hidrográficas e grupos taxonômicos, provavelmente devido à variabilidade geográfica na incidência das atividades de impacto humano e em função das diferenças nos requerimentos de nicho e vulnerabilidade das espécies a essas atividades.

Palavras-chave: Biodiversidade aquática; Conservação; Perda de hábitat..

Introduction

Freshwater ecosystems have been negatively impacted by various anthropogenic actions (Dudgeon et al. 2006). The most deleterious threats to freshwater fish are habitat modification, fragmentation and destruction, pollution, introduction of nonnative species, and climate change (Barletta et al. 2008; Arthington et al. 2016). As consequence, freshwater biota has suffered higher extinction rates than terrestrial and marine in the last decades (Jenkins 2003, Dirzo et al. 2014). The effects of these major threats are derived from human impacting activities (i.e. fine-scale threats) (Venter et al. 2016) whose impacts are context-dependent, since their occurrences and intensities show geographic variability (Vörösmarty et al. 2010). Furthermore, species also exhibit differential susceptibility to impacts according to their biological traits (e.g. reproduction, feeding strategies) (Olden et al. 2007, Castro & Polaz 2020). Therefore, intrinsic (i.e. biological traits) and extrinsic factors (e.g. type of impact) are important drivers of species vulnerability (Olden et al. 2007).

Growing agricultural expansion, the hydropower-based energy matrix and the disorderly growth of urban centers in Brazil (Martinelli et al. 2010, Soito & Freitas 2011, Cunico et al. 2012) expose fish to many types of threats. The intensity of these threats varies regionally, probably as the result of predominant economic activities in each region. For example, there are several hydropower plants planned for the Amazon basin in the coming years, representing an important potential threat for many species (Miesen et al. 2010, Fearnside 2012). Meanwhile, most of rivers of Paraná, Southeast and South Atlantic hydrographic regions are already severely impacted by dams long ago (Agostinho et al. 2007). In these regions, other threats have emerged as current main threats, such as urbanization, impacts derived from expansion of livestock and agriculture and introduction of non-native species (Pereira et al. 2017; Castro & Polaz 2020).

Many of biological traits important for the response of species to disturbance are phylogenetically conservative (Olden et al. 2007, Forero-Medina et al. 2009, Vilela et al. 2014). As consequence, the response to impacts can be similar among species of the same phylogenetic group. For example, species of Rivulidae that occur in temporary habitats, usually near urban centers or heavily mechanized agricultural areas, tend to be susceptible to habitat loss and pollution (Costa 2002, 2007, 2009, Castro & Polaz 2020). To cite another example, species of large-size of Siluriformes and Characiformes have been historically overexploited in some regions with several examples of local extirpation (Hoeinghaus et al. 2009).

In this study, we benefit from the national conservation status assessment conducted by the federal environmental agency, the Chico Mendes Institute for Conservation of Biodiversity - ICMBio, which assessed the risk of extinction for all valid freshwater fish species in Brazil (ICMBio 2018). We compiled information of broad and fine-scale threats reported as justification for the conservation status of all 311 threatened species and tested if these threats vary across hydrographic regions and taxonomic groups. We expected that the importance of different types of threats to threatened species varies among hydrographic basins, reflecting the differential incidence of types of anthropogenic interferences within regions. Additionally, we expect environmental impacts to be associated with specific taxonomic groups, reflecting differences in species susceptibility to different threats.

Materials and Methods

1. Data

We compiled information regarding threats, species range and taxonomic information for the 311 continental threatened Actinopterygii

species of Brazil listed in the Brazilian Red Book (ICMBio 2018). Threatened species included species classified as Vulnerable (VU), Endangered (EN), and Critically Endangered (CR) (IUCN 2012).

The political delimitation of the geographical area (Brazil) is justified by the availability of high-quality information on the extinction risk to species and respective major and fine-scale threats. These data were products from workshops conducted by the Chico Mendes Institute for Conservation of Biodiversity - ICMBio, which assessed the risk of extinction of all fish species in Brazil, supported by hundreds of specialists and published in its final version in the Brazilian Red Book of Threatened Species of Fauna (Chapter VI: Fishes) (ICMBio 2018). Moreover, the geographical area considered includes many river basins that correspond to the important ecoregions for aquatic biodiversity within the Neotropical region (Abell et al. 2008).

Information about the threats was obtained from the justification for the conservation status of each species available from ICMBio (2018). We assigned each species to one or more major threats: habitat loss, habitat fragmentation, pollution, harvesting, and introduced species (Table 1). We were able to identify at least one of the major threats for 308 (99%) of the 311 threatened species. Habitat loss and fragmentation and pollution can be consequences of several specific human impacting activities (*e.g.* urbanization, agriculture, damming) (Venter et al. 2006, Evans et al. 2011). In order to take these specific impacts into account, we also assigned threatened species in relation to fine scale categories of threats: agriculture, damming, deforestation, ecotourism, harvesting, introduced species, mineral extraction, siltation, urbanization, and water extraction (Table 1); this was possible for 295 (94.8%) of the 311 threatened species.

Species occurrences in river basins were obtained from the Catalog of Fishes database (http://researcharchive.calacademy.org) and ICMBio (2018). Species were then assigned to Brazilian hydrographic regions following the National Water Agency (ANA 2017).

2. Data analysis

We used a Chi-square test to test whether the proportion of species affected by different types of human activities varies across taxonomic groups. To represent the taxonomic group, we considered the order level. In order to test the association between the human activities influencing threatened species with the species occurrence in the hydrographic regions, we carried out a redundancy analysis (RDA). We used a matrix of presence of each species across hydrographic regions as response and a matrix of human activities representing the fine-scale threats as explanatory variable. We used the RDA instead canonical correspondence analysis (CCA) because the length of the gradient of the response variable was lower than four as estimated by detrended correspondence analysis (DCA) (ter Braak & Šmilauer 2002). We used Monte Carlo permutation test for significance at p < 0.05.

Results

The number of threatened species and the proportion of species of each order varied across hydrographic regions (Figure 1). Hydrographic regions with the highest number of threatened species were Southeast Atlantic, followed by Paraná, Tocantins-Araguaia, São Francisco, and Amazon. In relation to the taxonomic groups, Cyprinodontiformes was the order with highest number of threatened species (43.4% of

Table 1. Definition of major threats and human activities (fine-scale threats) negatively influencing Brazilian threatened fish specie	s.
Classification and definitions were partially derived from Venter et al. (2006) and Evans et al. (2011).	

Threats	Description
Major threats	
Habitat loss	Reduction or degradation of habitat due to deforestation, agricultural and livestock activities, urbanization, mining, siltation, infrastructure construction, extractive activities, and human disturbance
Habitat fragmentation	Reduction or interruption of connectivity between habitat patches, impairing fish movement and colonization
Pollution	Pesticides, herbicides, domestic and industrial effluents
Harvesting	Fishing and collection for aquarium purposes
Introduced species	Negative effects of nonnative species due to competition and predation
Human activities / fine-scale threats	
Agriculture	Crops, wood plantations, non-timber plantations, livestock (including ranching)
Damming	Construction of dams and impoundments
Deforestation	Logging of native vegetation within the catchment area
Ecotourism	Habitat alteration due to intensive tourist visitation
Mineral extraction	Mining and sand extraction
Siltation	Alteration of riverbed due to the deposition of terrestrial clastic material
Urbanization	Development of human settlements (urban, suburban, and rural), industrial and commercial buildings and roads
Water extraction	Draining, landfilling, depletion of groundwater and aquifers



Figure 1. Distribution of the threatened freshwater fishes listed in the Brazilian Red Book among orders and hydrographic regions. Number in parenthesis represent the total number of threatened species in the respective hydrographic region. Others orders comprise Atheriniformes, Batrachoidiformes, and Myliobatiformes.

all threatened species), followed by Siluriformes, Characiformes, Gymnotiformes, and Cichliformes (28.0, 18.3, 5.5, and 3.9% of all threatened species, respectively). Other orders represent 1.0% of the threatened species. Cyprinodontiformes, mainly species of Rivulidae, were dominant among the threatened species in all hydrographic regions, except in Amazon, Paraguay and Paraná, where Siluriformes (two formers) and Characiformes (latter) were dominants (Figure 1).

1. Major threats in relation to hydrographic regions and taxonomic groups

Of orders with more than one threatened species, habitat loss was the main threat (ranging from 87.3 to 100% of threatened species across orders), followed by habitat fragmentation (ranging from 0 to 43.8% of threatened species across orders), and pollution (ranging from 8.3 to 26.3% of threatened species across orders) (Table 2). harvesting and introduced species negatively influenced the conservation status of a lower number of species in seven of the eight orders (Table 2).

The threatened species are distributed across 11 of the 12 hydrographic regions of Brazil. Southeast Atlantic, Paraná, and the Tocantins-Araguaia were the hydrographic regions with the highest number of threatened species (55% of all threatened species). Of the major threats, habitat loss was the main threat in all basins (93.2% of all threatened species, ranging from 66.7 to 100% in the individual basins) (Table 3); followed by habitat fragmentation (24.4% of all threatened species, ranging from 0 to 64.3% in the individual basins), and pollution (18.3% of all threatened species, ranging from 0 to 66.7% in the individual basins) (Table 3). Harvesting and introduced species were identified as threats to 5.5 and 3.2% (ranging from 0 to 23.3% in the individual basins) of the threatened species, respectively (Table 3).

2. Fine-scale threats in relation to hydrographic regions and taxonomic groups

Most of species were associated with more than one human activity representing the fine-scale threats. Damming, agriculture, urbanization and deforestation affected the conservation status of the greatest number of species (91% of the threatened species) (Figure 2). Draining, siltation, mining and ecotourism combined were associated with 24.7% of the threatened species, but most of these species (74 of 77) were also

Orders	Threatened species	Habitat loss	Habitat fragmentation	Pollution	Harvesting	Introduced species
All orders	311	291 (93.6)	77 (24.7)	58 (18.6)	18 (5.8)	10 (3.2)
Cyprinodontiformes	135	129 (95.6)	25 (18.5)	19 (14.1)	11 (8.1)	3 (2.2)
Siluriformes	87	76 (87.3)	23 (26.4)	20 (23.0)	3 (3.4)	1 (1.1)
Characiformes	57	55 (96.5)	25 (43.8)	15 (26.3)	2 (3.5)	5 (8.8)
Gymnotiformes	17	17 (100)	0 (0)	3 (17.6)	0 (0)	0 (0)
Cichliformes	12	12 (100)	4 (33.3)	1 (8.3)	0 (0)	0 (0)
Atheriniformes	1	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
Batrachoidiformes	1	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Myliobatiformes	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)

Table 2. Distribution of the threatened freshwater fishes listed in the Brazilian Red Book among their respective orders. Number in parenthesis is the percentage in the respective order.

Table 3. Distribution of the threatened freshwater fishes listed in the Brazilian Red Book in relation to the major threats and hydrographic regions. Number in parenthesis is the percentage in the respective hydrographic region. Species may be associated with more than one threat, so that the sum of species across threats may exceed the number of species in each hydrographic region.

Hydrographic regions	Threatened species	Habitat loss	Habitat fragmentation	Pollution	Harvesting	Introduced species
All	311	290 (93.2)	76 (24.4)	58 (18.6)	17 (5.5)	10 (3.2)
Southeast Atlantic	61	53 (86.9)	23 (37.7)	23 (37.7)	3 (4.9)	3 (4.9)
Paraná	59	56 (94.9)	21 (35.6)	18 (30.5)	2 (3.4)	4 (6.8)
Tocantins-Araguaia	53	52 (98.1)	7 (13.2)	2 (3.8)	1 (1.9)	0 (0)
São Francisco	43	36 (83.7)	9 (20.9)	10 (23.3)	10 (23.3)	2 (4.7)
Amazon	42	42 (100)	1 (2.4)	0 (0)	2 (4.8)	0 (0)
South Atlantic	32	28 (87.5)	13 (40.6)	4 (12.5)	2 (6.3)	3 (9.4)
East Atlantic	24	23 (95.8)	10 (41.7)	7 (29.2)	2 (8.3)	3 (12.5)
Uruguay	14	14 (100)	9 (64.3)	1 (7.1)	0 (0)	0 (0)
Northwest Oriental Atlantic	4	4 (100)	1 (25.0)	1 (25.0)	0 (0)	0 (0)
Paraguay	3	2 (66.7)	0 (0)	2 (66.7)	0(0)	0 (0)
Northwest Occidental Atlantic	2	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)

influenced by the other more representative threats. The proportion of species of each order differed significatively for four threats (damming, agriculture, urbanization, and siltation). Damming was associated with a higher proportion of Siluriformes, Characiformes, Gymnotiformes, and Cichliformes, X^2 (5, n=308) = 123.9, p < 0.01. Threatened species of Cyprinodontiformes were mainly influenced by agriculture and urbanization, X^2 (5, n=308) = 12.5, p < 0.02 (Figure 2). Among the species influenced by siltation, Siluriformes species were the most represented, X^2 (5, n=308) = 20.8, p < 0.01 (Figure 2). The proportion of species of different orders did not differed for deforestation, draining, mining, and ecotourism, X^2 (5, n=308) = 10.7, p > 0.06.

Fine-scale threats influencing threatened species were associated with the hydrographic regions (RDA, p<0.01; $R^2=0.09$) (Figure 3). Damming was the main human activity associated with threatened species in Amazon; both damming and agriculture influenced the most of species in Tocantins-Araguaia, Northwest Oriental Atlantic, and Uruguay; Agriculture was the main threat in São Francisco, South Atlantic and Paraguay. Urbanization, deforestation and siltation were associated with threatened species in Paraná, Southeast Atlantic, East Atlantic, and Northwest Occidental Atlantic (Figure 3).

Discussion

We studied how threats influencing the conservation status of Brazilian threatened fish species are distributed across hydrographic regions and taxonomic groups. Habitat loss or degradation are by far the biggest threats to fish, affecting all representative taxonomic groups and hydrographic regions of Brazil. However, by assessing fine scale categories of threats, we found that specific human activities influencing threatened species vary across hydrographic regions, reinforcing the context-dependency of the spatial distribution of threats. The same pattern was observed for taxonomic groups, with some human activities being more influential on specific taxonomic groups, probably reflecting differential vulnerability of species.



Figure 2. Number of threatened species (bars) and cumulative number of species (line) of each order of fish associated with their respective fine-scale threats.



Figure 3. Biplot of Redundancy Analysis of human activities associated with fine-scale threats and hydrographic regions (gray dots). We omitted the name of the four hydrographic regions (Northwest Occidental Atlantic, Northwest Oriental Atlantic, Paraguay, and East Atlantic) which showed weak association with human activities (positioned at the center of biplot).

1. Major threats

Most of threatened species listed in the Brazilian Red Book (ICMBio 2018) have their conservation status justified due to habitat degradation and/or destruction. Damming, deforestation and the conversion of native vegetation into agriculture or urban areas are some of the most important sources of habitat degradation for threatened species. These activities result in changes in physical and chemical aspects of aquatic habitats (Arthington et al. 2016), negatively influencing conditions and resources required by species, especially those with specialized niches

with restricted distribution. In fact, many of the threatened species (most of the Critically Endangered and Endangered) are known from only a few locations, inhabiting specific biotopes which are exposed to potential impact from human activities.

Other major threats such as habitat fragmentation and pollution also affect a considerable number of species. Habitat fragmentation occurs due to the construction of dams, impoundments, road crossings and the draining of wetlands (Gido et al. 2016). As a consequence, the loss of connectivity among habitat patches affects fish movement and colonization dynamics, negatively influencing population persistence and even their capacity to deal with other impacts (Gido et al. 2016, Herrera-R et al. 2020). Pollution was one of the main threats for 18.6% of threatened species. These species usually inhabit small ponds or streams exposed to urban areas, agriculture or mining. The input of effluents from agriculture, industrial or urban areas usually represent additional negative effects for fish populations often already depressed by other threats (i.e. habitat loss and fragmentation).

Among the major sources of threats, harvesting and introduced species were those associated with a lower number of threatened species. Overfishing is a primary source of extinction risk for large species (Dudgeon et al. 2006), especially in marine systems (Dulvy et al. 2003). However, among the Brazilian threatened freshwater fish species, small-sized fishes captured for aquarium purposes (e.g. *Hypsolebias* spp. and *Hypancistrus zebra*) represent most of species which are jeopardized by harvesting. Despite large-sized fishes being preferable targets for fishing, most such species are broadly distributed, so that even though overfishing depresses local populations (Mateus & Penha 2007), persistence in other parts of their distribution results in lower risk under a national wide assessment (Castro & Polaz 2020). In relation to species affected by introduced species, few species were associated this threat (ICMBIO 2018). Altered habitats (e.g. artificial reservoirs) facilitate species introduction and concentrate the majority of introduced species

(Vitule et al. 2012, Pereira et al. 2017), where they are responsible for several impacts at the population, community, and ecosystem level (Vitule et al. 2009; Cucherousset 2011, Agostinho et al. 2015). However, habitat alterations preceding species introduction probably represent the primary drivers of decline of more sensitive species. This is likely the reason why introduced species are not identified as the main threat in many cases. However, it is worth considering that the difficulty of detecting the influence of biotic interactions as drivers of population trends, along with the scarcity of ecological studies on the effects of introductions may contribute to our underestimation of the effects of introduced species.

2. Human activities and fine-scale threats

Several human activities were listed as determinants of the conservation status of Brazilian threatened species. Most of these activities represent the source of the impacts underlying the habitat loss and degradation, the primary major threat for most of threatened species. Four of these human activities (damming, agriculture, urbanization, and deforestation) were associated with most of the threatened species (91%). However, the number of threatened species influenced by these activities is unevenly distributed across taxonomic groups and hydrographic regions. For Siluriformes, Characiforms, Gymnotiformes, and Cichliformes, dominant groups in Neotropical freshwaters (Nelson 2006), river damming is one of the main impacts that contribute to the risk of species extinction, especially for small-sized, specialized and rapids-dwelling species with restricted distributions. These species are highly vulnerable to hydrological alteration of their habitats due to dam construction (Liermann et al. 2012; Fitzgerald et al. 2018). To illustrate this process, Melanocharacidium nigrum Buckup 1993 and Harttia depressa Rapp Py-Daniel & Oliveira, 2001, both occurring in river rapids and rocky substrates, were locally extirpated due to the construction of dams within the Amazon basin (ICMBio 2018).

Despite the Neotropical ichthyofauna is dominated by Siluriformes and Characiformes (Castro 1999), Cyprinodontiforms is the order with the highest number of threatened species. This highlights the great vulnerability of this group, which is represented mainly by species of Rivulidae (92.6% of the threatened species of this order). Known as killifishes, these fishes inhabit permanent or temporary wetlands and many species are only known from a few populations (Costa 2002). Their high endemism and dependence on specific environmental characteristics and the regularity of rainfall regimes make this group particularly vulnerable to extinction (Berois et al. 2015). Several environmental impacts have been associated with this group, primarily habitat loss due to agricultural activities and urbanization. These activities are often also associated with the draining of wetlands which sometimes completely destroys aquatic habitats. Due to the great representativity of this group among the Brazilian threatened freshwater fish species, a nationwide conservation plan has been developed, the National Action Plan for the conservation of rivulid fish (ICMBio 2013).

Spatial distribution of human activities affecting threatened fish revealed some interesting patterns. The Amazon and Tocantins-Araguaia basins harbor high proportions of threatened species whose conservation status is associated with damming, mainly due to hydropower plant construction. River damming negatively affects fishes via several mechanisms. Damming dramatically changes the trophic structure and habitat, affecting mainly species with more specialized habits and reduces connectivity, affecting reproductive migration and dispersal (Greathouse et al. 2006, Albrecht et al. 2009). The Amazon and Tocantins-Araguaia basins correspond to great potential for hydropower production, which generates interest in the construction of new hydropower projects (Silvano et al. 2009). In addition to already installed hydropower plants, new dams are being planned in the coming years in these areas and their construction will seriously jeopardize many of the already threatened species (Kahn et al. 2014, Lees et al. 2016, ICMBio 2018).

The processes of urbanization, expansion of intensive agriculture and changes in the flow regimes of water resources reflect regional economic developments, which causes a significant amount of deleterious environmental impacts on soil, water, and air. The hydrographic regions of the Southeast Atlantic and Paraná had similar threats listed as being the most important. In fact, these basins are in economically developed regions, with some of the most populous cities and a high road density. For example, rivers and streams of the upper portion of Paraná basin (i.e. Upper Paraná ecoregion) has been historically impacted by deforestation, siltation, drainage, and agriculture (Fialho et al. 2008). These anthropogenic interferences increase the risk of extinction for most species and challenge the conservation of terrestrial and aquatic biodiversity (Helms et al. 2005, Peressin & Cetra 2014). Threatened species from the São Francisco, Tocantins-Araguaia, Uruguay, and South Atlantic hydrographic regions are influenced mainly by agricultural activities. These river basins have extensive agricultural areas (Mendonça 2006, Grützmacher et al. 2008, Balbinot Junior et al. 2009) and the damage caused by unsustainable agricultural practices increases the environmental impacts on soil and water. Moreover, the intensification of deforestation to expand agricultural activities may reduce the areas of native vegetation, especially riparian forest, directly affecting the maintenance of water quality and conservation of aquatic biota (Pusey & Arthington 2003).

It is worth mentioning that the conservation status assessment of species performed by nations, states and conservation organizations are based on the best knowledge available regarding the threats affecting species. Despite past and future projections on population trends are also part process, the conservation status assessment of species depends primarily on the current threats affecting each species. However, human activities affecting species vary temporally, reflecting economic activities and regional development at each moment in time. Thus, threats that are currently important in some regions, may not have been relevant in the past or will not become so in the future. Currently, damming is a primary threat for fish in Amazon and Tocantins-Araguaia, but this anthropogenic phenomenon already impacted all the large rivers in other regions long ago (e.g. Paraná, São Francisco, and Southeast Atlantic basins) (Agostinho et al. 2007). Most of the large and medium-sized fish were already extirpated from these basins (Hoeinghaus et al. 2009) and currently, other impacts have become primary threats in affecting remnant populations. Therefore, cycles of impacts are underway and environmental policies directed to avoid the associated cycles of extinction are urgent. In an optimistic scenario, one could consider even the reversibility of deleterious impacts, including, for example, dam removal (Pohl 2002) and restoration of degraded landscapes (Bowles & Whelan 1994), which could significantly to reduce the extinction debt (Strassburg et al. 2019).

In summary, our results show that habitat loss is a ubiquitous major threat jeopardizing the conservation status of the Brazilian fish fauna. However, different fine-scale threats mediate this process across hydrographic regions and taxonomic groups. Thus, regionally oriented management strategies and environmental policies may be required to mitigate the hazardous consequences of these geographically and biologically variable human impacts on biodiversity.

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Author Contributions

Murilo Luiz e Castro Santana: 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.

Fernando Rogério de Carvalho: Contribution to data analysis and interpretation. Contribution to critical revision, adding intelectual content.

Fabrício Barreto Teresa: Substantial contribution in the concept and design of the study. Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intelectual content.

Conflicts of Interest

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

Data Availability

We used data that are already public.

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Functional Feeding Group composition and attributes: evaluation of freshwater ecosystems in Atlantic Forest, Brazil

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Abstract: Benthic macroinvertebrates Functional Feeding Group (FFG) have been used to determine aquatic assemblage dynamics and as a biomonitoring tool. The main goals of this study were to assess the effects of stream variables on the abundance and richness of FFGs and evaluate ecosystem attributes (FFG ratios) as a tool to assess ecological conditions of Atlantic Rainforest streams. We sampled 146 sites with different impairment conditions in Rio de Janeiro, Brazil. Richness was significantly different among impairment conditions for all FFGs. Mixed-effect models show that aquatic macroinvertebrate FFGs differed in their responses to abiotic variables for abundance and richness. Also, they were reduced in the impaired sites when compared to intermediate and reference sites. The FFG ratio indicated significant differences along the impairment gradient. The FFG ratio analysis was shown to be a fast and cheap tool that can be used for monitoring aquatic ecosystems in the Atlantic Forest biome. However, further studies are required to calibrate the method specifically for the Atlantic Forest region. *Keywords:* Macroinvertebrate; streams; ecosystem attributes; impairment.

Composição e atributos de Grupos Funcionais Alimentares: avaliação de ecossistemas de água doce na Mata Atlântica, Brasil

Resumo: Os Grupos Funcionais de Alimentação (GFA) são utilizados para determinar a dinâmica da comunidade de macroinvertebrados bentônicos e como uma ferramenta de biomonitoramento. Os principais objetivos deste estudo foram: avaliar os efeitos de variáveis de riacho na abundância e riqueza de GFAs e os atributos do ecossistema (razão GFA) como uma ferramenta para avaliar as condições ecológicas dos córregos da Mata Atlântica. Foram amostrados 146 locais com diferentes condições de impacto no Rio de Janeiro, Brasil. A riqueza foi significativamente diferente com as condições de impacto entre todos os GFA. Os modelos de efeito misto mostraram que os GFA diferiam em suas respostas às variáveis abióticas quanto à abundância e riqueza. Além disso, eles diferem nas áreas impactadas quando comparados as áreas intermediária e de referência. A razão de GFA encontrou diferenças significativas ao longo do gradiente de impacto. A análise da razão de GFA evidenciou-se uma ferramenta rápida e barata, com potencial para ser utilizada no monitoramento de ecossistemas aquáticos no bioma Mata Atlântica. No entanto, mais estudos serão necessários para calibrar o método especificamente para a região da Mata Atlântica.

Palavras-chave: Macroinvertebrados; rios; atributos do ecossistema; impacto.

Introduction

Streams and rivers exhibit a high biological diversity and provide critical ecological functions and services. However, they are among the most threatened ecosystems due to anthropogenic activities, such as human settlements, industries pollution, and agriculture, which have led to increased habitat loss, higher pollution levels, invasions of exotic species, and the changing climate (Allan & Castilho 2007, Ceneviva-Bastos et al. 2017). Climate change tends to exacerbate anthropogenic stress due to increased water temperature, salinity, and changes in hydrological cycles, which results in shifting rainfall patterns and flow fluctuations (Durance 2007).

Biomonitoring has long been recognized as a tool to screen environmental health changes taking place in the environment (Barbour et al. 1999). Benthic macroinvertebrate is among the most used organisms to assess the ecological condition. Macroinvertebrates are a primary food source for fishes and other organisms (Rosenberg & Resh 1993). They are abundant in most streams, even small ones, have species at different trophic levels, with a wide range of pollution tolerance, and sampling is relatively easy at a low cost (Barbour et al. 1999, Bonada et al. 2006, Henriques-Oliveira & Nessimian 2010, Gieswein et al. 2019).

Two main approaches have been used in biomonitoring programs to assess freshwater macroinvertebrates: one uses richness and diversity indices. and the other uses functional attributes (Cummins 1973, Cummins & Klug 1979, Merritt et al. 1999, Merritt et al. 2002, Cummins 2018). The functional attributes based on morphology and feeding behavior. According to Dedieu et al. (2015), biological traits of freshwater organisms, such as feeding behavior, are useful tools for detecting change along gradients of anthropogenic disturbance. In freshwater ecology, macroinvertebrates Functional Feeding Groups (FFGs) have been used to conceptualizing community dynamics and assessing ecological status (Vannote et al. 1980). The attributes related to the structure and function give indicators of aquatic ecosystem conditions (Hawkins & Sedell 1981, Ceneviva-Bastos et al. 2017, Fugère et al. 2018). According to Vannote et al. (1980), trophic interactions can affect ecological processes by directly influencing flows of the distribution of energy and resources within the assemblage. Thus, functional analysis focuses on the type of food and food acquisition. FFGs are defined by the way organisms feed: (1) gathering collectors - feed on small organic matter particles deposited in the river bed; (2) filtering collectors - capture, by filtration, small organic matter particles suspended in the water column; (3) scrapers – scrape hard surfaces and feed on algae, bacteria, fungi, and dead organic matter adsorbed on substrates, (4) predators - swallow whole prey or body tissue fluids and (5) shredders - chew leaves or tissue from living vascular plant or dead wood and debris (Cummins 1973).

According to Merritt et al. (1996), the use of FFG ratio can estimate attributes related to the stream ecosystem. The FFG ratio serves as a surrogate for stream ecosystem attributes. These attributes include a trophic state (Autotrophy/Heterotrophy), the linkage between to functioning the riparian vegetation and the shredder, relative amounts of coarse and organic particles (transported and stored in the environment), the stability of the habitat, and ascendant control for predators to be driven by prey availability. The FFG ratio is a rapid and integrating technique used to establish a protocol for characterizing the ecological condition. This approach has been used to assess river conditions in Brazil. Cummins et al. (2005) used an FFG ratio to evaluate the sites ecological conditions in Southern Brazil, and Couceiro et al. (2011) assessed streams located in Brazil's Amazon forest. Multimetric and predictive indices for larger-scale protocols also used FFG components in South America (Baptista et al. 2007, Buss et al. 2015, Macedo et al. 2016, Oliveira et al. 2019, Souza et al. 2019).

This study's main goals were to evaluate all FFG categories and the effects of abiotic variables on abundance, richness, and FFG ratio to assess the ecological conditions of Atlantic Forest streams. In this context, this study used FFG and their ratios to assess the ecological conditions of Atlantic Forest streams.

Material and Methods

1. Study area

The Atlantic Forest region in Rio de Janeiro State is classified as the tropical state with a rainy summer season, with the most mountainous areas and plateaus classified as humid subtropical, with a hot summer and without a dry season or a dry winter (Alvares et al. 2013). Temperatures oscillate between 15°C and 28°C, and annual rainfall is around 1,000–1,500 mm.

The Rio de Janeiro state is composed of a group of coastal plains separated by hills and two mountain chains that run parallel to the ocean (Serra do Mar, ranging from altitudes 0–2000 ma s.l and Serra da Mantiqueira, ranging from 800 to 2500 m.a.s.l). The coastal plains are located at the piedmont of Serra do Mar mountain range, with altitudes about 200 ma.s.l.. It is a depositional zone formed by marine, lacustrine, and fluvial sedimentation processes (Brasil 1983). This region is affected by high impact by urban areas or agriculture and livestock grazing, making minimally impacted areas (reference) scarce. The mountain chains are located at higher altitudes (from >200 ma.s.l. to around 1,800 ma.s.l) with high slope and steep scarps. Most sites were sampled within or near protected areas (conservation units), which had a low to moderate impact on agricultural activities. For this reason, this region presents the most extensive riparian vegetation and forest fragments.

The Neotropical Atlantic Forest is one of the biodiversity hotspots worldwide. However, the biome has lost 88% of its original extent, and remnants are mostly spread throughout the higher parts of mountains, interspersed with agriculture and pasture (Ribeiro et al. 2011).

We selected sites based on the ad hoc indication and previous knowledge of the area to represent sites classified, *a posteriori*, as a reference, intermediate, or impaired. Sites classified as "reference" should meet all the following criteria: "optimal" or "good" environmental conditions according to the Habitat Assessment Protocol (HAP) (Barbour et al. 1999 – see rationale below); dissolved oxygen concentration ≥ 6 mg/l, pH between 6 and 8, absence of channelization, and <40% of the upstream area affected by urban areas. For a site to be classified as "impaired," the following criteria should be met: "poor" classification according to the HAP; dissolved oxygen <6 mg/l. Intermediate sites had characteristics between these two classes. We sampled 146 sites of the Atlantic Forest region in Rio de Janeiro State (74 references, 38 intermediates, and 34 impaireds) during the dry season. Sampled sites ranged from 1st to 5th order according to Strahler classification (Figure 1).

FFG for evaluation of freshwater



Figure 1. Map of the study area, showing the sampling sites in the Rio de Janeiro State, Brazil, indicating gradient of impairment (reference, intermediate and impaired).

2. Sample site evaluation

In each sampling site, the following physicochemical variables were recorded in the field: dissolved oxygen (DO; YSI 550A equipment), pH (LabConte MPA 210p), and Conductivity (Cond; using a LabConte MCA 150p). Water samples were preserved in sterile plastic bags (whirl-pak), according to APHA (2000). In the laboratory, the concentration of Ammonia (NH₂) was determined using a HACH (DR 2500). Chloride (Ch), total alkalinity (TA), total hardness (TH), and calcium (Ca) were determined by the titrimetric method following APHA (2000). Sampling sites were also classified in the field by the HAP (Barbour et al. 1999). The HAP has ten environmental parameters, such as substrate availability for colonization by benthic fauna, water velocity, embeddedness (pool variability for low-gradient streams), channel condition (sinuosity for low-gradient streams), sediment deposition, margin stability, and riparian vegetation. For each variable, a score between 0 and 20 was assigned. Sites were classified conforming to the mean score obtained, as follows: 0-5 "Poor," 5.1-9.9 "Regular," 10-14.9 "Good," and 15-20 "Excellent" environmental condition (Barbour et al. 1999).

3. Biological samples

Macroinvertebrates were sampled by using a kick-net with a mesh size of 500 μ m. For this, 20 samples (20 m²) were taken proportionally to the substrates available at each site, according to the multi-habitat method (Barbour et al. 1999). Samples were conserved in the field in 80% ethanol and taken to the laboratory. In the lab, samples were washed to remove coarse organic matter, such as leaves and twigs. The remaining material was deposited into a sampler (64×36 cm), divided into 24 quadrants, each measuring 10.5×8.5 cm (Fiocruz, Patent application number PCT/BR2011/000144). This method is used to assure the randomness of biological assessments, as it is less subject to the variability of team members (Oliveira et al. 2011).

4. Functional Feeding Group classification and ratios

Fauna and FFGs were attributed to each taxon based on keys from regional entomofauna studies (Nessimian 1997, Baptista et al. 2006, Henriques-Oliveira & Nessimian 2010, Fernandes 2015) in Neotropical studies (Velásques & Miserendino 2003, Tomanova et al. 2006, Brasil et al. 2014) and the USA reference (Merritt & Cummins 1996). Also, five FFG ratios, adapted from Merritt et al. (1996), were used (Table 1).

able 1. Functional Feeding Group (11 G) failes modified from Merrie & Cummins (1776).						
Ecosystem attributes	Symbols	FFG ratios	Criteria levels			
Autotrophy/Heterotrophy index	A/H	Scraper/shredder + total collector	Autotrophic >0.75			
Shredder index	CPMO/FPOM	Shredder/total collector	Shredder availability >0.25			
Filtering-collector index	TFPOM/BFPOM	Filtering collector/gathering collector	TFPOM higher than normal >0.50			
Habitat Stability index	HSI	Scraper + filtering collector/ shredder + gathering collector	Stable substrates >0.50			
Predator-prey index	Predator	Predator/ total collector + scraper + shredder	Predator to prey balance 0.10–0.20			

Table 1. Functional Feeding Group (FFG) ratios modified from Merritt & Cummins (1996).

The autotrophy and heterotrophy index (A/H) relates primary productivity to total community respiration. The A/H serves as indicated that the stream is autotrophy (autochthonous organic matter derived algae or rooted vascular aquatic plants) or heterotrophy (allochthonous organic matter resultant from the riparian zone). The ratio between coarse particulate organic matter and fine particulate organic matter (CPOM/FPOM) provides insights into the quality of the riparian zone cover and the availability of litter used by shredders. The ratio of fine particulate organic matter and benthic fine particulate organic matter (TFPOM/BFPOM) measure the availability of relative amounts of coarse and organic particles (transported and stored in the environment). The habitat stability index (HSI) indicates the abundance of bottom substrates for the colonization of macroinvertebrates such as stones, wood, and aquatic plants. The predator-prey index (Predator) reflects top-down control by predators.

5. Data analysis

4

Abundance and richness of the FFG in each sampled site were calculated to characterize the differences in community trophic structure along the gradient of impairment. Taxa that could be assigned to more than one FFG were equally divided among the possible groups (Mendes et al. 2017). Differences among these groups were estimated by contrasts of the expected mean marginal values obtained from multivariate mixed linear models fitted using the maximum likelihood estimator.

The fixed/systematic component of models included the impairment gradient, while the random component included the river basin of each sampled stream. Also, to eliminate the dependence among the closest sampled stream, a Gaussian spatial correlation structure was considered. For the adjusted models, a graphical analysis of residuals was performed to confirm their randomness. In analyses of model marginal mean estimates contrasts, adjustments of the confidence level were made by Sidak's method, and p-value adjustments were made by multiple comparisons using Tukey's method. Stepwise searches based on the minimization of the Akaike Information Criterion (AIC), in both forward and backward directions, were used to select the optimal, non-redundant, mixed-effect model (similar to the one described above) of abiotic variables (i.e., Stream Width, Altitude, DO, pH, Cond, NH₃, Ch, TH, TA, Ca and, HAP) on the abundance and richness of each FFG. The level of significance, alpha = 0.05, was used in the analyses. Analyses were performed in R software version 3.6.1 (R Development Core Team, 2018, http://www.r-project.org/) with functionalities augmented by the packages 'emmeans' (Russell & Lenth 2020), used in the obtainment of estimated marginal means of the fitted mixed models, and 'nlme' (Pinheiro et al. 2020), used in the fitting of those models.

Results

A total of 108,282 aquatic benthic macroinvertebrates distributed in 176 taxa were collected during the study. In general, contrasts after the multivariate mixed linear model estimated marginal means showed significant differences along with the impairment gradient sites (a, reference - intermediate; b, reference - impaired; and, c, intermediate - impaired).

Filtering collector was the most abundant FFG regardless of impairment classes, and *Simuliidae* was the dominant taxa along the impairment gradient (reference, intermediate, and impaired sites). Figure 2 shows that estimated marginal mean abundance differed along the impairment gradient for scrapers (b = 99.97, p<0.000001; and, c = 87.35, p=0.006) and shredders (a = 29.83, p=0.0005; and, b = 46.86, p<0.000001). Gathering and filtering collectors, and predators had their highest mean values at intermediate sites, while scrapers and shredders had higher mean values at reference sites.



Figure 2. Distributions of samples and estimatives for FFG abundance along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Box-and-whisker and strip plots (gray) representing samples distribution of FFG abundance. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.

A significant decrease for marginal mean estimates of all FFG richness was observed along the impairment gradient (Figure 3). All FFGs showed differences along the impairment gradient: gathering collector (a = 2.97, p=0.001; b = 8.72, p<0.000001; and, c = 5.76, p<0.000001); filtering collector (b = 1.60, p<0.000001; and, c = 1.15, p=0.0001); scraper (a = 2.62, p=0.002; b = 7.10, p<0.000001; and, c = 4.49, p<0.000001); predator (a = 1.93, p=0.01; b = 6.15, p<0.000001; and, c = 3.49, p<0.000001); and shredders (a = 1.57, p<0.000001; b = 3.49, p<0.00001; and, c = 1.91, p<0.000001). Similarly, mean richness numbers of all FFGs decreased along the impairment gradient.

As expected, abiotic variables, i.e., DO, pH, Cond, TH, TA, Ca, and HAP, were significantly different along the impairment gradient (Supplementary Material: Appendix 1). Stepwise searches showed the most relevant among these abiotic variables on the abundance and richness variance of aquatic macroinvertebrate FFG (Table 2). Overall, the coefficients of determination (R^2) of the optimal models selected were low for the abundance of macroinvertebrate FFG, ranging from 0.14 to 0.34 (p<0.001 for all), suggesting lower importance than anticipated of these abiotic variables on the FFG abundance variation among sites.

Estimated marginal means based on regression analyses (mixedeffect models) showed that the altitude was a significant abiotic variable for all FFG abundance (except for the filtering collector). Nonetheless, for the filtering collector and the predator, we observed a positive linear correlation between abundance along the impairment gradient (e.g., NH₃ and Calcium). Different results were found for abiotic variables and richness of FFGs (Table 3). Optimal models selected for the richness of FFG the R^2 were moderate, ranging from 0.36 to 0.52 (p<0.001 for all), suggesting higher importance of abiotic variables on the variation of FFG richness than for abundance variance. Reductions were correlated to the increase of NH₃ for all FFG. Calcium (Ca) also was negatively correlated with richness among predators and shredders. The better tendencies were also observed for Total Hardness (TH) for gathering collectors, filtering collectors, scrapers, and predators. As expected, these reductions in the richness of FFG were significantly between the intermediate and impaired sites and the reference sites (Figure 3).

In general, estimated marginal means for FFG ratios showed significant differences among the impairment gradient sites. Significant differences along the impairment gradient were found for the Autotrophic/Heterotrophic index (A/H) (Figure 4A; a = 0.16, p=0.02; b = 0.26, p<0.001). Regardless of the position along the impairment gradient, most sites were below the A/H level of 0.75 being Heterotrophic (91.1%), indicating the dependence of the stream food web on the availability of allochthonous riparian organic matter. For A/H numbers for reference, intermediate and impaired were 0.49, 0.33, and 0.23, respectively. Coarse Particulate Organic Matter/Fine Particulate Organic Matter index (CPOM/FPOM) is an indicator of the availability of food resources for shredders. For this index (Figure 4B) we found a significant difference along the impairment gradient (b = 0.17, p=0.006).



Figure 3. Distributions of samples and estimatives for FFG richness along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Boxand-whisker and strip plots (gray) representing samples distribution of FFG richness. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.



Figure 4. Distributions of samples and estimatives for FFG ratios along the impairment gradient (Ref – Reference, Int - Intermediate, and Imp - Impaired). Boxand-whisker and strip plots (gray) representing samples distribution of A/H (Autotrophy and Heterotrophy index), CPOM/FPOM (Coarse Particulate Organic Matter and Fine Particulate Organic Matter index), TFPOM/BFPOM (Transport of Fine Particles Organic Matter, and Benthic Fine Particles Organic Matter index), HSI (Habitat Stability index), and Predator (Predator-prey index) along the impairment gradient. Dots and lines (black) representing estimated means and 95% confidence intervals obtained after multivariate mixed linear models fitted using the maximum likelihood estimator.

Reference sites had a mean CPOM/FPOM ration of 0.23, close to the ratio level cut (> 0.25). Intermediate sites showed lower shredder interaction with the riparian vegetation (mean = 0.13), and, as expected, impaired sites had shredders very underrepresented (mean = 0.06). For the Transport of Fine Particles Organic Matter/Benthic Fine Particles Organic Matter index (TFPOM/BFPOM) no differences were found along the impairment gradient (Figure 4C). TFPOM/BFPOM indexes were of good quality independently of the impairment gradient (according to the criteria ratio level > 0.50), with estimated mean marginal values of 1.36, 1.66, and 1.27, for reference, intermediate, and impaired sites respectively. For the Habitat Stability index (HSI) (Figure 4D), a significant difference along the impairment gradient was found (b = 0.82, p < 0.001), with estimated mean values of 2.11 and 1.92 for reference, and intermediate sites, respectively, which indicates an abundance of stable substrates. The estimated marginal mean value was 1.29 for impaired sites above the ratio level cut (> 0.50) for a stable substrate.

 Table 2. Stepwise searches based on the Akaike information criterion (AIC) minimization, in both forward and backward directions, of abiotic variables on the abundance of macroinvertebrate Functional Feeding Groups (FFG).

FFG	Variables	β	Std.Error	DF	t.value	p.value
Gathering-collector	(Intercept)	372.957	90.637	134	4.115	< 0.001
AIC=1828.64;	Width (m)	2.225	1.145	134	1.944	0.054
$R^2 = 0.17;$	Altitude (m)	0.117	0.027	134	4.300	< 0.001
L. Ratio= 22.99;	pH	-31.791	12.112	134	-2.625	0.010
p< 0.001	Log10_Ch (mg/L)	-190.588	76.806	134	-2.481	0.014
Filtering-collector	(Intercept)	-280.649	169.998	131	-1.651	0.101
AIC=2109.13;	Width (m)	8.631	2.217	131	3.894	< 0.001
$R^2 = 0.24;$	$Log10_NH_3 (mg/L)$	1945.715	370.471	131	5.252	< 0.001
L. Ratio= 63.75;	DO (mg/L)	44.067	13.699	131	3.217	0.002
p<0.001	Log10_Ca (mg/L)	1276.571	269.336	131	4.740	< 0.001
	Log10_TA	-287.848	198.065	131	-1.453	0.149
	HAP	-14.916	2.813	131	-5.302	< 0.001
	Log10_Cond (S/cm)	468.123	127.368	131	3.675	< 0.001
Scraper	(Intercept)	286.510	79.610	131	3.599	< 0.001
AIC= 1801.01;	Width (m)	1.671	0.698	131	2.394	0.018
$R^2 = 0.14;$	Altitude (m)	0.092	0.033	131	2.766	0.006
L. Ratio= 52.07;	DO (mg/L)	-13.712	4.358	131	-3.146	0.002
p<0.001	pН	-24.231	9.417	131	-2.573	0.011
	Log10_Ch	-117.153	51.677	131	-2.267	0.025
	HAP	3.897	1.091	131	3.571	< 0.001
	Log10_Cond (S/cm)	91.922	51.444	131	1.787	0.076
Predator	(Intercept)	328.199	122.679	133	2.675	0.008
AIC= 1929.17;	Altitude (m)	0.087	0.044	133	1.978	0.050
$R^2 = 0.21;$	$Log10_NH_3 (mg/L)$	712.903	360.219	133	1.979	0.050
L. Ratio= 33.52;	pH	-34.548	16.472	133	-2.097	0.038
p<0.001	Log10_Ca (mg/L)	479.868	121.618	133	3.946	< 0.001
	Log10_Cond (S/cm)	-142.137	69.245	133	-2.053	0.042
Shredder	(Intercept)	-59.243	17.263	132	-3.432	0.001
AIC= 1466.86;	Altitude (m)	0.030	0.009	132	3.504	0.001
$R^2 = 0.34;$	$Log10_NH_3$ (mg/L)	-132.232	67.989	132	-1.945	0.054
L. Ratio= 69.46;	DO (mg/L)	3.375	1.461	132	2.311	0.022
p<0.001	Log10_Ca (mg/L)	-46.292	29.530	132	-1.568	0.119
	Log10_TA (mg/L)	60.436	17.327	132	3.488	0.001
	HAP	2.898	0.609	132	4.758	< 0.001

AIC- Akaike Information Criterion, R²– Coefficient of Determination, L.Ratio– Likelihood Ratio, p-value– p-value after Likelihood Ratio Test, DF – Degrees of Freedom. Log10_NH₃ - Ammonia; Log10_TH- Total Hardness, Log10_Ch– Chloride, HAP- Habitat Assessment Protocol DO- Dissolved Oxygen and Log10_TA-Total Alkalinity and Log10_Ca– Calcium.

Finally, for the predator-prey index (Predator) (Figure 4E) significant differences were found along the impairment gradient (b = -0.29, p<0.000001; c = -0.30, p<0.000001). Impaired sites showed the highest estimated marginal mean value, 0.52, compared to the reference (0.23)

and intermediate sites (0.23). Most ratios were within the range of criteria levels for reference and intermediate sites and an overabundance of predators at impaired sites.

Table 3. Stepwise searches based on the Akaike information criterion (AIC) minimization, in both	th forward and backward d	irections, of abiotic	variables on the
richness of macroinvertebrate Functional Feeding Groups (FFG).				

FFG	Variables	β	Std.Error	DF	t.value	p.value
Gathering- collector	(Intercept)	9.743	1.078	133	9.034	<0.001
AIC= 804.63; R2= 0.52;	Altitude (m)	0.002	0.001	133	2.325	0.022
L. Ratio= 111; p<0.001	log10_NH3 (mg/L)	-22.917	7.174	133	-3.194	0.002
	log10 TH (mg/L)	-4.755	0.991	133	-4.797	< 0.001
	log10 Ch (mg/L)	-4.530	2.188	133	-2.070	0.040
	HAP	0.503	0.056	133	8.987	< 0.001
Filtering- collector	(Intercept)	3.998	0.375	133	10.657	<0.001
AIC= 440.02; R2= 0.36;	Width (m)	-0.013	0.004	133	-3.263	0.001
L. Ratio= 81.26; p<0.001	log10_NH ₃ (mg/L)	-8.742	2.004	133	-4.362	<0.001
	log10_TH (mg/L)	-0.627	0.397	133	-1.579	0.117
	log10_Ch (mg/L)	-1.409	0.646	133	-2.180	0.031
	HAP	0.104	0.016	133	6.642	< 0.001
Scraper	(Intercept)	7.896	1.625	132	4.861	< 0.001
AIC= 788.85; R2= 0.52;	log10_NH ₃ (mg/L)	-30.211	6.164	132	-4.901	<0.001
L. Ratio= 107.12; p<0.001	DO (mg/L)	-0.232	0.125	132	-1.860	0.065
	log10_TH (mg/L)	-6.407	1.490	132	-4.300	< 0.001
	log10_Ch (mg/L)	-6.062	2.112	132	-2.870	0.005
	log10_TA (mg/L)	4.935	1.897	132	2.602	0.010
	HAP	0.600	0.043	132	14.044	< 0.001
Predator	(Intercept)	7.116	0.939	133	7.578	< 0.001
AIC= 772.52; R2= 0.42;	Altitude (m)	0.003	0.001	133	3.366	0.001
L. Ratio= 75.08; p<0.001	log10_NH ₃ (mg/L)	-12.633	6.259	133	-2.018	0.046
	log10_Ca (mg/L)	-7.705	1.705	133	-4.520	< 0.001
	log10_Ch (mg/L)	-4.168	2.128	133	-1.959	0.052
	HAP	0.329	0.052	133	6.321	< 0.001
Shredder	(Intercept)	1.876	0.391	134	4.803	< 0.001
AIC= 557.39; R2= 0.51;	Altitude (m)	0.001	0.000	134	2.188	0.030
L. Ratio=104.05; p<0.001	log10_NH ₃ (mg/L)	-14.241	3.124	134	-4.558	<0.001
	log10_Ca (mg/L)	-2.305	1.397	134	-1.649	0.101
	HAP	0.216	0.025	134	8.739	< 0.001

AIC- Akaike Information Criterion, R^2 - Coefficient of Determination, L.Ratio- Likelihood Ratio, p-value p-value after Likelihood Ratio Test, DF – Degrees of Freedom. Log10_NH₃ - Ammonia; Log10_TH- Total Hardness, Log10_Ch- Chloride, HAP- Habitat Assessment Protocol, DO- Dissolved Oxygen, Log10_TA-Total Alkalinity and Log10_Ca- Calcium.

Discussion

Anthropogenic activities may impact stream ecosystems by causing habitat fragmentation, degradation, sedimentation, which consequently increase the abundance of tolerant species and a decrease of sensitive species (Mangadze et al. 2019). In our study, the abundance of gathering and filtering collectors and predators were higher at intermediate sites. In contrast, abundance and richness of scrapers and shredders were negatively correlated with the impairment gradient. Gathering collectors are often recognized as generalists and are considered dominant along the impairment gradient (Leslie & Lamp 2017). They can also transform the decomposition of Fine Particulate Organic Matter (FPOM) and carbon availability within the sediment (Leslie & Lamp 2017). In our study, filtering collectors were the most abundant FFG along the impairment gradient, due to large numbers of Simuliidae in urban areas. These organisms are reasonably resistant to disturbance (Feld et al. 2002). Furthermore, significant differences were found for scraper abundance between reference, intermediate, and impaired sites. According to Jun et al. (2011), scrapers are sensitive to impairment because they mainly consume attached algae that grow on the cobbles and pebbles of undisturbed streams. As for predators, we found lower mean abundance at impaired sites than the intermediate site. Predator abundance appears to be driven mainly by prey availability and have studies found an increased abundance in streams affected by human activities (Rawer-Jost et al. 2000). As expected, the abundance of shredders was reduced along the stream impairment gradient because of reduced quantities of riparian vegetation. Similar results were found by Oliveira & Nessimian (2010) that reported lower relative participation of this FFG on impaired sites. Although shredder abundance was very low, we found significant differences between reference and impaired sites.

Richness numbers displayed the best response and highest sensitivity to detect impairment. Richness numbers of all FFG were significantly reduced along the impairment gradient stream. Richness can have an essential function in characterizing stream ecosystem condition (Kaboré et al. 2016, Couceiro et al. 2011, Drover et al. 2020).

Estimated marginal means based on regression analyses (mixed-effect models) showed that the altitude was a significant abiotic variable for all FFG abundance, except for the filtering collector. According to Jacobsen (2008), one possible explanation is that at higher altitudes, streams tend to have lower temperatures and higher slopes, contributing to higher concentrations of DO. Tomanova et al. (2007) showed that altitude combined with position along the longitudinal gradient is an important factor controlling the FFG assemblages of stream macroinvertebrates in neotropical streams. In this study, the richness of all FFGs was reduced along the impairment gradient. These reductions were correlated to the increase of NH₂ for all FFG. According to Camargo (2019), physicochemical alterations, such as the increase of ammonia (NH₂), can be toxic to sensitive macroinvertebrate taxa. Also, it is well known that large-scale agriculture and urbanization may decrease water quality leading to alterations as the loss of riparian vegetation with a significant effect on the FFG structure and function (Gieswein et al. 2019). Streams in this region are subject to different pressures, including intensive urbanization and untreated sewage discharges. Most sites suffered the influence of multiple chemical and physical anthropogenic stressors. Agriculture and urban land-use practices reduce water quality due to inputs of fine sediments, nutrients, and pesticides. Alterations to the river channel's physical structure may cause a loss in riparian vegetation, which would be expected to produce a significant effect on FFG structure (Fu et al. 2016).

FFG ratios showed a variable response along the impairment gradient. The Autotrophic/Heterotrophic index (A/H) serves as a surrogate of Production/Respiration (P/R), which was significantly different along the impairment gradient. P/R has been used as the relative importance of energy fixed by primary producers (Vannote et al. 1980), i.e., P/R ratios among ecosystems are proxies of allochthonous ratio/autochthonous organic matter. According to this criterium, even though reference sites had higher estimated marginal mean values, almost sites in our study were classified as heterotrophic, independent of their position along the impairment gradient. These results follow Cummins et al. (2005), who also found that all sampled sites on their study of the Atlantic Forest stream of Southern Brazil could be characterized as heterotrophic. Other tropical/subtropical streams in Kenya also classified close-canopy streams as heterotrophic (Masese et al. 2014). According to the Coarse Particulate Organic Matter/Fine Particulate Organic Matter index (CPOM/FPOM), a shallow shredder interaction with riparian vegetation was found in impaired sites. According to Cummins et al. (1989), this decline is most probably related to the removal of riparian vegetation from agricultural and urban areas. We also found a TFPOM/BFPOM index unresponsive to the impairment gradient, which agrees with Couceiro et al. (2011). Moreover, the Habitat Stability index (HSI) indicated stable substrates that were more abundant in intermediate sites. One explanation for these results would be the intermediate disturbance hypothesis (Ward & Stanford 1983, Ward et al. 1999). Intermediate sites were submitted to constant sewage discharges. It seems to generate moderate mortality in the species not in such numbers that a recovery is impossible, but at the same time, sufficient to limit the growth of competitive species. For the Predator index, it was observed a low top-down control in reference and intermediate sites. Almost all FFG ratios showed significant differences along the impairment gradient. This observation does not agree with Kaboré et al. (2016), which found inconsistent results in different land use in West Africa.

FFG ratios as a surrogate of the ecosystem attributes may reduce the time and costs of the evaluation being fast, cheaper, and an integrated tool based on morphological and behavioral mechanisms of food acquisition. Moreover, this study evaluated marginal mean estimates for FFG ratio, abundance, and mostly for richness, as a useful tool to assess the ecological conditions of Atlantic Forest streams. Despite the almost FFG ratio being able to discriminate along the impairment gradient, further studies would be necessary to calibrate the method specifically for the Atlantic Forest region.

Supplementary Material

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The following online material is available for this article: Appendix 1 - Summary of the abiotic variables (mean value ± standard error) investigated along the impairment gradient. Appendix 2 - Functional Feeding Groups (FFG) for Atlantic Forest taxa.

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Author Contributions

Priscilla da Silva Pereira: 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.

Natália Freitas de Souza: Contribution to manuscript preparation and adding intellectual content.

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

Marcelo Ribeiro-Alves: Substantial contribution to manuscript preparation and critical revision, adding intellectual content, contribution to data analysis and interpretation.

Helena Lúcia Carneiro Santos: Substantial contribution to manuscript preparation and critical revision, adding intellectual content.

Daniel Forsin Buss: Contribution to manuscript preparation and to critical revision, adding intellectual content.

Conflicts of interest

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

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Lizards and snakes of Refúgio de Vida Silvestre Matas do Siriji, an Atlantic Forest hotspot of the Pernambuco Endemism Center, Northeastern Brazil

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Abstract: The Atlantic Forest north of the São Francisco River, known as the Pernambuco Endemism Center (PEC), comprises small, poorly-known and, consequently, highly threatened forest remnants, such as Refúgio de Vida Silvestre (RVS) Matas do Siriji, a montane forest located in the municipality of São Vicente Férrer, state of Pernambuco, Northeast Brazil. We provide the results of the first inventory of the squamate fauna of the region with comments on the conservation status of some species, comparisons with other locations in Northeast Brazil and a brief discussion of biogeography. Time-constrained transects, pit-fall traps, occasional encounters and third-party records registered 18 lizard species and 25 snake species, with the rarefaction curve of the former tending to stability. The RVS Matas do Siriji possesses 39.81 % of the lizard and snake species known for the state of de Pernambuco, being the third richest area in species in the State, with a composition similar to that of other areas within PEC. Based on the lists of SEMAS, ICMBio and IUCN, some of the registered species are considered vulnerable to extinction while others have yet to be evaluated. The RVS Matas do Siriji includes a rich, threatened and underestimated fauna of squamate reptiles, indicating that more restrictive protection measures must be adopted in this Conservation Unit.

Keywords: Northeastern Atlantic Forest; state of Pernambuco; Squamata; Endangered species.

Lagartos e serpentes do Refúgio de Vida Silvestre Matas do Siriji, um hotspot da Mata Atlântica do Centro de Endemismo Pernambuco, Nordeste do Brasil

Resumo: A Mata Atlântica ao norte do Rio São Francisco, conhecida como Centro de Endemismo Pernambuco (CEP), é composta pelos menores, menos conhecidos e, consequentemente, mais ameaçados remanescentes florestais, como o Refúgio de Vida Silvestre (RVS) Matas do Siriji, uma floresta serrana localizada no município

de São Vicente Férrer, Pernambuco, Nordeste do Brasil. Aqui apresentamos o primeiro inventário sobre a fauna de Squamata dessa região, com comentários sobre o status de conservação de algumas espécies, similaridade na composição com outras localidades do Nordeste do Brasil e uma breve discussão biogeográfica. Foram utilizados transectos limitados por tempo, armadilhas de queda, encontros ocasionais e registro por terceiros. Registramos 18 espécies de lagartos e 25 de serpentes com a curva de rarefação de lagartos próxima da estabilidade. O RVS Matas do Siriji possui 39,81 % das espécies de lagartos e serpentes do Estado de Pernambuco, ocupando o terceiro lugar em número de espécies no Estado e apresentando uma composição similar a de outras áreas do CEP. Além disso, com base nas listas da SEMAS, ICMBio e IUCN, algumas das espécies registradas encontram-se em estado vulnerável a extinção e outras não possuem avaliação. Portanto, o RVS Matas do Siriji comporta uma rica, ameaçada e subestimada fauna de répteis Squamata e medidas de proteção mais restritivas devem ser adotadas nessa Unidade de Conservação.

Palavras-chave: Mata Atlântica nordestina; Estado de Pernambuco; Squamata; Espécies ameaçadas.

Introduction

Centuries of successive economic cycles and constant occupation of Atlantic Forest environments has seriously compromised the ecological balance of the unique ecosystems of the domain (Silva & Casteleti 2005). The Atlantic Forest originally covered an area of 1,480,000 km², which corresponds to 17% of the national territory (Freitas et al. 2019). Today, only 160,000 km² (12.4%) of the coverage of the Atlantic Forest domain remains (SOS Mata Atlântica & INPE 2019), which houses at least 1% to 8% of the world's biodiversity (Silva & Casteleti 2005), earning it recognition as a global biodiversity *hotspot* (Myers et al. 2000).

Northeast Brazil has the lowest coverage of remaining of Atlantic Forest and the lowest amount of protected areas (Lobo-Araújo et al. 2013, Vale et al. 2018), even though it has great biological importance due to high endemism, especially north of the São Francisco River. Among the biogeographic subunits that make up the Northeastern Atlantic Forest is the Pernambuco Endemism Center (PEC) (Rizzini 1997, Cavalcanti & Tabarelli 2004), a region that extends from the state of Alagoas to the state of Rio Grande do Norte (Prance 1982, Santos et al. 2007). The region possesses high endemism, with emphasis on plants (Prance 1982, Santos et al. 2007), butterflies (Brown 1979, Tyler et al. 1994) and birds (Silva et al. 2004, Lobo-Araújo et al. 2013, Vale et al. 2018), and is considered to have been an important refuge for Atlantic Forest species north of the São Francisco River during Quaternary climate changes (see Carnaval et al. 2009, Porto et al. 2013, Costa et al. 2017).

Thus far, a total of 84 lizard species (Tozetti et al. 2017) and 142 snakes species (Marques et al. 2019) have been documented for the Atlantic Forest and although such estimates have yet to be made for PEC, some works have already contributed to the knowledge of its reptile fauna (Silva et al, 2006, Santana et al. 2008, Moura et al. 2012, Moura et al. 2015, Roberto et al. 2015, Oliveira et al. 2016, Roberto et al. 2017, Melo et al. 2018, Mesquita et al. 2018). Nonetheless, the herpetofauna of many areas remains to be sampled and evaluated, especially with regard to conservation (Roberto et al. 2017). Thus, it is extremely necessary and urgent that the herpetofauna of remnants that make up the PEC be surveyed, as the results of such studies are essential to better target conservation action plans (Margules & Pressey 2000). At least 15 species of squamate reptiles present in PEC were contemplated by the Plano Nacional para Conservação da Herpetofauna Ameaçada da Mata Atlântica Nordestina (PAN) (ICMBio 2019), four Endangered (EN): Coleodactylus natalensis, Leposoma baturitensis, Atractus caete and Bothrops muriciensis; two Vulnerable (VU): Echinanthera cephalomaculata and Amerotyphlops paucisquamus; four Near

Threatened (NT): Amphisbaena carvalhoi, A. heathi, A. lumbricalis and Coleodactylus elizae; and five Data Deficient (DD): Ophiodes striatus, Atractus maculatus, A. potschi, Dendrophidion atlantica and Liotyphlops trefauti.

The purpose of the present study was to inventory the species that comprise the squamate taxocenosis of Refúgio de Vida Silvestre (RVS) Matas do Siriji, a forest of PEC, and to characterize the taxocenosis, discuss the conservation status of its species and compare its squamate composition with that of other areas of Northeast Brazil.

Material and Methods

1. Study area

The RVS Matas do Siriji (Figure 1) (7°37'S, 35°30'W), also known as Mata do Siriji or Mata do Estado, is located between 600 and 640 m in altitude in the municipality of São Vicente Férrer, state of Pernambuco (Ferraz & Rodal 2006), Northeast Brazil. The climate is hot and humid according to the Köppen classification, with a dry summer and a rainy season that starts in January/February and ends in September but can extend until October (Beltrão et al. 2005). The RVS encompasses 600 ha (6.3 Km²) (Figure 1B) of montane forest (Rodal et al. 1998) composed of Dense and Montane Ombrophilous Forest (Figure 1C) (Pietrobom & Barros 2002).



Figure 1. Location of the study area – Refúgio de Vida Silvestre Matas do Siriji, in the state of Pernambuco (A; dark grey), municipality of São Vicente Férrer (B), Northeastern Brazil. The numbered circles (I, II and III) represent the sampled subareas. (C) Landscape of an environment found in the area.
Three subareas were selected for sampling (Figure 1B) and assessed for their structure and environmental conditions:

— Subarea I (07°37'00.4"S, 035°30'17.3"W, 575 m). With a history of growing manioc 50 years ago, and its subsequent abandonment, Subarea I currently possesses a shrub-tree stratum with little open space and is marked by a large number of young A. Banana (*Musa* spp.) monoculture surrounds the subarea almost completely and human presence is more frequent in this subarea than in the others. The terrain has little declivity (8.2%) and is considered undulating. It has, on average: 74% canopy coverage; 6.03 cm litter height; five trees per every 16 m² of 15 m in height and 31.20 cm circumference at breast height (CBH); air and soil temperature of 22.5°C and 24°C, and air and soil humidity of 81% and 85%, respectively.

— Subarea II (07°36'49.9''S, 035°30'25.9''W, 566 m). Marked by great declivity (40.1%), this subarea area was classified as strongly undulating. It is composed of a predominantly arboreal stratum that is slightly more spaced than in subareas I and III. No livestock or agriculture areas have been developed in the subarea but there are some trails. There is a source of water and an artificial pond at the beginning of the path of this subarea, while at the halfway mark there is a marked presence of rocks of varying sizes and many jackfruit (*Artocarpus heterophyllus*) from seedlings to large individuals of 15 m. The subarea has, on average: 80% canopy cover; 5.92 cm litter height; four trees per every 16 m² of 14 m in height and 27.98 cm CBH; air and soil temperature both 25.5°C; air and soil humidity of 78% and 83%, respectively.

— Subarea III (07°36'58.0"S, 035°30'37.1"W, 537 m). This subarea is more heterogeneous because it is markedly arboreal, including the presence of trees with tabular roots. It has a part with "bare" soil and a 20 m² rocky outcrop while the end of the path has a dense forest in better condition of preservation with natural streams, presenting gently undulating declivity (7.4%). This subarea has, on average: 85% canopy coverage; 6.24 cm litter height; four trees per every 16 m² of 15 m in height and 34.61 cm CBH; air and soil temperature of 24.5°C and 23.7°C; and air and soil humidity of 79% and 82%, respectively.

2. Sampling

Data collection was performed monthly from April 2018 to September 2019 (with the exception of May, June, August and September 2018), during field expeditions of seven or eight consecutive days (total of 102 days in the field).

Time-constrained visual searches of 1 hr 30 min were performed systematically along transects in each subarea (Crump & Scott Jr. 1994) by five observers each period (morning, afternoon and night) of the day for a total of 2,205 hours (1,530 during the day / 675 at night; 441 hours/ observer). *Pitfall traps* (Cechin & Martins 2000) were also used with two linear arrays, separated by at least 50 m, of five buckets each — a 60 l bucket at each end and three 20 l buckets in between, all separated by 5 m — per subarea for a total of 864 hours/bucket. Were also included occasional encounters and third-party records, which consists of photos provided during the study by people who live in the area.

Collected specimens (Appendix) were identified using Freitas (2015), Roberto et al. (2017) and consultation with experts. Still in the field were fixed, and finally deposited in the collection of the Laboratório de Herpetologia da Universidade Federal de Campina Grande (LHUFCG), in the municipality of Patos, state of Paraíba, Brazil and in the Coleção Paleoherpetológica e Herpetológica da Universidade Federal Rural de Pernambuco (CPH-UFRPE) and in the Coleção de Zoologia Didática da Universidade Federal Rural de Pernambuco (CZD-UFRPE), in the municipality of Recife, state of Pernambuco, Brazil. All procedures were carried out under permanent licenses for the collection of zoological material (SISBIO number. 11218-1 and 66285-1), as well as authorization from *Secretaria de Meio Ambiente e Sustentabilidade* (CPRH) management (process number 014349/2018).

3. Data analysis

In describing the taxocenosis, the number of sightings of a species was considered equivalent to its abundance since abundance itself was not assessed. Dominance (d) (Berger-Parker index), equitability (J) (Pielou index) and diversity of the taxocenosis were obtained through the Shannon-Wiener index (H') (Magurran 1988) using PAST 3.25 software (Hammer et al. 2001). An abundance distribution diagram was created and tested using relative frequency [(number of samples with a record of a species/total number of samples) x 100] and relative abundance [(number of individuals of the same species/total number of individuals collected in the area) x 100] of each species (Dajoz 2005), based on the adequacy of theoretical models of distribution and abundance (broken-stick, geometric, log-series or log-normal) (Melo 2008, Mesquita et al. 2013).

Values for the species rarefaction curves were obtained with PAST 3.25 (Hammer et al. 2001) using the rarefaction system with 10,000 randomizations (using sampling days as sample units). The values were then transferred to Microsoft Excel (2016), where graphs more representative of the curves' behavior were produced to evaluate the efficiency of the sampling effort (Gotelli & Colwell 2001). Species richness for lizards and snakes were estimated using the non-parametric estimators ICE and Jacknife II (Colwell & Coddington 1994) in Estimates 9.1.0 software (Colwell & Elsensohn 2014).

Classical cluster analyses were performed for lizards (79 spp.) and snakes (122 spp.) separately using the Jaccard Index (presence (1) / absence (0)) (Magurran 2004) in PAST 3.25 (Hammer et al. 2001). Records of squamate species at 36 locations in the Northeastern Atlantic Forest and associated ecosystems, including RVS Matas do Siriji, were used: 19 montane forests, including forest enclaves in the semiarid region - Serra do Urubu (Moura et al. 2011, Roberto et al. 2017), municipalities of Arcoverde, Belo Jardim and Sertânia (Freitas et al. 2019a), in Pernambuco state; Agrestina, Arara, Bananeiras, Bezerros, Brejo dos Cavalos, Brejo de Madre de Deus and Mata do Pau-Ferro (Pereira-Filho & Montingelli 2011; Pereira-Filho et al. 2020), in Pernambuco and Paraíba states; Reserva Biológica Pedra Talhada (Roberto et al. 2015), Pernambuco state; Chapada do Araripe (Borges-Nojosa & Caramaschi 2003, Ribeiro et al. 2008, Ribeiro et al. 2012), Ceará state; Parque Estadual Pico do Jabre (Pereira-Filho & Montingelli 2011, Arruda 2017), Paraíba state; and Planalto do Ibiapaba (including Parque Nacional do Ubajara) and (Borges-Nojosa & Caramaschi 2003, Loebmann & Haddad 2010, Castro et al. 2019) Serra do Maranguape, Serra da Aratanha and Maciço do Baturité (Borges-Nojosa & Caramaschi 2003, Borges-Nojosa 2007), Ceará state; 14 low altitude fragments of Atlantic Forest near the coast - Estação Ecológica do Tapacurá (Moura et al. 2012), rainforest fragment Tejipió (Oliveira et al. 2016) and Parque Estadual Dois Irmãos (Santos et al. 2017, Melo et al. 2018), Pernambuco state, Área de Preservação Permanente Mata

do Buraquinho (Santana et al. 2008) and Reserva Biológica Guaribas (Mesquita et al. 2018), Paraíba state, Reserva Madeiras (Moura et al. 2015), Reserva Particular Usina Porto Rico (Queissada 2009) and Mata do Engenho Coimbra, Alagoas state (Gonçalves 2008), Refúgio de Vida Silvestre Matas do Junco (Morato et al. 2011), Sergipe state, Área de Proteção Ambiental Lagoa Encantada (Dias et al. 2014), of northeastern coast the Bahia (Marques et al. 2017), Recôncavo Baiano (Freitas 2014), Serra da Jiboia (Freitas et al. 2018) and Serra do Timbó (Freitas et al. 2019b), Bahia state; and three areas of mesic caatinga — Parque Nacional Chapada Diamantina (Magalhães et al. 2015), Bahia state, Parque Nacional Serra das Confusões (Vechio et al. 2016) and Parque Nacional Serra da Capivara (Cavalcanti et al. 2014), Piauí state. The aim was to assess similarities among these areas and determine if they form groups based on the three types of formations, and if so, to which does RVS Matas do Siriji belong.

Dendrograms were produced for lizards and snakes with, respectively, 28 (12 montane forests, 13 low-altitude Atlantic Forest fragments and three areas of mesic caatinga) and 31 (15 mountain forests, 13 low-altitude Atlantic Forest fragments and three areas of mesic caatinga) localities because only lizards (four locations) or snakes (eight locations) were inventoried at some locations.

Species registered only through third-party records were included only in the species list and in the classical cluster analysis.

4. Species conservation status

Conservation status was assessed using the list published by Secretaria de Meio Ambiente e Sustentabilidade (SEMAS 2017) of the state of Pernambuco, Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (ICMBio 2018) and the Red List of Threatened Species of the International Union for the Conservation of Nature (IUCN 2020). Only Hemidactylus mabouia was not evaluated since it is exotic. Taxonomic nomenclature follows Costa and Bérnils (2018).

Results

4

A total of 43 species were recorded during the study period, of which 18 were lizards belonging to 10 families and 25 were snakes belonging to six families (Table 1; Figures 2–7). Most species were recorded through occasional encounters (n=26), 16 of which (37.21% of all species) were exclusively detected by this method (Table 1). Time-constrained visual searches recorded the second most species (n=24), 12 of which (27.91%) were only recorded by this method (Table 1). *Pitfall traps* collected seven individuals of four species, two of which (4.65%) were recorded exclusively by this method — the lizard *Dryadosaura nordestina* and the snake *Amerotyphlops arenensis*. Third-party records added two species (4.65%), which were both snakes (Table 1). Another species (*Oxyrhopus petolarius digitalis*) was registered in the area by third parties after the end of the study, and was added to the list.

The rarefaction curve for lizards approached the asymptote and ICE and Jacknife II estimators predicted 18.7 (\pm 0) and 20 (\pm 0) species, respectively (Figure 8A). Thus, these estimators predicted between one and two more species than were recorded by our sampling effort (18). The rarefaction curve for snakes did not cease increasing and ICE and Jacknife II estimators predicted 41.49 (\pm 0) and 41.76 (\pm 0) species (Figure 8B), both of which are 20 more species than were recorded by our sampling effort (22). Among the lizards, *Enyalius* aff. *catenatus* and *Gymnodactylus darwinii* were the most frequently seen, representing 17% (n=41) and 12% (n=28) of the total number of sightings (n=239) of the entire taxocenosis. Among snakes, *Dipsas variegata* and *Lachesis muta* were most recorded, representing 4% (n=10) and 3% (n=8) of the total number of sightings of the whole taxocenosis (Table 1).

The reptile taxocenosis showed low dominance (d= 0.139) and high equitability (J= 0.824), indicating that it is stable (H'= 3.080). It fit the log-normal model (Figure 9), as there was no significant difference between the distribution of abundances of the taxocenosis and the model (X^2 = 2. 907; *p*= 0.234).

Among the species found in RVS Matas do Siriji, three had a conservation status of Vulnerable (*Strobilurus torquatus, Dipsas sazimai* and *Lachesis muta*), according to at least one of the three lists consulted (Table 1). *Dendrophidion atlantica* is considered Data Deficient (DD) by ICMBio (2018) and was not evaluated by the other lists (SEMAS 2017 and IUCN 2020), whereas only *Amerotyphlops arenensis* was not evaluated by any of the lists (Table 1).

The similarity dendrogram for lizards (Figure 10A) formed two large groups, represented by different phytophysiognomies, with Group I (upper part) comprising 12 areas with a predominance of mesic caatinga with small remnants of seasonal Atlantic Forest; and Group II (bottom part) comprising 15 areas of Atlantic Forest, with montane or lowland, dense or open ombrophilous forest. In this dendrogram, RVS Matas do Siriji is inserted in Group II, sharing greater composition (59%) with Parque Estadual de Dois Irmãos (PEDI-PE), although also sharing just over 50% with Área de Proteção Permanente Mata do Buraquinho (APPMB-PB), Reserva Particular Usina Porto Rico (RPUPR-AL), Reserva Madeiras (RM-AL) and Mata do Engenho Coimbra (MEC-AL).

The similarity dendrogram for snakes (Figure 10B) revealed that RVS Matas do Siriji shared a maximum of 40% of its composition with Serra do Urubu (SU-PE) and 30% with other areas with montane or plain, dense or open ombrophilous forest. Groupings with considerable similarity with RVS Matas do Siriji and other areas were not observed. A grouping of montane forests located more centrally and northwest of the Caatinga, including Parque Estadual Pico do Jabre (PEPJ-PB), Arcoverde (AV-PE), Sertânia (SE-PE), Belo Jardim (BJ-PE) and Chapada do Araripe (CA-CE), remained in both dendrograms, sharing 34% of their compositions in the snake dendrogram and 54% in the lizard dendrogram (Figure 10).

Discussion

To date, there is an estimated 41 species of lizards and 67 species of snakes in the state of Pernambuco (SEMAS 2017). Thus, RVS Matas do Siriji possesses 39.81% of the lizard and snake species of the state, with more species than RPPN Pedra D'antas and RPPN Frei Caneca (which make up Serra do Urubu) with 37.04% (Roberto et al. 2017), and fewer than Estação Ecológica do Tapacurá (47.22%) (Moura et al. 2012) and Parque Estadual de Dois Irmãos (48.15%) (Santos et al. 2017, Melo et al. 2018), all of which are areas of extreme importance for the state of Pernambuco and for PEC as a whole.

Most species had a similar number of sightings, and thus the taxocenose had high equitability and low dominance and did not differ significantly from the log-normal model (Figure 9). According to Dajoz (2005), a fit to this model is common for communities controlled by

Table 1. List of lizard and snake species recorded in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019, with respective collection methods, relative frequency, conservation status (according SEMAS, ICMBio and IUCN) and record type. TCVS = time-constrained visual search, OE = occasional encounter, PT= pitfall trap, TPR = third-party records, TPR*= third-party records after study, LC= Least Concern, DD= Data Deficient, VU= Vulnerable, AF= restricted to the Atlantic Forest, PEC= restricted to the Atlantic Forest and endemic to PEC.

Family/Species	Collection method	Relative frequency (%)	SEMAS 2017/ICMBio 2018/IUCN 2019	Record type
Lagartos				
Dactyloidae				
Dactyloa punctata (Daudin, 1802)	TCVS	9.54	LC/LC/not evaluated	Collected
Norops fuscoauratus (D'Orbigny, 1837 in Duméril & Bibron, 1837)	TCVS	7.05	LC/LC/ not evaluated	Collected
Norops ortonii (Cope, 1868)	TCVS/EO	1.24	not evaluated /LC/ not evaluated	Collected
Gekkonidae				
Hemidactylus agrius Vanzolini, 1978	OE	2.07	LC/LC/LC	Collected
Hemidactylus mabouia	TCVS/OE	0.83	-	Collected
(Moreau de Jonnés, 1818)				
Gymnophthalmidae				
Dryadosaura nordestina Rodrigues, Freire, Pellegrino & Sites Jr., 2005 AF	PT	1.24	LC/LC/LC	Collected
Leiosauridae				
<i>Enyalius</i> aff. catenatus AF	TCVS/PT	17.01	LC/LC/LC	Collected
Phyllodactylidae				
Gymnodactylus darwinii	TCVS/PT	11.62	LC/LC/LC	Collected
(Gray, 1845) Ar				
Polychrotidae				
Polychrus marmoratus (Linnaeus, 1758)	OE	1.66	LC/LC/LC	Collected
Polychrus acutirostris Spix, 1825	TCVS	0.83	LC/LC/LC	Photographic record
Scincidae				
Copeoglossum nigropunctatum (Spix, 1825)	OE	3.32	LC/LC/LC	Collected
Brasiliscincus heathi (Schmidt & Inger, 1951)	OE	0.83	LC/LC/LC	Photographic record
Sphaerodactylidae				
Coleodactylus meridionalis (Boulenger, 1888)	TCVS	2.49	LC/LC/LC	Photographic record
Teiidae				
Ameiva ameiva ameiva (Linnaeus, 1758)	TCVS	2.49	LC/LC/LC	Photographic record
Salvator merianae Duméril & Bibron, 1839	OE	1.24	LC/LC/LC	Photographic record
Kentropyx calcarata Spix, 1825	TCVS/OE	0.41	LC/LC/LC	Photographic record
Tropiduridae				
Strobilurus torquatus Wiegmann, 1834 AF	OE	1.24	VU/LC/LC	Collected
Tropidurus hispidus	TCVS	7.47	LC/LC/LC	Collected
<u>(Spix, 1825)</u>				
Serpentes				
Boidae				
Boa constrictor Linnaeus, 1758	TPR	-	LC/LC/ not evaluated	Photographic record
Epicrates assisi Machado, 1945	OE	0.83	LC/LC/ not evaluated	Photographic record
Colubridae				
Chironius flavolineatus Jan, 1863	OE	0.41	LC/LC/LC	Photographic record
Dendrophidion atlantica Freire, Caramaschi & Gonçalves, 2010 PEC	OE/TCVS	1.66	DD/DD/ not evaluated	Collected
<i>Echinanthera cephalomaculata</i> Di Bernardo, 1994 PEC	TPR	-	not evaluated /VU/ not evaluated	Photographic record

Continue...

Continuation...

<i>Echinanthera cephalostriata</i> Di Bernardo, 1996 ^{AF}	TCVS	1.24	not evaluated /LC/LC	Collected
Oxybelis aeneus (Wagler in Spix, 1824)	OE	0.83	LC/LC/LC	Collected
Spilotes pullatus pullatus (Linnaeus, 1758)	TCVS	0.41	LC/LC/LC	Collected
Tantilla melanocephala (Linnaeus, 1758)	OE	0.41	LC/LC/LC	Collected
Dipsadidae				
Dipsas variegata (Duméril, Bibron & Duméril, 1854)	OE/TCVS	4.15	LC/LC/LC	Collected
Dipsas sazimai Fernandes, Marques & Argôlo, 2010 AF	OE	0.41	VU/LC/LC	Collected
Erythrolamprus taeniogaster (Jan, 1863)	OE/TCVS	0.83	LC/LC/LC	Collected
Philodryas nattereri Steindachner, 1870	OE	0.41	LC/LC/LC	Photographic record
Philodryas olfersii (Lichtenstein, 1823)	OE	0.41	LC/LC/LC	Photographic record
Imantodes cenchoa (Linnaeus, 1758)	TCVS/OE	2.49	LC/LC/LC	Collected
Oxyrhopus guibei Hoge & Romano, 1978	OE	1	LC/LC/LC	Collected
Oxyrhopus petolarius digitalis (Reuss, 1834)	TPR*	-	LC/LC/LC	Photographic record
<i>Oxyrhopus trigeminus</i> Duméril, Bibron & Duméril, 1854	OE	0.83	LC/LC/LC	Collected
Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	TCVS	0.83	LC/LC/LC	Photographic record
Taeniophallus affinis (Günther, 1858) AF	OE	1	not evaluated /LC/LC	Collected
Xenodon rabdocephalus rabdocephalus (Wied-Neuwied, 1824)	OE/TCVS	1.66	not evaluated /LC/ not evaluated	Collected
Elapidae				
Micrurus lemniscatus carvalhoi Roze, 1967	OE	1	DD/LC/LC	Photographic record
Typhlopidae				
Amerotyphlops arenensis Graboski, Pereira- Filho, Silva, Prudente & Zaher, 2015 PEC	PT	1.24	not evaluated / not evaluated / not evaluated	Collected
Viperidae				
Bothrops leucurus Wagler in Spix, 1824 AF	OE/TCVS	2.49	LC/LC/ not evaluated	Collected
Lachesis muta (Linnaeus, 1766)	TCVS	3.32	VU/LC/VU	Collected

several ecological factors, which leads to a balance in the sharing of available resources or, in other words, less competition, which is a commonly observed pattern for tropical forests.

The richness estimators ICE and Jacknife II indicated that, in 102 days of sampling, we recorded between 90% and 96% of the maximum expected lizard richness and 53% of the maximum expected snake richness (without considering the three snake species obtained by third-party records). Curves that do not reach an asymptote (Figure 8) demonstrate a need for continued sampling (Gotelli & Colwell 2001), which is reinforced by the species recorded by the third-party records during and after the study. In addition, species richness is proportional to sampling effort (Melo et al. 2003), and so it would take a greater sampling effort to record the rest of the species, especially the snakes. Thus, the present list of species is an initial milestone in the study of reptiles of RVS Matas do Siriji.

The methods that contributed the most to determining richness and abundance in the present study were, respectively, occasional encounters and time-constrained visual searches (Table 1). The efficiency of these methods for registering reptiles has been reported in many studies in different biomes (Carvalho et al. 2005, Freitas & Silva 2007, Quintela et al. 2010, Roberto et al. 2017), including Pedrosa et al. (2014) who reported that such methods are better for sampling snakes. On the other hand, *pitfall traps* represented a less efficient method, especially for snakes. According to Cechin & Martins (2000), *pitfall traps* are less efficient in environments with high vertical structure, that is, forest environments in which many species have an arboreal habit. However, the importance of this method (How & Shine 1999, Henderson et al. 2016) in recording fossorial species, such as *Amerotyphlops arenensis* and *Dryadosaura nordestina* (Roberto et al. 2017), and especially lizards and snakes with active foraging, is undeniable.

The third-party records are also of paramount importance, as has been seen in the literature for snake inventories, due to its fortuitous character (Cunha & Nascimento 1978, Cechin & Martins 2000). Indeed, the voluntary contribution of residents of RVS Matas do Siriji to the present study led to the inclusion of three additional species of snakes (*Boa constrictor, Echinanthera cephalomaculata* and *Oxyrhopus petolarius digitalis*), one of which is considered VU according to ICMBio. These findings confirm the efficiency of using a combination of different methods to optimize the sampling of snakes (Caldas et al. 2016).

Although most of the species observed in the present study are considered Least Concern (LC) by SEMAS (2017) (27 species), ICMBio



Figure 2. Lizard species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) Dactyloa punctata (LHUFCG 2224), (B) Norops fuscoauratus (unvouchered, juvenile), (C) Norops ortonii (CPH-UFRPE 5461), (D) Hemidactylus agrius (unvouchered, juvenile), (E) Hemidactylus mabouia (unvouchered), (F) Dryadosaura nordestina (LHUFCG 2316), (G) Enyalius aff. catenatus (unvouchered, female) (H) Enyalius aff. catenatus (CPH-UFRPE 5479, male).

(2018) (34 species) and IUCN (2020) (26 species), some considerations are needed with regard to species in the VU and DD categories, as well as the lack of evaluation for some species (Table 1):

The snake *Echinanthera cephalomaculata* is an endemic species of PEC, with its first record being at its type locality in the state Alagoas (Di-Bernardo 1994, Roberto et al. 2015) and recent records for three locations in the state of Pernambuco (Freitas et al. 2019b). Its registration in RVS Matas do Siriji, therefore, expands its distribution 74 km to the north of its nearest record (municipality of Gravatá, state of Pernambuco). Since the expansion of its distribution to the state of Pernambuco was done after the publication of the SEMAS list, the absence of its ranking is justifiable; nonetheless it is in urgent need of evaluation, such as by IUCN. The other species of the genus, *E. cephalostriata*, also recently had its distribution expanded in the state of Pernambuco (Dias et al. 2019), which was also its second record for PEC – the first record was done by Roberto et al. (2015), and was also not evaluated by SEMAS.

The snake species *Taeniophallus affinis* is also not listed by SEMAS due to the fact that its two records in the state of de Pernambuco (Roberto et al. 2017, Santos et al. 2017) were published after (September and December, respectively) the list (May 2017). The snake *Xenodon rabdocephalus rabdocephalus* was not evaluated by SEMAS, although it had already been registered in Reserva Biológica Pedra Talhada (Roberto et al. 2015) located on the border between the states of Pernambuco and Alagoas. The recent expansion of its distribution to PEC (Andrade Lima et al. 2020) reveals sampling gaps in that



Figure 3. Lizard species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) *Gymnodactylus darwinii* (LHUFCG 2269), (B) *Polychrus marmoratus* (unvouchered, male), (C) *Polychrus acutirostris* (unvouchered), (D) *Copeoglossum nigropunctatum* (unvouchered), (E) *Brasiliscincus heathi* (unvouchered), (F) *Coleodactylus meridionalis* (unvouchered), (G) *Ameiva ameiva ameiva* (unvouchered, male), (H) *Salvator merianae* (unvouchered).

region and makes the assessment of its extinction risk more accurate. Therefore, these two species need urgent evaluation by SEMAS. The arboreal lizard *Norops ortonii* was registered by Oliveira et al. (2016) in the state of Pernambuco prior to the publication of the SEMAS list but was not evaluated.

Although Fernandes et al. (2004), considered that there are no subspecies of *Lachesis muta* (*L. m. muta* and *L. m. rhombeata*), both the IUCN and SEMAS categorized *L. m. rhombeata* as VU, considering only the populations of the Atlantic Forest distributed from the state of Ceará to the south of the state of Rio de Janeiro (Campbell & Lamar 2004). Interestingly, this was the second most sighted species of snake within RVS Matas do Siriji, as it was for another location in the state of Pernambuco (Serra do Urubu; Roberto et al. 2017). The main reason for its classification as VU is habitat loss due to intense destruction of the Atlantic Forest (Alves et al. 2014), which is the reality for almost all fragments of the biome in Brazil, but especially those of PEC. Even if they are not considered subspecies, evaluation of the populations of the Atlantic Forest and the Amazon separately by ICMBio would more accurately represent the conservation status of the populations.

The snake *Amerotyphlops arenensis*, which only occurs in the states of Paraíba, Alagoas and Pernambuco (Graboski et al. 2015, Roberto et al. 2015, 2017), was recently described. This species has not been evaluated by any of the three consulted lists and needs to be urgently. The species was probably not on the SEMAS list because its first record for the state of Pernambuco (Roberto et al. 2017) was published four months after the publication of the list, although there was already a



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Figure 4. Lizard and snake species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) *Kentropyx calcarata* (unvouchered), (B) *Strobilurus torquatus* (unvouchered), (C) *Tropidurus hispidus* (unvouchered), (D) *Boa constrictor* (unvouchered), (E) *Epicrates assisi* (unvouchered), (F) *Chironius flavolineatus* (unvouchered), (G) *Dendrophidion atlantica* (unvouchered), (H) *Echinanthera cephalomaculata* (unvouchered).

record on the border between the states of Pernambuco and Alagoas (Roberto et al. 2015).

It should be noted that the classification of DD (e. g. *Dendrophidion atlantica* and *Micrurus lemniscatus carvalhoi*) should not be taken as any less of a concern since little-known species may be at a higher degree of threat than VU or Endangered (EN) species. This is especially true for species with disjunct distributions and low densities and that are restricted to only one region, such as PEC, which, according to Ribeiro et al. (2009), retains about 12.1% of its original coverage, of which only 1% is protected.

The clear formation of two groups in the lizard dendrogram (Figure 10A) seems to reflect the phytophysiognomies of the localities: Group I with seasonal (dry) forests west of Planalto da Borborema, which, despite some of them having remnants of Atlantic Forest (montane forests), are inserted in the Caatinga biome and, thus, are strongly influenced by it; and Group II (in which RVS Matas do Siriji is inserted) with predominantly ombrophilous (humid) forests east of Planalto da Borborema, typical of Atlantic Forest, including other montane forests. Some studies involving plants (Santos et al. 2007, Rodal & Sales 2008, Rodal et al. 2008) and snakes (Pereira-Filho et al. 2020) as models, have demonstrated the division of montane forests into "wet" and "dry", with Planalto da Borborema being indicated as responsible for the division.

We emphasize that the lizard composition of localities was more efficient at showing the formation of these groups of "wet" and "dry" forests than snake composition. This finding probably does not reflect a true random distribution of snakes, but instead the difficulty in their sampling.



Figure 5. Snake species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) *Echinanthera cephalostriata* (CPH-UFRPE 5841), (B) *Oxybelis aeneus* (LHUFCG 2374, juvenile), (C) *Spilotes pullatus pullatus* (unvouchered), (D) *Tantilla melanocephala* (LHUFCG 2451), (E) *Dipsas variegata* (CPH-UFRPE 5273, juvenile), (F) *Dipsas sazimai* (CPH-UFRPE 5492, juvenile), (G) *Erythrolamprus taeniogaster* (unvouchered), (H) *Philodryas nattereri* (unvouchered).

In general, the lizard and snake composition of RVS Matas do Siriji was found to be similar to six other Atlantic Forest fragments of PEC (PEDI-PE, APPMB-PB, RM-AL, RPUPR-AL, MEC-AL and SU-PE), which are all east of Planalto da Borborema and share species frequently recorded in Atlantic Forest environments, such as Dryadosaura nordestina, Dactyloa punctata, Enyalius catenatus, Gymnodactylus darwinii, Norops fuscoauratus, Norops ortonii, Polychrus marmoratus, Strobilurus torquatus, Bothrops leucurus, Dendrophidion atlantica and Lachesis muta. This finding may be a reflection of the proximity of RVS Matas do Siriji to these locations, as well as its phytophysiognomic similarity with the dense ombrophilous forests of these areas. On the other hand, the presence of seasonal montane forest (located in the center and northwest of the Caatinga; west of Planalto da Borborema) (AV-PE, SE-PE, BJ-PE, PEPJ-PB and CA-CE) in the two dendrograms, even while maintaining a lizard fauna more similar to those of the three areas of Caatinga (PNSC-PI, PNSCO-PI and PNCD-BA), demonstrates that the lizard and snake fauna of this type of montane forest (e.g., Gymnodactylus geckoides Spix, 1825, Hemidactylus brasilianus (Amaral, 1935), Lygodactylus klugei (Smith, Martin & Swain, 1977), Ameivula ocellifera (Spix, 1825), Phyllopezus pollicaris (Spix, 1825), Psychosaura agmosticha (Rodrigues, 2000), Tropidurus semitaeniatus (Spix, 1825), Vanzosaura multiscutata (Amaral, 1933), Apostolepis cearensis Gomes, 1915, Boiruna sertaneja Zaher, 1996, Bothrops erythromelas Amaral, 1923, Epictia borapeliotes (Vanzolini, 1996), Thamnodynastes almae Franco & Ferreira, 2003 and T. sertanejo Bailey, Thomas & Silva-Jr, 2005) is strongly influenced by the Caatinga.



Figure 6. Snake species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) *Philodryas olfersii* (unvouchered), (B) *Imantodes cenchoa* (unvouchered), (C) *Oxyrhopus guibei* (LHUFCG 2286, juvenile), (D) *Oxyrhopus petolarius digitalis* (unvouchered), (E) *Oxyrhopus trigeminus* (CPH-UFRPE 5275), (F) *Pseudoboa nigra* (unvouchered), (G) *Taeniophallus affinis* (LHUFCG 2271), (H) *Xenodon rabdocephalus rabdocephalus* (unvouchered).



Figure 7. Snake species registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. (A) *Micrurus lemniscatus carvalhoi* (unvouchered), (B) *Amerotyphlops arenensis* (LHUFCG 2265), (C) *Bothrops leucurus* (LHUFCG 2368), (D) *Lachesis muta* (unvouchered).

Although it is part of PEC, some authors (Pôrto et al. 2004, Ferraz & Rodal 2006, Santos et al. 2007) consider RVS Matas do Siriji to be a "Brejo de Altitude" ("Brejo Nordestino") — another biogeographic subunit of the Atlantic Forest located north of Rio São Francisco being a mosaic of phytophysiognomies with minimal influence from the Caatinga biome on its northwest side and stronger influence by the Atlantic Forest to the south-southeast, including being considered a "área de tensão ecológica" (Caatinga – Atlantic Forest ecotone) (Santos & Tabarelli 2004). The literature argues that "Brejos de Altitude" are



Figure 8. Rarefaction curves (red) with its standart deviation (dashed lines) and ICE (light grey) and Jacknife II (dark grey) curves for lizards (A) and snakes (B) registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Férrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019.



Figure 9. Abundance distribution for species of lizards and snakes registered in Refúgio de Vida Silvestre Matas do Siriji, municipality of São Vicente Ferrer, state of Pernambuco, Northeastern Brazil, between April 2018 and September 2019. Bars = relative frequency, line = relative abundance. The diagram fits the log-normal model (X^2 = 2.907; p= 0.234).

places where fauna from the Caatinga and Atlantic Forest can coexist (e.g., Borges-Nojosa & Caramaschi 2003, Pereira-Filho & Montingelli 2011, Ribeiro et al. 2012, Castro et al. 2019, Freitas et al. 2019). Only four (9.30%) typical Caatinga species (*Brasiliscincus heathi*, *Hemidactylus agrius*, *Polychrus acutirostris* and *Philodryas nattereri*) were recorded in RVS Matas do Siriji demonstrating that "Brejos de Altitude" possess different compositions throughout their distribution (e. g. Ribeiro et al. 2012, Roberto et al. 2015, Roberto et al. 2017; Castro et al. 2019, Freitas et al. 2019), with strong or weak influences from the Caatinga.



Figure 10. Jaccard similarity dendrograms for lizards (A), involving 29 locations with 12 montane forests (1), 13 low-altitude Atlantic Forest fragments (2) and three areas of mesic caatinga (3); and snakes (B), involving 32 locations with 16 montane forests, 13 low-altitude Atlantic Forest fragments and three areas of mesic caatinga, all in Northeastern Brazil. RVSMS (indicated by the arrow)=Refúgio de Vida Silvestre Matas do Siriji, SU=Serra do Urubu, AR=Arcoverde, BJ=Belo Jardim, SE=Sertânia, AG=Agrestina, BE=Bezerros, CA=Cavalos, MD=Brejo Madre de Deus, EET=Estação Ecológica do Tapacurá, FMAT=Fragmento de Mata Atlântica Tejipió, PEDI=Parque Estadual Dois Irmãos (áreas no Estado de Pernambuco-PE), RBPT=Reserva Biológica Pedra Talhada, RM=Reserva Madeiras, RPUPR=Reserva Particular Usina Porto Rico, MEC=Mata do Engenho Coimbra (áreas no Estado de Alagoas-AL), AR=Arara, BA=Bananeira, MPF=Mata do Pau-Ferro, PEPJ=Parque Estadual Pico do Jabre, APPMB=Área de Preservação Permanente Mata do Buraquinho, RBG=Reserva Biológica Guaribas (áreas no Estado da Paraíba-PB), CA=Chapada do Araripe, PI=Planalto do Ibiapaba, SM=Serra do Maranguape, AS=Serra da Aratanha, MB=Maciço do Baturité, PNU=Parque Nacional do Ubajara (áreas no Estado do Ceará-CE), RVSMJ=Refúgio de Vida Silvestre Matas do Junco (área no Estado de Sergipe-SE), APALE=Area de Proteção Ambiental Lagoa Encantada, FACN=Floresta Atlântica Costeira a Nordeste, RBFAN=Recôncavo Baiano Floresta Atlântica do Norte, SJ=Serra da Jiboia, ST=Serra do Timbó, PNCD=Parque Nacional Chapada Diamantina (áreas no Estado da Bahia-BA), PNSCO=Parque Nacional Serra das Confusões, PNSC=Parque Nacional Serra da Capivara (áreas no Estado do Piauí-PI). Grupo 1 = mesic or xeric caatinga; Grupo 2 = Atlantic Forest with dense ombrophilous forest.

Among the species recorded in the present study, 11 (25.58%) are exclusive to the Atlantic Forest, with three (*Arenensis arenensis*, *Dendrophidion atlantica* and *Echinanthera cephalomaculata*) being found only in PEC, confirming the importance of RVS Matas do Siriji for the maintenance of populations of endemic species in this sector of the Atlantic Forest. Another 28 species (65.12%), including *Polychrus marmoratus*, *Kentropyx calcarata*, *Dipsas variegata*, *Oxyrhopus petolarius digitalis*, *Xenodon rabdocephalus rabdocephalus* and *Lachesis muta*, are shared with the Amazon, as is true for other areas of the PEC (Moura et al. 2012, Roberto et al. 2015, Roberto et al. 2017, Santos et al. 2017, Mesquita et al. 2018), evidencing the meeting of the great Atlantic Forest and Amazon biomes during part of the Cenozoic (Prance 1982, Andrade-Lima 1982, Teixeira et al. 1986, Santos et al. 2007), and demonstrating the irreplaceable role that PEC has played in the reconstruction of the history of these two biomes.

Species distribution patterns and historical relationships between morphoclimatic domains are two major biogeographical enigmas that are frequently investigated in Brazil (Prance 1982, Santos et al. 2007). Accordingly, different groups of animals, such as reptiles, seem to be efficient models for understanding the remote relationships between the Amazon and Atlantic Forest and, more recently, between the Atlantic Forest and the Caatinga in the biogeographic regions of PEC and "Brejos de Altitude" (montane forests) (e. g. Rodrigues et al. 2014; Prates et al. 2018). However, it is known that many areas in these regions have yet to be sampled (Borges-Nojosa & Arzabe 2005, Roberto et al. 2017), making the need to inventory these places urgent, not only for biogeographic reasons but especially for the conservation of existing biodiversity.

In general, the squamate fauna of RVS Matas do Siriji possesses typical elements of the Northeastern Atlantic Forest, with species that only occur in the PEC, and is even similar to Parque Estadual de Dois Irmãos (Santos et al. 2017). Thus, RVS Matas do Siriji reaffirms the capacity of PEC to harbor high endemism of several groups, with Squamata being no exception. Additionally, RVS Matas do Siriji possesses endangered species (Lima et al. 2020) and species with few recent records for Pernambuco (Dias et al. 2019a, 2019b; Freitas et al. 2019b), including five species that are not included in the list of species for the state (SEMAS 2017), highlighting the need to update the list to include such species mainly for the assessment of their conservation status. Although RVS Matas do Siriji has been a full protection Conservation Unit since 2014 and identified as a priority conservation area in PEC for 20 years (MMA 2000), it still suffers from the removal of vegetation, giving way to vast monocultures of sugarcane and bananas. Therefore, RVS Matas do Siriji is home to a rich, threatened and underestimated reptile fauna, and still contributes abundantly to the maintenance of biodiversity, not only of PEC, but the Atlantic Forest as a whole, reasons that call for the protection of this neglected Conservation Unit.

Supplementary Material

The following online material is available for this article: Appendix

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Author Contributions

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Conflicts of Interest

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

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Feeding habits of the Robber Frog *Pristimantis paulodutrai* (Bokermann, 1975) in northeastern Brazil

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Abstract: Studying feeding habits is crucial to understand complex predator-prey interactions. Even though anurans play a fundamental role in the control of arthropods populations, the diet of several Neotropical species is poorly known. We describe the frequency and occurrence of prey items and their dry mass in stomach contents of the Robber Frog *Pristimantis paulodutrai* in the north east of the state of Bahia, Brazil. Based on the stomach contents of 30 individuals, Araneae, Isopoda, and Formicidae were found to be the most important food items. The generalist diet of this frog, which seems to be phylogenetically conserved among *Pristimantis*, is likely to be linked to its ecological dominance in the habitats surveyed. Our study corroborates the high trophic relevance of Robber Frogs in tropical forests as generalist predators.

Keywords: Amphibia; Anura; Bahia; Craugastoridae; diet; predator-prey interaction.

Hábitos alimentares da rã *Pristimantis paulodutrai* (Bokermann, 1975) no nordeste do Brasil

Resumo: Estudar hábitos alimentares é crucial para entender as complexas interações predador-presa. Embora os anuros tenham papel fundamental no controle das populações de artrópodes, a dieta de várias espécies neotropicais é pouco conhecida. Descrevemos a frequência e a ocorrência de presas e sua massa seca no conteúdo estomacal da rã *Pristimantis paulodutrai*, no nordeste do estado da Bahia, Brasil. Com base no conteúdo estomacal de 30 indivíduos, Araneae, Isopoda e Formicidae foram os itens alimentares mais importantes. A dieta generalista desta rã, a qual parece ser conservada filogeneticamente entre os *Pristimantis*, provavelmente está ligada à sua dominância ecológica nos habitats pesquisados. Nosso estudo corrobora a alta relevância trófica dos *Robber Frogs* em florestas tropicais como predadores generalistas.

Palavras-chave: Amphibia; Anura; Bahia; Craugastoridae; dieta; interação predador-presa.

Introduction

While studies of feeding habits of anurans have been increasing in number (e.g., Ortega et al. 2005, Forti et al. 2011, Solé et al. 2019), the relationship between frogs and their preys is still little known. Descriptive studies are fundamental to reveal complex predator-prey interactions and to determine the role of anurans in ecosystems (Hocking & Babbitt 2014). The diet of frogs is particularly affected by their foraging behaviour and the characteristics of microhabitats (Toft 1995).

The genus *Pristimantis* Jiménez de la Espada, 1870 comprises 566 species, distributed from southern Central America to the Brazilian Atlantic Forest (Frost 2021). Most members of this genus are generalist predators (Arroyo et al. 2008, Garcia-R et al. 2015, Gutiérrez-Cárdenas et al. 2016). Nevertheless, knowledge about the natural history of these direct-developing frogs is still incipient, and the diet of several species, among them *Pristimantis paulodutrai* (Bokermann, 1975), which is the subject of our study, remains unknown.

The frog *P. paulodutrai* is restricted to the Atlantic Forest in Bahia state (Trevisan et al. 2020), and it is considered the most abundant and spatially distributed anuran species from coastal forests of northern Bahia state (Bastazini et al. 2007). In this study we aimed to identify the diet composition of this species, and given its ecological dominance among other amphibian species, we expected to find a rich assemblage of consumed preys. Yet, under an exploratory approach, we seek spatially structured feeding patterns for *P. paulodutrai* considering the main environmental landscape units in the study area.

Material and Methods

We examined 90 adult specimens of P. paulodutrai deposited in the Natural History Museum of the Federal University of Bahia (UFBA), collected by Bastazini et al. (2007) and previously used for ecologic, taxonomic, morphometric, and/or cytogenetic studies on the genus Pristimantis (e.g., Bastazini et al. 2007, Napoli et al. 2009, Dabés et al. 2012, Trevisan et al. 2020). The specimens were collected in a coastal Atlantic Forest area (a 500 hectares private conservation unit named Reserva Sapiranga, 12°33'59"S, 38°02'18"W, 20 m a.s.l, WGS84 datum) and in coastal sandplains (restinga environment at Praia do Forte, 12°34'12"S, 38°00'04"W, 10 m a.s.l., WGS84 datum), both in the municipality of Mata de São João, state of Bahia, Brazil. The specimens were sampled in four distinct habitats (Bastazini et al. 2007): in Reserva Sapiranga, forested environments containing (1) springs, streams, and rivers, and (2) areas without bodies of water on the ground and with less than 20 tank bromeliads in a sample unit of 60 x 25 m; in the restinga of Praia do Forte, (3) sandy soil covered by many shrubs and terrestrial tank bromeliads under direct sunlight (> 100 bromeliads per sample unit), which is contiguous with (4) a riparian forest that surrounds a lake formed by the freshwater Timeantube river, the latter filled with emergent vegetation. Detailed description of the environment, sampling units, sampling methods, and vouchered specimens were provided by Bastazini et al. (2007). During fieldwork voucher specimens of P. paulodutrai (referred by the authors as Eleutherodactylus ramagii) were kept alive in containers with ice up to six hours to reduce the speed of digestion of stomach contents (T.F.S.S. Alves-dos-Santos, personal observation). Prey availability was not measured during the sampling period and, therefore, we did not calculate electivity of prey categories (comparison between consumed and available food items). Specimens

http://www.scielo.br/bn

examined are housed in the amphibian collection of the Natural History Museum of the Federal University of Bahia (UFBA) (Appendix 1).

We dissected individuals and examined their stomach content using an Olympus stereomicroscope. Only adult males were evaluated because females and juveniles were rare in the samples. The prey items were grouped into orders (except for Annelida, Gastropoda, and Formicidae). We followed the methods of Magnusson et al. (2003) to determine the dry mass and to estimate the contribution of each prey item. The total dry mass of each prey item was recorded to the nearest 0.1 mg, and each food item was dried in a 50°C stove for two hours. We assigned a mass value of 0.1 mg for prey categories if their dry mass was less than the precision of the instrument (1 mg) to record the prey category in the diet without influencing the contribution of the most important prey categories.

We calculated the proportion of biomass (M) and frequency (F) of prey categories for each stomach individually and then averaged these proportions for the stomachs that were full. This procedure resulted in proportional data, which avoids the bias originating from the anurans' uneven body size (Evans & Lampo 1996) and the biomass of the stomach content and confers the same importance to all specimens (Smith et al. 2004). The proportion of occurrence (O) of each consumed prey category was calculated as the number of stomachs with a prey category averaged for the total number of full stomachs. Items with the highest index of relative importance (IRI) was considered the most important prey categories in the diet of P. paulodutrai (Pinkas et al. 1971). We used the IRI (%) values in a hierarchical clustering method, under an exploratory approach, to seek and display the strongest structure of feeding patterns for P. paulodutrai considering four habitats identified by Bastazini et al. (2007), using the Ward's method solution (Ward's minimum variance method; Ward 1963) and Euclidean distance measure as distance metric. The source data matrix consists of four lines (environment types) and 11 columns (prey categories). The analysis was performed using the software Statistica ver. 13 (TIBCO 2018).

Results and Discussion

We obtained 30 adult males with full stomachs, containing 49 food items ($\bar{x} = 1.6$ item/stomach; total mass = 0.10724 g) belonging to 11 prey categories (Figure 1, Table 1). The following prey categories had the largest (over 10%) proportions of occurrence (O): Araneae (43.3%), Formicidae (23.3%), Isopoda (20.0%), and Coleoptera (16.7%). Orthoptera, Isopoda, Araneae, and "Larvae" had the largest M (24.2%, 21.5%, 16.7%, and 12.5%, respectively), although the former was consumed by only three specimens of *P. paulodutrai* (10%) and Araneae by 13 specimens (43.3%). Coleoptera and Isopoda contributed with similar frequencies (16.7% and 20.0%, respectively), but with different mass contributions (8.3% and 21.5%, respectively); the O of "larvae" was smaller than in Coleoptera (10% and 16.7%, respectively), but with higher M (12.5% and 8.3%, respectively). The most important prey categories (index of relative importance > 10%) consumed by *P. paulodutrai* were Araneae (46.6%), Isopoda (16.1%), and Formicidae (12.4%) (Figure 1d, Table 2).

The high number of empty stomachs from adult males of *P. paulodutrai* (66%) could be explained due to all individuals have been captured while reproductively active. The impact of calling behavior on male foraging success was already reported for *E. coqui*, which considerably reduce its foraging activity while exhibit to potential



Figure 1. Prey categories in the diet of *Pristimantis paulodutrai* from a restinga area in the municipality of Mata de São João, state of Bahia, Brazil. (a) percentage of occurrence of each consumed alimentary category (number of stomachs with an alimentary category averaged for the total number of non-empty stomachs); (b) average percentage of prey categories; (c) average percentage of biomass contribution; and (d) IRI%, index of relative importance (Pinkas 1971). Numbers at the top of columns represent non-transformed values

sexual partners (Woolbright & Stewart 1987). In this species, males usually start foraging after midnight, when calling activity is ceased (Woolbright 1985, Woolbright & Stewart 1987). Therefore, temporal sampling bias could also contribute to explain empty stomachs in part of *P. paulodutrai* adult males because all of them were captured by active search at night from 1800 to 2400 h (Bastazini et al. 2007), assumed here as the period of their highest calling activity.

The hierarchical clustering analysis using IRI (%) values was able to express structure in feeding patterns for *P. paulodutrai* along the landscape (Figure 2, Table 2), gathering two habitat groups: (1) a group composed of forested environments with water bodies on the ground (habitats 1 and 4), and (2) a group of environments deprived of water bodies on the ground, combining forested zones and open restinga habitats with terrestrial tank bromeliads (habitats 2 and 3). Foraging success in amphibians is known to be dependent of local climatic conditions (seasonal and/or daily fluctuations) and one possible consequence is the reduction of movement and foraging activity due to water-conserving posture in situations of osmotic stress (Pough et al. 1983, Woolbright & Stewart 1987, Wells 2007). Therefore, we suppose that variations in qualitative and/or quantitative prey availability throughout the day, combined with periods of low motility and foraging,

Table 1. Prey categories in the diet of *Pristimantis paulodutrai* within four habitats of a restinga landscape in the municipality of Mata de São João, state of Bahia, Brazil. Habitats are ordered following clustering structure resulted from Ward's method solution (Figure 2). An empty cell indicates a non-available prey category in habitat. Values in parentheses correspond to non-transformed data. Reserva Sapiranga: habitat 1, forested environments containing springs, streams, and rivers; habitat 2, forested environments without bodies of water on the ground and with less than 20 tank bromeliads in a sample unit of 60 x 25 m. Praia do Forte: habitat 3, sandy soil covered by many shrubs and terrestrial tank bromeliads under direct sunlight (> 100 bromeliads per sample unit); and habitat 4, riparian forest that surrounds a lake formed by the freshwater Timeantube river, the latter filled with emergent vegetation.

		Habitat 4			Habitat 1			Habitat 2			Habitat 3	
	%0	%M	%F	%0	%M	%F	% 0	%M	%F	%0	%M	%F
Araneae	25.0	14.4	25.0	44.4	9.9	25.0	42.9	40.1	33.3	66.7	21.5	31.3
	(2)	(0.00250)	(2)	(4)	(0.00580)	(4)	(3)	(0.00630)	(3)	(4)	(0.00331)	(5)
Formicidae	12.5	4.6	12.5	22.2	0.9	18.8	14.3	7.0	11.1	50.0	11.7	25.0
	(1)	(0.00080)	(1)	(2)	(0.00051)	(3)	(1)	(0.00110)	(1)	(3)	(0.00181)	(4)
Coleoptera	37.5	44.8	37.5				28.6	7.1	22.2			
	(3)	(0.00780)	(3)				(2)	(0.00111)	(2)			
Isopoda	25.0	36.2	25.0	44.4	28.6	25.0						
	(2)	(0.00630)	(2)	(4)	(0.01680)	(4)						
Dermaptera				11.1	1.5	6.3				33.3	26.6	12.5
				(1)	(0.00090)	(1)				(2)	(0.00410)	(2)
Hemiptera							28.6	20.4	22.2	16.7	19.5	6.3
							(2)	(0.0032)	(2)	(1)	(0.00300)	(1)
Larva				22.2	21.8	12.5				16.7	3.9	6.3
				(2)	(0.01280)	(2)				(1)	(0.00060)	(1)
Orthoptera				22.2	37.3	12.5	14.3	25.5	11.1			
				(2)	(0.02190)	(2)	(1)	(0.00400)	(1)			
Acari										16.7	1.3	6.3
										(1)	(0.00020)	(1)
Diplopoda										16.7	7.1	6.3
										(1)	(0.00110)	(1)
Gastropoda										16.7	8.4	6.3
										(1)	(0.00130)	(1)

Table 2. Index of relative importance - IRI (Pinkas et al. 1971) calculated for alimentary categories eaten by *Pristimantis paulodutrai* within four habitats of a restinga landscape in the municipality of Mata de São João, state of Bahia, Brazil. Habitats are ordered following clustering structure resulted from the Ward's method solution (Figure 2). An empty cell indicates a non-available prey category in habitat. Reserva Sapiranga: habitat 1, forested environments containing springs, streams, and rivers; habitat 2, forested environments without bodies of water on the ground and with less than 20 tank bromeliads in a sample unit of 60 x 25 m. Praia do Forte: habitat 3, sandy soil covered by many shrubs and terrestrial tank bromeliads under direct sunlight (>100 bromeliads per sample unit); and habitat 4, riparian forest that surrounds a lake formed by the freshwater Timeantube river, the latter filled with emergent vegetation

	Overall	Restinga	Habi	tat 4	Habi	tat 1	Habi	tat 2	t 2 Habitat	
	IRI	IRI%	IRI	IRI%	IRI	IRI%	IRI	IRI%	IRI	IRI%
Araneae	1961.80	46.59	984.20	16.92	1550.18	24.51	3147.22	52.61	3514.38	44.80
Isopoda	675.71	16.05	1530.17	26.31	2382.90	37.68				
Formicidae	520.39	12.36	213.72	3.68	435.97	6.89	258.76	4.33	1836.90	23.41
Coleoptera	308.54	7.33	3087.28	53.09			836.79	13.99		
Orthoptera	302.74	7.19			1106.71	17.50	522.47	8.73		
Larva	186.18	4.42			762.27	12.05			169.02	2.15
Hemiptera	119.04	2.83					1216.90	20.34	428.42	5.46
Dermaptera	107.85	2.56			86.48	1.37			1302.96	16.61
Gastropoda	10.84	0.26							244.68	3.12
Diplopoda	10.22	0.24							223.06	2.84
Acari	7.42	0.18							125.78	1.60

could contribute to different prey consumption by *P. paulodutrai* within distinct habitats. Furthermore, distinct composition and abundance of prey items between habitats could be argued as an explanation for the obtained results but these data were not acquired and, therefore, we cannot test if prey availability or frog's prey preference are able to explain our results.

Some *Pristimantis* species and other Brachycephaloidea are considered sit-and-wait foragers and not ant specialists (Toft 1981). In our study, *P. paulodutrai* had a varied diet (11 prey categories), which is more typical of an active forager's profile (Duellman & Trueb 1994), with Formicidae being only its third most important prey category. Such high diversity of consumed preys followed the general feeding pattern of related species (e.g., Arroyo et al. 2008, Garcia-R et al. 2015, Gutiérrez-Cárdenas et al. 2016). We conclude that *P. paulodutrai*, as other common species, is not a diet specialist frog, which supports its large dominance within the studied landscape.

The numerical and spatial dominance of *P. paulodutrai* over other species in the study area could be partially explained by the ability of this common frog to prey on different groups of arthropods. While the spatial distribution of specialist predators is affected by limited prey availability, generalists may have a flexible distribution, with the availability of prey being a weaker spatial predictor (Giaretta et al. 1998, Santos et al. 2004). Although prey availability does not seem to be an important factor in restricting the spatial distribution of *P. paulodutrai*, this hypothesis must be tested in the future using an electivity analysis.

Pristimantis paulodutrai is a plastic species concerning water absorption under different osmotic stresses (e.g., low rates of dehydration and high rates of rehydration from water), which may favour its dominance in dry environments (Dabés et al. 2012). In addition, these frogs have direct development and terrestrial breeding (Napoli et al. 2009), which do not limit their distribution to the proximity of water bodies. The combination of these features with a generalist diet can explain the wide spatial distribution of *P. paulodutrai*.

These results contribute to our knowledge about the feeding habits of a Neotropical frog species, expand the scientific comprehension of



Figure 2. Hierarchical clustering analysis (Ward's method) on the Index of Relative Importance (IRI %) (Pinkas 1971) calculated for alimentary categories eaten by *Pristimantis paulodutrai* in four different habitats of a restinga area in the municipality of Mata de São João, state of Bahia, Brazil. Reserva Sapiranga: habitat 1, forested environments containing springs, streams, and rivers; habitat 2, forested environments without bodies of water on the ground and with less than 20 tank bromeliads in a sample unit of 60 x 25 m. Praia do Forte: habitat 3, sandy soil covered by many shrubs and terrestrial tank bromeliads under direct sunlight (> 100 bromeliads per sample unit); and habitat 4, riparian forest that surrounds a lake formed by the freshwater Timeantube river, the latter filled with emergent vegetation

the interaction between anurans and their preys, and provide additional insights to the spatial distribution of *P. paulodutrai*.

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Author Contributions

Thales Francisco S. S. Alves-dos-Santos: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Lucas Rodriguez Forti: Contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Marcelo Felgueiras Napoli: 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 declares that they have no conflict of interest related to the publication of this manuscript.

Ethics

The specimens were collected with authorizations from the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA/RAN, license numbers 02006.002336/03-26 and 0210.000812/05-84).

Data availability

All specimens used in this study are housed at the Amphibian Collection of the Natural History Museum of the Federal University of Bahia, Salvador, Bahia, Brazil, a scientific collection of a public institution (Appendix 1). Quantitative data used in hierarchical clustering analysis are available in Table 2.

Supplementary Material

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

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Aquatic larval of the genus Arrenurus (Trombidiformes: Parasitengonina: Arrenuridae) associated with Odonata species from Pampa Biome, Brazil

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Abstract: Many studies have reported that the interaction between water mite larvae and their Odonata hosts affects mating success, flight, and longevity. Males and females of Odonata species collected in the steppes and coastal plains (Pampa Biome) of Rio Grande do Sul were analyzed. Mites were removed when present and the prevalence and intensity of parasites was calculated. The aim of this study was to search and report new Odonata hosts species that are parasitized by water mite larvae and also to evaluate the prevalence and intensity rates; the differences in mite occurrence and frequency between males and females, and between thorax and abdomen of the dragonflies and damselflies in the southern Pampa biome located in Rio Grande do Sul. A total of 162 larval mites were found associated to two Odonata families: Coenagrionidae (Acanthagrion lancea Selys, 1876, Ischnura capreolus Hagen, 1861 and Ischnura fluviatilis Selys, 1876) and Libelullidae (Micrathyria ocellata Martin, 1897 and Perithemis mooma Kirby, 1889). All mites were identified as Arrenurus (Arrenurus) sp. (Arrenuridae) and showed high numbers when attached to I. capreolus (55.5%), I. fluviatilis (33.3%), followed by low numbers on M. ocellata (6.1%), A. lancea (3.7%), and P. mooma (1.2%). Mites were found on males and females of I. capreolus and *I. fluviatilis*, females of *A. lancea* and *P. mooma* and in *M. ocellata* only in males. As the parasitized Odonata species are generalist and abundant in all water body types, traits associated with mating and oviposition or larval behavior are believed to explain the frequency of parasitism in these species.

Keywords: water mites; lentic systems; dragonfly; damselfly; parasitism

Larvas aquáticas do gênero Arrenurus (Trombidiformes: Parasitengonina: Arrenuridae) associada a espécies de Odonata do bioma Pampa, Brasil

Resumo: Muitos estudos relatam que a interação entre as larvas parasitas e seus hospedeiros Odonata afetam o sucesso do acasalamento, o voo e a longevidade. Foram analisados machos e fêmeas de espécies de Odonata coletados nas estepes e planícies costeiras do bioma Pampa do Rio Grande do Sul. Os ácaros foram removidos quando presentes e a prevalência e intensidade dos parasitas foram calculadas. O objetivo deste estudo foi pesquisar e relatar novas espécies hospedeiras de Odonata que estão parasitadas por larvas de ácaros aquáticos, avaliar as taxas de prevalência e intensidade; avaliar a diferença na ocorrência e frequência de ácaros em fêmeas e machos e entre o tórax e abdômen de libélulas e libelulinhas no bioma Pampa meridional localizado no Rio Grande do Sul. Um total de 162 ácaros foram encontrados associados a duas famílias de Odonata: Coenagrionidae (Acanthagrion lancea Selys, 1876, Ischnura capreolus Hagen, 1861 e Ischnura fluviatilis Selys, 1876) e Libelullidae (Micrathyria ocellata Martin, 1897 e Perithemis mooma, 1889) Kirby. Todos os ácaros foram identificados como Arrenurus (Arrenurus) sp. (Arrenuridae) e apresentaram números elevados quando anexados a I. capreolus (55,5%), I. fluviatilis (33,3%), seguido por números baixos em M. ocellata (6,1%), A. lancea (3,7%) e P. mooma (1,2%). Os ácaros foram encontrados em machos e fêmeas de I. capreolus e I. fluviatilis, fêmeas de A. lancea e P. mooma e em M. ocellata apenas em machos. Como as espécies de Odonata parasitadas são generalistas e abundantes em todos os tipos de corpos d'água, acredita-se que características associadas ao acasalamento e oviposição ou comportamento larval explicam a frequência de parasitismo nessas espécies.

Palavras-chave: ácaros aquáticos, sistema lêntico, libélulas, libelulinhas, parasitismo

Introduction

The hyporder Parasitengonina is characterized by mites that have parasitic larva and predatory nymphs and adults; the resting stages provide an adaptation to avoid unfavorable conditions in unstable environments, and larval parasitism on flying insects provides substantial advantages, ensuring dispersal and rapid exploitation of new habitats (Smith et al. 2001, Smith et al. 2010, Proctor et al. 2015). Hydrachnidiae, or water mites, are a highly diverse group of Parasitengonina, comprising over 6,000 described species and reported in all regions of the world, except for Antarctica (Cook 1974, Viets 1987). They are found in lotic and lentic habitats, as well as in springs, interstitial waters, wetlands, temporary pools, marine habitats, torrential waterfalls, ponds, streams, and lakes (Smith & Cook 1991, Smith et al. 2001, Goldschmidt 2016).

A very ordinary family of Hydrachnidiae is Arrenuridae, and in the preparasitic phase they are very fast and active, searching out their hosts under the water surface walking on the substrate or swimming. The survival period for free-swimming larvae in the preparasitic phase ranges from 4 days to 6 weeks (Smith & Oliver 1986, Smith 1988, Smith et al. 2010). Their larvae parasitize species of Diptera, Coleoptera, and Odonata orders, all groups with the final instar active in the water or flying (Smith & Cook 1991, Smith et al. 2001, Zawal 2008, Smith et al. 2010, Gerecke et al. 2016). The predominant water mite genus that uses Odonata larvae and adults as hosts is Arrenurus Dugès (stricto sensu) (Arrenuridae) (Andrew et al. 2012), and at least 55 species have been described as Odonata ectoparasites (Davids 1997, Zawal & Dyatlova 2006, Baker et al. 2007, Zawal 2008). The genus Arrenurus is one of the most species-rich of the Hydrachnidiae, with about 1000 species present in most of the zoogeographic regions (Smit 2020). However, the genus lacks cosmopolitan species, and each region supports its own set of species (Zawal 2008). A list of South American water mites species was published by Rosso de Ferradás and Fernández (2005) who listed 150 Arrenurus species. The number of reported South America Arrenurus reaches now 153 species (Smit 2020). Some species listed for Brazil are A. clavipes Lundblad, 1941, A. corniger Koenike, 1894, A. epimerosus Marshall, 1919, A. ludificator Koenike, 1905, A. nitidus K. Viets, 1937, A. quadrituberculatus K. Viets, 1937, A. triconicus Marshall, 1919 and A. undulatus Lundblad, 1937 (Smit 2020).

Arrenurus s. str. has not been precisely determined, mainly due to the lack of possibility to identify mite larvae (Smith,1988, Zawal 2008, Smith et al. 2010, Zawal & Buczyński 2013). Contemporary taxonomical knowledge on water mites is based primarily on males, but the description of the female should also be provided as they show significant morphological differences as well; the larval and nymphal stages are considerably less known (Zawal 2008, Smith et al. 2010, Smit 2020).

Larvae of many subfamilies of water mites exhibit strong selectivity in their attachment to particular parts of the host body (Smith & Oliver 1986). Larvae of *Arrenurus* s. str species have preferences for either thoracic or abdominal sites and are less seen in the head or wings of odonate hosts (Smith et al. 2010). Studies indicate that mite parasitism can affect longevity, flight, and fecundity (Åbro 1979, Åbro 1982, Forbes 1991, Forbes & Baker 1991).

Many odonate species respond to attached mites by aggregating their haemocytes at the sites of puncture and by producing melanotic encapsulation of feeding tubes, but *Arrenurus* genus have a developed powerful mouthpart specialized in anchor to the host's body; the pedipalps have a well-developed claw that fixes in the cuticle and then with its chelicerae saber pierces the body till finds the hemolymph; then the larva produces a feeding device characterized by a narrow gelatinous resilient blind sac called stylostome, which seems to inject cytotoxins into the wound of the damselfly, thus, producing a paralysis which allows sufficient time to develop a stylostome to absorb nutrients, and this makes the damselfly's defensive apparatus ineffective to cope with the stylostome (Åbro 1979).

Heavy mite infestation brings several wounds in close proximity, accompanied by loss of more or less extensive areas of the epidermis. Despite Odonata wound repair by congregating hemocytes, local lack of epidermis seems to enfeeble the host, presumably owing to desiccation, thus, the infestation contributes to reduced longevity (Åbro 1982).

Through this form of feeding, Reinhardt (1996) observed that ectoparasitic mites have a negative influence on flight ability one meter was the longest distance flown by 35.2 % of the infested adults immediately after release while none flew farther than five meters; in the group of the non-parasitized damselflies, 75% flew more than one meter, and this pattern was consistent in both sexes. Oviposition injuries were observed by Rolff (1999) which tested *Arrenurus cuspidator* (O. F. Müller, 1776) on *Coenagrion puella* Linnaeus, 1758 and found that the number of eggs laid by the damselfly decreased with increased ectoparasite abundance.

Rodrigues et al. (2013) were the first to report larval of the *Arrenurus* genus parasites on Odonata species *Ischnura fluviatilis* Selys, 1876 (Coenagrionidae) and *Miathyria marcella* Selys, 1857 (Libellulidae) in Brazil.

The present study had the main objective to search and report new Odonata hosts for parasitic larvae of Arrenurus genus and also to evaluate the prevalence and intensity rates; the differences in mite occurrence and frequency between males and females, and between thorax and abdomen of the dragonflies and damselflies in the Pampa biome located in the Rio Grande do Sul state, Brazil.

Material and Methods

The present study was the first one performed in the Pampa biome. This biome covers the southern half of the state of Rio Grande do Sul and extends to Argentina and Uruguay. This biome constitutes the Brazilian portion of the South American Pampas, which are classified as steppes by the international phytogeographic system. The Pampa is limited by the Atlantic Forest biome to the north, and by the Chaco and the Patagonian steppes to the west. The steppes in the Pampa region have no dry season. However, they undergo high thermal amplitude and intense drying cold fronts, which increase evapotranspiration, and consequently, cause occasional droughts. This factor limits arboreal flora and riverbanks, valley bottoms, and protects lands from cold fronts; in other areas, grassy-woody species predominate. Steppes have been undergoing an intense anthropization process, due to cattle raising, grain cultivation, and fires (IBGE 2019). The coastal area (Coastal Plains) comprises sedimentary land of both fluvial and marine origin, flattened or depressed areas, generally with sandy soils. Pioneer Formations are predominant in this area. This vegetation occupies unstable land and is in constant ecological succession (IBGE 2019).

1. Study area

Samplings were conducted between 2016/17 in the municipalities of Caçapava do Sul, Manoel Viana, Mata, Quaraí, Rosário do Sul, Santa Margarida do Sul, Santana da Boa Vista, São Francisco de Assis, São Gabriel, São Pedro do Sul, São Sepé, São Vicente do Sul, and Uruguaiana (steppe) in temporary waters, rivers, streams lakes and wetlands. Samplings in coastal plains were conducted only in wetlands (the most common water body type in that phytophysiognomy) in the municipalities of Arroio Teixeira, Capão da Canoa, Cidreira, Curumin, Pinhal, Torres, Tramandaí and Xangri-lá between 2016/18.

2. Sampling methods

The material referred to Renner et al. (2017) and Renner et al. (2018) was used in the present study. Odonata specimens were preserved in 96% alcohol in glass pots with lids and identification labels. Specimens are deposited at the Natural Science Museum (MCNU) of Univates and the collection authorization process was issued by IBAMA, via the SISBio system under the number 50624-1.

3. Laboratory activities and identification

Odonata specimens were observed using a Zeiss 435063-9010-100 Stemi 305 Stereo Microscope and photographed using Zen software. Mites were removed from dragonflies and damselflies with the help of histological needle and tweezers, and stored in Eppendorf tubes in Koenike's fluid (10mL acetic acid; 40mL distilled water; 50mL glycerin) (Walter & Krantz 2009).

Subsequently, mites were mounted on microscopic slides in Hoyer's medium and dried at 60-70°C for seven days. After this period, slides were sealed with crystal varnish to prevent contamination, and then, they were stored at the Laboratory of Acarology - Univates collection, where air humidity is controlled for proper storage of the material. Larval mites were analyzed using a Zeiss Imager Z2 optical microscope with phase contrast and were photographed using the Zen software. Mites were identified to the genus level using the most recent key provided by Smith et al. (2010). Odonata specimens were identified according to Garrison et al. (2006; 2010) and Lencioni (2006).

4. Data analysis

Two indices were calculated: 1. Prevalence (number of parasitized individuals/total number of analyzed individuals X 100), 2. Intensity (total number of parasite/number of parasitized individuals).

In order to analyze the differences in mite occurrence between: (1) thorax and abdomen and (2) females and males was performed a G-test ($p\leq0.05$), using Bioestat 5.0 software (Ayres et al. 2007).

Results

A total of 3134 specimens divided of 100 species were analyzed (Suplementary material) but only 44 specimens of five species had larval mites attached; they were found in lakes, rivers, and temporary waters of São Francisco de Assis, and in wetlands of São Pedro do Sul, Mata, Caçapava do Sul and Santa Margarida do Sul (steppe); in the coastal area, mites were found in wetlands of Tramandaí, Pinhal, and Cidreira (Figure 1). Four new Odonata species are reported to the host list for *Arrenurus* s.str: *Ischnura capreolus* Hagen, 1861, *Acanthagrion lancea* Selys, 1876, *Perithemis mooma* Kirby, 1889 and *Micrathyria ocellata* Martin, 1897. (Table 1).



Figure 1. Map with collection points in Rio Grande do Sul state where *Arrenurus* sp. larvae were and were not present.

A total of 162 *Arrenurus (Arrenurus)* sp. larvae (Table 2; Figure 2 A-B) were found to parasitizing five Odonata species: *M. ocellata* (Figure 3 A-B) and *P. mooma* (Figure 3 C-D) (Libellulidae) and *A. lancea* (Figure 4 A-B), *I. capreolus* and *I. fluviatilis* (Coenagrionidae).

A high number of mites occurred when they were attached to *I. capreolus* (55.5%), *I. fluviatilis* (33.3%), followed by low numbers when attached to *M. ocellata* (6.1%), *A. lancea* (3.7%), and *P. mooma* (1.2%). Mites found in the steppes were associated to the five Odonata species listed above; whereas only *I. fluviatilis* and *I. capreolus* were found in the coastal area. The collection points of the steppe did not had urbanization, that results in a higher diversity of Odonata species, and also more hosts for the parasitic mites. In the Coast, higher levels of urbanization were found around the collection points, thus, decreasing the number and diversity of species.

Mites attached to *A. lancea*, *M. ocellata*, and *P. mooma* were only found on the thorax, while mites attached to *I. capreolus* and *I. fluviatilis* were found both on the thorax and abdomen (Table 3). Significant difference was observed in the body's part where water mite larvae was found in the species from steppe (p = 0.0005), but not for the coastal species (p = 0.8776).

Larvae attached to *A. lanceae* and *P. mooma* were found only in females; when attached to *M. ocellata*, they were found only in males, and when attached to *I. capreolus* and *I. fluviatilis*, they were found in both females and males. No significant differences between host's sex preferences were found in *I. capreolus* and *I. fluviatilis* (p=0.1413).

Table 1. Municipalities and coordinates where	Arrenurus (A.) sp. was found	along with the water body a	nd Odonata species collected at each site.
1		0	1

	Municipality	W	S	Water body	Species
	São Pedro do Sul	54°50'22.2"	29°65'96"	Wetland	Acanthagrion lancea
	Mata	54°42'85.8"	29°65'29"	Wetland	Ischnura capreolus
				Wetland	Ischnura fluviatilis
	Caçapava do Sul	53°27'7.1"	30°53'35.4"	Wetland	Ischnura capreolus
	Santa Margarida do Sul	53°84'94.4"	30°34'32"	Wetland	Acanthagrion lancea
				Wetland	Perithemis mooma
				Wetland	Ischnura fluviatilis
	São Francisco de Assis	55°17'07.6"	30°34'32"	Lake	Ischnura capreolus
S4				Lake	Ischnura fluviatilis
Steppe				Lake	Micrathyria ocellata
				Lake	Perithemis mooma
	São Francisco de Assis	55°19'20.4"	30°34'32"	Lake	Ischnura capreolus
				Lake	Ischnura fluviatilis
				Lake	Micrathyria ocellata
				Lake	Perithemis mooma
	São Francisco de Assis	55°08'54.7"	29°36'28.2"	River	Micrathyria ocellata
				River	Perithemis mooma
	São Francisco de Assis	55°07'7.3"	29°35'43,1"	Temporary waters	Ischnura fluviatilis
	Tramandaí	50°10'26"	30°05'37"	Wetland*	Ischnura fluviatilis
				Wetland*	Ischnura capreolus
	Cidreira	50°12'03"	30°09'26"	Wetland*	Ischnura fluviatilis
				Wetland*	Ischnura capreolus
Const	Cidreira	50°13'14"	30°10'57"	Wetland*	Ischnura fluviatilis
Coasi				Wetland*	Ischnura capreolus
	Pinhal	50°17'31.7"	30°12'50.1"	Wetland	Ischnura fluviatilis
				Wetland	Ischnura capreolus
	Tramandaí	50°09'01"	30°01'16"	Wetland*	Ischnura fluviatilis
				Wetland*	Ischnura capreolus

* = modified environment (high level of urbanization)

Table 2. Occurrence of Arrenurus (A.) sp. larvae in Odonata species from the Pampa Biome, Brazil.

Ecosystem	Odonata species	Analyzed individuals	Parasitized individuals	Prevalence of infestation (%)	Total of parasites found	Intensity of infestation
	I. capreolus	19	9	47.4	38	4.2
	I. fluviatilis	53	12	22.6	15	1.3
Steppe	A. lancea	11	4	36.4	6	1.5
	M. ocellata	11	1	9.1	10	10
	P. mooma	12	1	8.3	2	2
Carat	I. capreolus	37	7	18.9	52	7.4
Coast	I. fluviatilis	49	10	20.4	39	3.9

Discussion

The present study demonstrates that despite having a range of Odonata species for colonization (about 130 different species evaluated) only five of them were found with parasites. The species that were found being parasitized are very common and generalist in our state, they can be found either in lentic and lotic environments, with presence or absence of luminosity, and saturated O_2 or not. The parasitized species in the steppe areas of the Pampa were found in all types of water bodies present in the region: lakes, rivers, wetland and temporary waters, and in the coastal region only in wetlands (which occur most frequently in this area), indicating that there is no preferred location for infestation to



Figure 2. Ventral view of *Arrenurus* (*A*.) sp. parasitic larvae. A Pedipalps, Coxal Plates and Legs; B Excretory Pore



Figure 3. A *Micrathyria ocellata* male (in lateral view) with larvae on thorax; B *Micrathyria ocellata* close up of thorax, ventral view; C *Perithemis mooma* female (in lateral view); D *Perithemis mooma* close up of thorax, lateral view.



Figure 4. A. Acanthagrion lancea male with larvae attached on abdomen (in the circle) and arrow pointing to larvae attached in the thorax; **B**. Close up in the circle.

occur. The mites did not seem to have any preferences either for thoracic or abdominal attachment on the host's body, and either for females or males, and that may indicate that attachment happens by chance.

One important aspect from the analyzed species is that they lay eggs directly in the water, and sometimes in plants and small pieces of wood floating in surface; those species can also mate with more than one partner.

	Parasites on	host´s body	Specimens pa	rasitized by sex
Steppe	Abdomen	Thorax	Ŷ	3
A. lancea	0	6	4	0
I. capreolus	22	16	2	7
I. fluviatilis	10	5	4	8
M. ocellata	0	10	0	1
P. mooma	0	2	0	1
Coast	Abdomen	Thorax	Ŷ	3
I. capreolus	31	21	3	4
I. fluviatilis	23	16	2	8
I. capreolus I. fluviatilis	31 23	21 16	3 2	4 8

In traits and general behavior shown in Corbet (1999), Córdoba-Aguilar (2008) and Dalzochio et al. (2018), A. lancea exhibit larval climbing behavior and their oviposition is epiphytic, which means that egg laying occurs on leaves, woods, or rocks, whether or not submerged in water. I. capreolus and I. fluviatilis are also climbers but they exhibit endophytic oviposition pattern, i.e. damselflies lay eggs inside plant tissue in the waters surface. On the other hand, Libelullidae larvae, e.g. P. mooma and M. ocellata, are sprawlers and exophytic, which means egg laying occurs directly into the water. All these species spend a long period near the water, and it is easier for larval mites to attach to the host than those in Aeshnidae and Gomphidae, which never climb, are very active, and difficult to collect. Thus, species with terrestrial behavior are less prone to being parasitized (Smith et al. 2010). A general rule in Coenagrionidae is that adult males spend more time near water bodies searching for mating while adult females disperse in the vicinity and return to the water to breed (Corbet 1999, Córdoba-Aguilar 2008). This may explain the numbers of mites found on Ischnura spp. males, as these individuals spend a long time in the water and are very abundant in all kinds of water bodies, and that agrees with some of the Ilvonen et al. 2016, Ilvonen & Suhonen, 2016 found, where many Coeanagrionidae are reported being parasitized by Arrenuridae mites.

Ilvonen et al. (2016) found no differences in infestation by water mites between damselfly males and females, which conflicts with the findings of Rob and Forbes (2005), who observed a higher infestation by water mites on *Lestes disjunctus* Selys, 1862 (Lestidae) females. Ilvonen & Suhonen 2016 tested Odonata immune responses to water mites; mass was significantly different between sexes, females being heavier than males; between species, the encapsulation response was different, but not between sexes; it was also found considerable differences in the encapsulation response between different odonate species, e.g. *I. elegans* (Coenagrionidae) had the lowest encapsulation rate, whereas dragonfly *Leucorrhinia dubia* Vander Linden, 1825 (Libellulidae) had the highest. These defense mechanisms add up to an effective immune system, capable of defending against parasites and thus prolonging the host's lifespan and reproductive success. In their study, also found that damselflies had also much higher water mite prevalence than dragonflies.

In Brazil, studies on parasitism of aquatic mites on odonates and other host species are scarce, and many species can only be identified as morphospecies of specific genera due to the lack of taxonomic studies for the Neotropics. It is important to collect adults and conduct oviposition studies with females in order to correctly associate the larvae with their parents. Also, full descriptions of Hydrachnidiae must have males, females and larvae. In addition, molecular tools (e.g. barcoding) can be useful for creating a database for the identification of species and even larvae. Thus, further studies must be carried out in lotic and lentic environments in order to find and describe larvae, and adults either females and males to report the existing and describe the new species in Brazil; studies on the damage caused on dragonflies and damselflies by mites should be performed in order to discover whether flight, longevity and oviposition are really affected. This type of analysis was already performed by Reinhardt (1996) who observed that ectoparasitic mites have a negative influence on flight ability of Nehalennia speciosa Charpentier, 1840 (Coenagrionidae); one meter was the longest distance flown by 35.2 % of the infested adults immediately after release, whereas none flew farther than five meters; in the group of the non-parasitized damselflies, 75% flew more than one meter and this pattern was consistent in both sexes. Advanced adults of Coenagrion hastulatum Charpentier, 1825 and Enallagma cyathigerum Charpentier, 1840 heavily loaded with parasites had often lost the typical agility to move and could be easily catched (Åbro 1981), this flight injuries is due to the mites attachment in the thoracic region where there is more hemolymph stream and consequently more consumed energy due to the time that Odonata spends flapping their wings (Corbet 1999). Oviposition test was performed by Rolff (1999) which tested Arrenurus cuspidator (O. F. Müller, 1776) on C. puella Linnaeus, 1758 and found that the number of eggs laid by the damselfly decreased with increased ectoparasite abundance. New hosts should also be sought to report new host-parasite interactions and continue to build knowledge on aquatic mite fauna for Rio Grande do Sul state.

Supplementary Material

The following online material is available for this article: **Table S1** - Analyzed species

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Author Contributions

Gabriel Lima Bizarro: Substantial contribution in the concept, design of the study and to manuscript preparation.

Marina Dalzochio: Contribution to data collection.

Eduardo Périco: Contribution to data analysis and interpretation. Guilherme Liberato: Contribution to data analysis and interpretation. Marina Dalzochio: Contribution to data analysis and interpretation. Liana Johann: Contribution to data analysis and interpretation. Noeli Juarez Ferla: Contribution to data analysis and interpretation.

Conflicts of interest

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

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Herpetofauna of Matas de Água Azul, an Atlantic Forest remnant in Serra do Mascarenhas, Pernambuco state, Brazil

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Abstract: The northern most portion of Atlantic Forest is currently considered as the most vulnerable and threatened in this ecoregion, while also harboring the ecoregion's least studied biota. Herein we present results of a herpetofaunistic survey in Refúgio de Vida Silvestre (Wildlife Refuge) Matas de Água Azul (RVSMAA), in the mountain range Serra do Mascarenhas, northeastern state of Pernambuco, Brazil, one of the largest forest remnants in the northern Atlantic Forest. The sampling was carried out in four expeditions using methods of visual searching and pitfall traps. A total of 43 species of amphibians and 40 species of reptiles were registered, including anurans, caecilians, lizards, snakes and chelonians. The richness recorded represented 56% of amphibians and 20% of reptiles registered in the Atlantic Forest north of the São Francisco River. The RVSMAA holds a high diversity of amphibians and reptiles, representing one of the highest levels of richness in the northern Atlantic Forest. The same of the highest levels of richness in the northern Atlantic Forest. The record of seven species included in some of the threatened species lists reinforces the importance of the area for the conservation of the Atlantic Forest herpetofauna and adds relevant information to our knowledge of northern Atlantic Forest biodiversity and aids in its assessment of conservation.

Keywords: Amphibians; Hotspot; Inventory; Conservation; Reptiles.

Herpetofauna das Matas de Água Azul, um remanescente de Mata Atlântica na Serra do Mascarenhas, estado de Pernambuco, Brasil

Resumo: Atualmente, a porção norte da Mata Atlântica é considerada a mais vulnerável, ameaçada e com menos estudos sobre a biota em toda sua extensão. Apresentamos aqui os resultados do inventário herpetofaunístico realizado no Refúgio de Vida Silvestre Matas de Água Azul (RVSMAA), localizada na cadeia de montanhas da Serra do Mascarenhas, estado de Pernambuco, Brasil, um dos maiores remanescentes florestais da porção norte da Mata Atlântica. A amostragem da área foi realizada em quatro expedições onde foram utilizados os métodos de busca visual e estações de armadilhas de interceptação e queda. Um total de 43 espécies de anfíbios e 40 de répteis foram registradas, incluindo anuros, cecílias, lagartos, serpentes e quelônios. A riqueza registrada representa 56% dos anfíbios e 20% dos répteis registrados para a Mata Atlântica ao norte do Rio São Francisco. O RVSMAA possui elevada diversidade de anfíbios e répteis, representando uma das maiores riquezas do norte da Mata Atlântica. O registro de sete espécies incluídas em alguma das listas de espécies ameaçadas para a região reforça a importância da área para a conservação da herpetofauna do Nordeste e acrescenta informações relevantes ao conhecimento e conservação da biodiversidade da Mata Atlântica ao norte do Rio São Francisco.

Palavras-chave: Anfíbios; Hotspot; Inventário; Conservação; Répteis.

Introduction

The Atlantic Forest ecoregion represents the second largest forest block in the Neotropical region, originally occupying an extensive area along the eastern coast of South America, from Argentina to the northeastern region of Brazil (Galindo-Leal & Câmara 2003, Ribeiro et al. 2009). The heterogeneity of phytophysiognomies, together with historical and geographical factors, have been determinant in establishing a unique biodiversity (Moura et al. 2016), with this ecoregion demonstrating high richness and endemism (Myers et al. 2000, Roll et al. 2017).

The Atlantic Forest presents an extensive latitudinal amplitude, which generates a considerable variation in temperature, precipitation and humidity, resulting in broad climatic heterogeneity throughout its territory (Ribeiro et al. 2009, Alvares et al. 2013). Topographic variation (0-2700 meters) together with vegetation composition (Pinto & Brito 2003), have played a fundamental role in the diversification of Neotropical biota, resulting in very distinctive biotas throughout its area, demonstrating a noticeable break in fauna and flora compositions when comparing northern and southern portions of the Atlantic Forest (Costa 2003, Carnaval et al. 2014).

Nevertheless, the Atlantic Forest has been severely threatened since the start of the European colonization in Brazil, accelerating the anthropogenic pressure during the last century mainly due to deforestation and the conversion of natural environments into plantations and cattle farming areas. This anthropogenic disruption is mainly caused by the advance of agricultural frontiers, resulting in its current highly relictual coverage, covering no more than 11% of its original area (Ribeiro et al. 2009). The high richness, endemism and the strong threats this area suffers, confer to the Atlantic Forest the status of a worldwide hotspot for biological diversity (*sensu* Myers et al. 2000), and it is therefore, considered as a priority area for the conservation of neotropical biota (Rossa-Feres et al. 2017).

The Atlantic Forest may be divided into eight biogeographical subregions based on endemism areas and transitional regions (Silva & Casteleti 2003, Ribeiro et al. 2011). The northernmost subregion is the Pernambuco Biogeographic Sub-Region (BSR Pernambuco; *sensu* Ribeiro et al. 2009), extending latitudinally from the far north of Rio Grande do Norte state to Alagoas state (05°15'S - 10°30'S), whose southern limit is defined by the São Francisco River, and is currently the most vulnerable and threatened subregion. The BSR Pernambuco is, currently, a mosaic of a few isolated forest remnants within large areas of sugarcane monocultures and pastures (Tabarelli et al. 2006).

As a result, this subregion is classified as the least protected by official conservation units (less than 1% of the existing remnants) and is home to the lowest number of studies on biodiversity in the Atlantic Forest (Ribeiro et al. 2009). Studies that aim to catalog the diversity and distribution of amphibian and reptile species in the BSR Pernambuco are restricted to punctual and short-term inventories, mostly in forest remnants close to urbanized areas (e.g., Santana et al. 2004, Roberto et al. 2017, Melo et al. 2018, Dubeux et al. 2020a). Despite this, 79 species of amphibians and 196 species of reptiles are currently registered for the BSR Pernambuco, of which 23 are endemic (Moura et al. 2016, Almeida et al. 2016, Costa & Bérnils 2018, Mesquita et al. 2018, Dubeux et al. 2020b).

The Serra do Mascarenhas mountain range is one of the largest continuous remnants of Atlantic Forest in the BSR Pernambuco. Located in Pernambuco state, it includes two main forest fragments, "Matas de Água Azul" and "Mata de Xixá", an area of high biological importance for biodiversity conservation in the northern Atlantic Forest and also represents one of the most preserved and least altered forested areas in the state (Pietrobom & Barros 2007, SEMAS 2014). Nevertheless, as with other regions in the northern Atlantic Forest, this area is undergoing an intense process of fragmentation due to the advance of plantations (sugar cane) and other strong anthropogenic pressures (SEMAS 2014).

Aiming to preserve the forest remnants in the northern Atlantic Forest of Pernambuco state, in 2014 a state conservation unit of integral protection was created within the areas of "Matas de Água Azul" and "Mata de Xixá", the second largest continuous remnants of Atlantic Forest in Pernambuco state (Pernambuco 2014), with an area of approximately 3800 ha: the Refúgio de Vida Silvestre (Wildlife Refuge) Matas de Água Azul (RVSMAA) (SEMAS 2014). Despite the importance of RVSMAA as a protected area in the northern Atlantic Forest, there are few studies on its biota which are mostly restricted to an inventory of Pteridophytes and technical reports on birds and plants (Pietrobom & Barros 2007, Lucena 2009, Pereira 2009). Information involving the herpetofauna of the region is limited to a preliminary list of amphibians presented by Santos & Carnaval (2002), which recorded 18 anuran species.

Thus, herein we present the results of a herpetofaunistic inventory in the Serra do Mascarenhas mountain range, specifically within the limits of the conservation unit Refugio de Vida Silvestre Matas de Água Azul, aiming to fill important gaps in the poorly known fauna of the BSR Pernambuco and in particular, of the virtually unknown herpetofauna of Serra do Mascarenhas. As such, we expect that the records reported herein can be used to provide information to supply biogeographical studies and to elaborate conservation strategies for this highly threatened environment.

Material and Methods

1. Study area

The forest remnants present in the Serra do Mascarenhas mountain range are mainly concentrated in the area currently defined as the integral conservation unit, Refúgio de Vida Silvestre Matas de Água Azul. The area of the unit covers 3800 ha in the municipalities of Vicência, Timbaúba and Macaparana, in northeastern Pernambuco state, Brazil (Figure 1- 2). The area is characterized by the presence of a series of hills, with altitudes varying from 150 to 500 meters a.s.l. within ridges and valleys that stand out within the Borborema Plateau (SEMAS 2014). The majority of Serra do Mascarenhas is drained by abundant springs that flow into the main tributaries of the left bank of Siriji River, and large dams such as the one in the Engenho Água Azul (Figure 2F) and the Mascarenhas reservoir (SEMAS 2014). The vegetation is characterized by subdeciduous and deciduous forests, varying up to dense Ombrophilous and mountainous rainforest, featuring large forest blocks and series of smaller surrounding fragments (Figure 1C) (Pietrobom & Barros 2003, Lucena 2009).

The sampling of amphibians and reptiles was conducted during four expeditions that explored different areas in two distinct seasons. The data on average rainfall and temperature, considered to characterize each season, are for the *Zona da Mata* mesoregion in Pernambuco state, where the Serra do Mascarenhas is located. The climatic data encompass



Figure 1. A = Location of Pernambuco state within South America (red rectangle). B = Location of the area including Serra do Mascarenhas within Pernambuco state (red rectangle). C = Serra do Mascarenhas area; black line corresponds to the limits of Refúgio de Vida Silvestre Matas de Água Azul (RVSMAA). Gray circles correspond to Pitfall Traps (PF); blue circles correspond to visited water bodies (WB); blue lines correspond to hydrography of the area. Light green = original covering of the Atlantic Forest; dark green = remaining Atlantic Forest.



Figure 2. Landscapes and sampling sites in the Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A-D = Vegetation in study areas. E-F = Examples of water bodies. G-H = Examples of pitfall traps.

the time of expeditions and was provided by the state climatic agency (APAC 2020). The region presents a tropical Atlantic (As) climate, with a dry season during the summer, according to Koppen's climate classification (Alvares et al. 2014), and two marked seasons defined by

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rainfall pattern: dry season (October-March) and rainy season (April-September). The first and third expeditions were carried out during the dry season from February 7th to 22th 2017 (average monthly rainfall of ~78.2 mm and temperatures ranging from 20.5°C to 34.6 °C), and from January 8th to February 19th 2018 (average monthly rainfall 53.4 mm, and temperatures ranging from 20.3°C to 31.7°C), respectively. The second and fourth expeditions were carried out during the rainy season from July 10th to 19th 2017 (average monthly rainfall of ~133.5 mm, and temperatures ranging from 19.1°C to 26.4°C), and from July 26th to August 21th 2018 (average monthly rainfall of 152.7 mm, and temperatures ranging from 18.8°C to 29.9°C), respectively.

The first expedition was conducted at the eastern portion of RVSMAA (7°36'S 35°19'W, elevation ~300-400 m) (all coordinates were taken under DATUM WGS84). Four water bodies (WB) were sampled: three small human-built water reservoirs with water at low levels (maximum depths less one meter) and with areas of 366 m² (WB1), 695 m² (WB3) and 560 m² (WB4); all were located in open areas with herbaceous vegetation around their edges (see Table 1 for coordinates). Water body 2 is a dam, with an area of 3,727 m² and a maximum depth of three meters, also located in an open area and with herbaceous vegetation around its edges. Three pitfall traps (PFs) were installed (see sampling methods below). PF1 and PF2 were installed in areas of secondary forest, in a steep area with tall trees (~22-30 m) and some rocky outcrops in the forest; PF3 was installed in a flat area with spaced out trees and ground covered by a dense layer of leaf litter, without rocky outcrops.

The second expedition was carried out in a locality known as Mata do Engenho Água Azul (7°36'S 35°22'W, elevation ~300-400 m) in the central portion of RVSMAA. Three water bodies were sampled: WB5 (reservoir of Engenho Água Azul) had an area of 400 m² and a maximum depth of six meters, with part of its shores connected to the forest and other parts to areas with tall grass, accumulated fallen tree trunks and areas with herbaceous vegetation. The WB6 has an area of 1,140 m² with a maximum depth of two meters and tall grasses inside and some scattered fallen tree trunks at its shores. Water body 7 is a swamp, with an area of approximately 7,600 m² and a maximum depth of two meters, full of tall grasses and aquatic plants. Three pitfall traps were installed: PF4, parallel, and PF6, perpendicular, to a stream that flows into the WB5, ~100 meters inside the forest from the nearest border, in an area presenting dense vegetation and where the ground was covered by a dense layer of leaflitter. Pitfall trap 5 was installed in a steep area, 470 meters inside the forest from the closest border, in an area presenting dense vegetation and where the ground was covered by a dense layer of leaflitter.

The third and fourth expeditions were carried out in a locality know as Mata de Xixá (7°36'S 35°24'W, elevation ~300-500 m) in the western portion of RVSMAA. Only one water body was sampled. The WB8 is a small human-built water reservoir, with an area of approximately 79 m² and a maximum depth of two meters, located in an open area and presenting herbaceous vegetation around its edges. Nine Pitfall traps were installed: PF10-15 were installed inside the continuous fragment of Mata do Xixá; PF10, PF12 and PF14 were installed ~200 meters inside the forest from the closest edge of fragment, and P11, PF13 and PF15 were installed ~400 meters inside the forest from the edge of the fragment. All areas have dense vegetation composed mainly of primary vegetation with large trees, and where the ground is covered with a dense

Abbreviation	Coordinates	Locality	
PF1	7°36'35"S 35°19'19"W		
PF2	7°36'37"S 35°19'31"W		5
PF3	7°36'47"S 35°19'52"W	Mata do Engenho Água Azul. Timbaúba	irst
WB1	7°36'55"S 35°19'34"W	Mata do Engenho Água Azul, Vicência	cam
WB2	7°36'52"S 35°19'38"W	Vicência	paig
WB3	7°36'51"S 35°19'29"W		n,
WB4	7°37'00"S 35°19'38"W		
PF4	7°36'31"S 35°22'54"W		10
PF5	7°36'36"S 35°22'47"W		Jeco
PF6	7°36'47"S 35°22'45"W	Mata do Engenho Água Azul, Timbaúba	nd c
WB5	7°36'24"S 35°22'41"W	Mata do Engenho Água Azul, Vicência	amp
WB6	7°36'38"S 35°22'32"W		Daig
WB7	7°36'54"S 35°22'21"W		в
PF7	7°35'32"S 35°23'15"W		
PF8	7°35'52"S 35°23'50"W		Т
PF9	7°35'29"S 35°24'18"W		hird
PF10	7°36'14"S 35°23'57"W		and
PF11	7°36'17"S 35°24'10"W	Mata da Vivá Timbovha	fou
PF12	7°36'05"S 35°24'10"W	Mata de Aixa, Timbauba	rthe
PF13	7°36'14"S 35°24'20"W		amp
PF14	7°36'02"S 35°24'35"W)aigi
PF15	7°36'15"S 35°24'29"W		SU
WB8	7°35'50"S 35°24'31"W		

Table 1. List of sampling points with geographical coordinates and the locality names in the Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. Pitfall Trap (PF) and Water Bodies (WB).

layer of leaflitter, although also presenting sparse clearings. The areas where PF14-15 were installed, presented some rocky outcrops in the forest. Pitfall traps 7-9 were installed inside of three different, smaller and isolated fragments from the largest forest fragments. In these three fragments the vegetation was drier and more open when compared to the continuous forest in Mata do Xixá fragment.

2. Sampling methods

Two different and complementary sampling methods were used. Visual encounter surveys without time constraints (VES) were used to sample areas where no pitfall traps were placed, inside the forest and close to water bodies (Figure 1) aiming to cover the largest area possible (Foster 2012). All pitfall traps were installed in straight line with buckets of 30 liter and five-meter drift fences between each bucket, at specific sites (Foster 2012). Sampling efforts were distinct in each expedition. The number of buckets and pitfalls changed in each expedition and area. Logistical issues such as locations with rocky soil, uneven terrain or areas with reduced access were determinant in the choice for the amount of buckets. In the first expedition, effort for VES without time constraints was 11 people/day with diurnal and nocturnal searches for 15 days and three pitfall traps were installed, two with 19 buckets each and one with 34 buckets, that remained active for 14 days, totaling a sampling effort of five buckets/day. In the second expedition, the VES effort consisted of seven people/day with diurnal and nocturnal searches

for nine days; we also used three pitfall traps, this time with 30 buckets each, which remained active for 10 days with a sampling effort of nine buckets/day. During the third and fourth expeditions the VES consisted of eight people/day with diurnal and nocturnal searches for 28 days; nine pitfall traps with 11 buckets each were installed and remained active for 30 days, with a sampling effort of three buckets/day.

3. Data collection and analysis

The specimens collected were euthanized using lidocaine 2% and a sample of liver or muscle tissue was collected from each specimen and stored in alcohol. The specimens were fixed in formaldehyde 10% in accordance with the permits emitted by environmental agencies (ICMBio permit nº 46368; CPRH process nº 03840/2016) and the authorization of the Ethics Committee for the Use of Animals of the Federal University of Pernambuco (CEUA-UFPE nº 0006/2017). All specimens and tissues samples collected were or will be incorporated into the Herpetological Collection of the Federal University of Pernambuco (CHUFPE) under the acronyms CHUFPE and PMSN and PDM (CHUFPE field numbers). Due to closures of the Universities during the COVID-19 pandemic, some specimens have still to be incorporated into the collection and have kept their field numbers associated with their collection numbers, allowing for the correct identification of each individual specimen. The complete voucher list is available in Supplementary Material 1. The taxonomic nomenclature followed Frost (2020) and Uetz et al. (2019).

Values of relative abundance were not obtained for any of the expeditions, however, for several species, mainly reptiles, samples were composed of singletons or doubletons (Table 2 and 3). Estimates of the effectiveness of sampling effort for amphibians and lizards were performed for the complete data set. Snakes and chelonians were not included in this analysis due to the low rate of capture. As encounters with these animals were occasional, the resulting list may be underestimated, which may compromise the results. The data matrices were constructed based on individuals per species (Gotelli & Colwell 2001), including VES and PF data. Species accumulation curves were generated through 1000 randomizations without substitution using only taxa recorded with collected specimens (taxa sampled by auditory or visual records were not included). Additionally, for the same datasets, species richness was estimated using non-parametric tests of Chao 1, since this estimator uses abundance data considering the presence of species represented by only one individual (singletons) or two individuals (doubletons) in the sample. All the analyzes were performed using the EstimateS 9.1.0 software (Colwell et al. 2012).

4. Conservation status

The conservation status of each species followed global, national and local assessments. The Red Book of Endangered Species of the International Union for the Conservation of Nature and Natural Resources (IUCN 2020) was used for the global assessment, the *Livro Vermelho da Fauna Brasileira Ameaçada de Extinção* of *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio 2018) was used for the national assessment, and the *Lista Estadual Oficial de Espécies da Fauna Ameaçada de Extinção – Anfibios* (SEMAS, 2015) and *Lista Estadual Oficial de Espécies da Fauna Ameaçada de Extinção – Répteis* (SEMAS, 2017) were used for the local assessment (Pernambuco state) of the conservation status of each species.

Results

A total of 708 specimens were sampled during the four field expeditions, 43 amphibian and 40 reptile species. The amphibian species belongs to 22 genera and 10 families of anurans and one family of Gymnophiona. The family Hylidae was the richest with 17 species (40%

Table 2. List of amphibian species recorded in the Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. $C1 = 1^{st}$ Campaign; $C2 = 2^{nd}$ Campaign; C3-4: 3^{rd} and 4^{th} Campaigns; the numbers in these columns correspond to specimens collected in each campaign. Specimens recorded by Santos & Carnaval (2002) are discriminated in the fifth column. Sampling methods abbreviations: AS = Active Search; PF = Pitfall Traps; AR = Auditory Records; VR = Visual Records. Conservation status abbreviations: VU = Vulnerable;<math>LC = Least Concern; DD = Data Deficient; EN = Endangered; NA = Not Available; (-) Recorded species with no individuals collected.

				Santos &	C	Conservation status		
AMPHIBIAN SPECIES	C1	C2	C3-4	Carnaval (2002)	methods	IUCN	ICMBio	State list
ANURA								
Aromobatidae								
Allobates olfersioides (Lutz, 1925)			2		AS, PF	VU	VU	EN
Bufonidae								
Rhinella crucifer (Wied-Neuwied, 1821)	5	8	14	х	AS, PF	LC	LC	LC
Rhinella granulosa (Spix, 1824)	6	4	2	х	AS	LC	LC	LC
Rhinella jimi (Stevaux, 2002)	5	1	-		AS, VR	LC	LC	LC
Craugastoridae								
Pristimantis sp.	9	8	18		AS, PF	LC	LC	LC
Eleutherodactylidae								
Adelophryne baturitensis Hoogmoed, Borges & Cascon, 1994	5		6		PF	VU	LC	NA
Hylidae								
Boana albomarginata (Spix, 1824)	12	5		Х	AS	LC	LC	LC
Boana crepitans (Wied-Neuwied, 1824)	8				AS	LC	LC	LC
Boana exastis (Caramaschi & Rodrigues, 2003)		-	1		AS, VR	DD	LC	EN
Boana faber (Wied-Neuwied, 1821)	9		2		AS	LC	LC	LC
Boana raniceps (Cope, 1862)	5	1	1	х	AS	LC	LC	LC
Boana semilineata (Spix, 1824)		15	1	х	AS, AR	LC	LC	LC
Corythomantis greeningi Boulenger, 1896			2		AS	LC	LC	LC
Dendropsophus branneri (Cochran, 1948)	4	8	1	Х	AS	LC	LC	LC
Dendropsophus elegans (Wied-Neuwied, 1824)		5	-	Х	AS, VR	LC	LC	LC
Dendropsophus minutus (Peters, 1872)	1	5		х	AS	LC	LC	LC
Dendropsophus oliveirai (Bokermann, 1963)		9	1	Х	AS	LC	LC	LC
Dendropsophus soaresi (Caramaschi & Jim, 1983)	1				AS	LC	LC	LC
Scinax auratus (Wied-Neuwied, 1821)		5			AS	LC	LC	LC

Continue...

Continuation								
Scinax eurydice (Bokermann, 1968)	1	4	3		AS	LC	LC	LC
Scinax gr. ruber (Laurenti, 1768)	7	1	3		AS	LC	LC	LC
Scinax nebulosus (Spix, 1824)		4		х	AS	LC	LC	LC
Trachycephalus mesophaeus (Hensel, 1867)			5		AS	LC	LC	LC
Leptodactylidae								
Adenomera aff. hylaedactyla (Cope, 1868)		1	1		AS, PF	NA	NA	NA
Leptodactylus fuscus (Schneider, 1799)	3		1	х	AS, PF	LC	LC	LC
Leptodactylus macrosternum Ribeiro, 1926	11	3	1		AS, PF	LC	LC	LC
Leptodactylus cf. mystaceus (Spix, 1824)	1	1			AS	LC	LC	LC
Leptodactylus natalensis A. Lutz, 1930	1	6			AS, PF	LC	LC	LC
Leptodactylus troglodytes A. Lutz, 1926	7	1	3	х	AS, PF	LC	LC	LC
Leptodactylus vastus A. Lutz, 1930	4	3	2		AS, PF	LC	LC	LC
Physalaemus cuvieri Fitzinger, 1826	5	1	11	х	AS, PF	LC	LC	LC
Pseudopaludicola mystacalis (Cope, 1887)			-		VR	LC	LC	DD
Microhylidae								
Chiasmocleis alagoana Cruz, Caramaschi & Freire, 1999	2		3		AS, PF	DD	EN	DD
Dermatonotus muelleri (Boettger, 1885)	1		1		AS	LC	LC	LC
Elachistocleis cesarii (Miranda-Ribeiro, 1920)				х	-	NA	LC	LC
Stereocyclops incrassatus Cope, 1870		2	11		AS, PF	LC	LC	LC
Odontophrynidae								
Macrogenioglottus alipioi Carvalho, 1946			14		PF	LC	LC	NA
Proceratophrys cristiceps (Müller, 1883)			1			LC	LC	LC
Proceratophrys renalis (Miranda-Ribeiro, 1920)	2	1	26		AS, PF	NA	LC	LC
Phyllomedusidae								
Hylomantis granulosa (Cruz, 1989)		1	4	х	AS	LC	VU	VU
Pithecopus gonzagai Andrade, Haga, Ferreira, Recco-Pimentel,	12	0	1		15	NIA	NIA	NIA
Toledo & Bruschi, 2020	12	9	1	Х	AS	NA	INA	NA
Ranidae								
Lithobates palmipes (Spix, 1824)	21	1	1	х	AS	LC	LC	LC
GYMNOPHIONA								
Siphonopidae								
Siphonops sp.			-		VR	NA	NA	NA

of total richness), followed by Leptodactylidae (9 spp.), Microhylidae (4 spp.), Bufonidae and Odontophrynidae (3 spp. each), Phyllomedusidae (2 spp.), and Aromobatidae, Craugastoridae, Eleutherodactylidae, Ranidae and Siphonopidae (1 species each) (Figures 3-5). The species list of the anurans recorded at each site, including number of collected specimens, information about collection method and global, national and local conservation status is provided in Table 2.

Forty species of reptiles, within 35 genera and 19 families of Squamata and one genus of Testudines, were recorded. Within the Squamata, 18 species of lizards were collected, with Teiidae being the most diverse family with three species recorded, followed by Dactyloidae, Polychrotidae, Scincidae and Tropiduridae (2 spp. each), Diploglossidae, Gekkonidae, Gymnophthalmidae, Iguanidae, +, Phyllodactylidae and Sphaerodactylidae (1 species each) (Figure 6). Twenty-one snake species were recorded, with Dipsadidae being the most diverse family with nine species, followed by Colubridae (5 spp.), Typhlopidae and Viperidae (2 spp. each), Boidae, Elapidae, and Leptotyphlopidae (1 species each) (Figure 7). Within the Testudines, only one species was recorded, belonging to the family Chelidae (Figure 6A). The species list of reptiles recorded at each site, including number of collected specimens, information about collection method and global, national and local conservation status is provided in Table 3. The accumulation curves for Anura and for the pooled herpetofauna datasets reach an asymptote and are close (pooled Herpetofauna) (N = 56; Chao 1 = 57.43 ± 5.14) or meet (Anura) (N = 41; Chao 1 = 41.20 ± 3.56) the curve of Chao 1 estimator, whereas the lizard's curve does not reach an asymptote or reach the estimator curve (N = 15; Chao 1 = 15.99 ± 2.87) (Figure 8).

Discussion

The virtually unknown herpetofauna from Serra do Mascarenhas is composed of 43 species of amphibians and 40 species of reptiles. In addition, Serra do Mascarenhas has five species of herpetofauna considered as threatened according to local lists. The species list elaborated herein reveals a rich fauna corresponding to 56% of amphibians and 20% of all reptile species (36% of lizard and 26% of snake species) reported for the BSR Pernambuco (Costa & Bérnils 2018, Mesquita et al. 2018, Dubeux et al. 2020b). The richness recorded herein, represents 44% of the herpetofauna known for Pernambuco state,

Herpetofauna of Matas de Água Azul

Table 3. List of reptile species recorded in the Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. $C1 = 1^{st}$ Campaign; $C2 = 2^{nd}$ Campaign; $C3-4 = 3^{rd}$ and 4th Campaigns; the numbers in these columns correspond to specimens collected in each campaign. Sampling Methods: AS = Active Search; PF = Pitfall Traps; VR = Visual Record. Conservation status: VU = Vulnerable; LC = Least Concern; DD = Data Deficient; NA = Not Available; (-) Recorded species with no individuals collected.

				Sampling	Con	servation st	atus
REPTILE SPECIES	C1	C2	C3-4	methods	IUCN	ICMBio	State list
TESTUDINATA							
Chelidae							
Phrynops geoffroanus (Schweigger, 1812)		1		AS	NA	LC	LC
SQUAMATA							
Dactyloidae							
Dactyloa punctata (Daudin, 1802)	5	5	7	AS, PF	NA	LC	LC
Norops fuscoauratus (D'Orbigny, 1837)	11		4	AS	NA	LC	LC
Diploglossidae							
Diploglossus lessonae Peracca, 1890			1		LC	NA	LC
Gekkonidae							
Hemidactylus mabouia (Moreau de Jonnès, 1818)	6	-	-	AS	NA	NA	NA
Gymnophthalmidae							
Dryadosaura nordestina Rodrigues, Freire, Pellegrino &	2			DE	IC	LC	LC
Sites, 2005	Z			PF	LC	LC	LC
Iguanidae							
Iguana iguana (Linnaeus, 1758)		1	-	AS, VR	LC	LC	LC
Leiosauridae							
Enyalius aff. catenatus (Wied, 1821)	26	2	24	AS, PF	LC	LC	LC
Phyllodactylidae							
Gymnodactylus darwinii (Gray, 1845)	7	1	10	AS, PF	LC	LC	LC
Polychrotidae							
Polychrus acutirostris Spix, 1825		2		AS	LC	LC	LC
Polychrus marmoratus (Linnaeus, 1758)		7	2	AS	LC	LC	LC
Scincidae							
Psychosaura agmosticha (Rodrigues, 2000)			1	PF	LC	LC	LC
Psychosaura macrorhyncha (Hoge, 1946)			1	PF	LC	LC	NA
Sphaerodactylidae							
Coleodactylus meridionalis (Boulenger, 1888)	5	9	13	AS, PF	LC	LC	LC
Teiidae							
Ameiva ameiva (Linnaeus, 1758)			1	PF	LC	LC	LC
Kentropyx calcarata Spix, 1825			6	PF	LC	LC	LC
Salvator merianae Duméril & Bibron, 1839			-	VR	LC	LC	LC
Tropiduridae							
Strobilurus torquatus Wiegmann, 1834	2		3	PF	LC	LC	VU
Tropidurus hispidus (Spix, 1825)	2	1		AS	LC	LC	LC
Boidae							
Corallus hortulana (Linnaeus, 1758)		1		AS	LC	LC	LC
Colubridae							
Chironius flavolineatus Jan, 1863	1	6		AS	LC	LC	LC
Dendrophidion atlantica Freire, Caramaschi &	1				27.4	DD	DD
Gonçalves, 2010	1			AS	NA	DD	DD
Oxybelis aeneus (Wagler in Spix, 1824)		1		AS	LC	LC	LC
Tantilla melanocephala (Linnaeus, 1758)	1		3	AS	NA	LC	LC
Spilotes pullatus (Linnaeus, 1758)	-			VR	LC	LC	LC
Dipsadidae							
Dipsas neuwiedi (Ihering, 1911)	1			AS	LC	LC	LC
Helicops angulatus (Linnaeus, 1758)			4	AS	LC	LC	LC
Imantodes cenchoa Linnaeus, 1758	5			AS	LC	LC	LC

Continue...

Continuation...

Oxyrhopus trigeminus Duméril, Bibron & Duméril, 1854	1	1	AS	LC	LC	LC
Pseudoboa nigra (Duméril, Bibron & Duméril, 1854)	1		AS	LC	LC	LC
Taeniophallus affinis (Günther, 1858)	2		AS	LC	LC	NA
Thamnodynastes pallidus (Linnaeus, 1758)	1	1	AS	LC	LC	LC
Xenodon merremi (Wagler in Spix, 1824)	1	1	AS	LC	LC	LC
Xenodon rabdocephalus (Wied-Neuwied, 1824)	2	2	AS	LC	LC	LC
Elapidae						
Micrurus aff. ibiboboca (Merrem, 1820)		1	AS	NA	DD	DD
Leptotyphlopidae						
Epictia borapeliotes (Vanzolini, 1996)		1	PF	NA	LC	LC
Typhlopidae						
Amerotyphlops arenensis Graboski, Pereira-Filho, Silva, Prudente & Zaher, 2015	2	2	PF	LC	LC	LC
Amerotyphlops brongersmianus (Vanzolini, 1976)	1	1	PF	LC	LC	LC
Viperidae						
Bothrops leucurus Wagler, 1824		2	AS	NA	LC	LC
Lachesis muta (Linnaeus, 1766)		1	AS	NA	LC	VU



Figure 3. Amphibians of Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A- Allobates olfersioides (CHUFPE-A 1103, adult male, SVL 13.74 mm); B - Rhinella crucifer (CHUFPE-A 0969, adult male, SVL 55.80 mm); C - R. granulosa (PMSN 333, adult male, SVL 53.54 mm); D - R. jimi (CHUFPE-A 0656, adult male, SVL 279.60 mm); E - Pristimantis sp. (unvouchered specimen); F - Adelophryne baturitensis (unvouchered specimen); G - Boana albomarginata (PMSN 579, adult male, SVL 46.24 mm); H - B. crepitans (PMSN 344, adult female, SVL 58.80 mm); I - B. exastis (PDM 05, adult male, SVL 70.50 mm); J - B. faber (CHUFPE-A 0746, adult female, SVL 89.27 mm); K - B. raniceps (PMSN 542, adult male, SVL 64.76 mm); M - Corythomantis greeningi (CHUFPE-A 1041, adult male, SVL 72.83 mm); N - Dendropsophus branneri (unvouchered specimen); O - D. elegans (unvouchered specimen).

Figure 4. Amphibians of Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A - Dendropsophus minutus (unvouchered specimen); B - D. oliveirai (PMSN 315, adult male, SVL 17.51 mm); C - D. soaresi (CHUFPE-A 0769, adult male, SVL 34.65 mm); D - Scinax auratus (unvouchered specimen); E - S. eurydice (PMSN 687, adult male, SVL 52.72 mm); F - Scinax gr. ruber (unvouchered specimen); G - S. nebulosus (unvouchered specimen); H - Adenomera aff. hylaedactyla (unvouchered specimen); J - L. macrosternum (unvouchered specimen); K - Leptodactylus fuscus (CHUFPE-A 0728, adult male, SVL 44.50 mm); J - L. macrosternum (unvouchered specimen); K - Leptodactylus aff. mystaceus (PMSN 411, adult male, SVL 27.30 mm); L - L. natalensis (unvouchered specimen); M - L. troglodytes (CHUFPE-A 0756, adult male, SVL 46.38 mm); N - L. vastus (PMSN 264, adult male, SVL 25.73 mm); O - Physalaemus cuvieri (PMSN 234, adult female, SVL 26.70 mm).



Figure 5. Amphibians of Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A - *Pseudopaludicola mystacalis* (unvouchered specimen); B - *Chiasmocleis alagoana* (PMSN 329, adult male, SVL 21.66 mm); C - *Dermatonotus muelleri* (PMSN 371, adult female, SVL 67.28 mm); D - *Stereocyclops incrassatus* (CHUFPE-A 1038, adult male, SVL 54.57 mm); E - *Proceratophrys cristiceps* (unvouchered specimen); F - *P renalis* (CHUFPE-A 767, adult male, SVL 42.93 mm); G - *Hylomantis granulosa* (CHUFPE-A 1093, adult male, SVL 36.20 mm); H - *Pithecopus gonzagai* (PMSN 462, adult male, SVL 31.60 mm); I - *Lithobates palmipes* (CHUFPE-A 0952, adult female, SVL 46.32 mm).

including at least 64% of amphibian and 32% of reptile species, one of the largest assemblages of amphibians and reptiles ever registered for the state (e.g., Santos & Carnaval 2002, Moura et al. 2010, Roberto et al. 2017). When exclusively considering the richness reported for the Atlantic Forest of Pernambuco, the assemblage reported here comprises approximately 41% of the snake, 69% of the lizard and 67% of the amphibian species registered for this ecoregion.

In comparison with the herpetofauna recorded in adjacent Atlantic Forest fragments in Pernambuco state, the richness found in RVSMAA is the fourth largest in the state, only falling behind that recorded for the *Estação Ecológica de Tapacurá* (36 species of amphibians and 56 of reptiles; Moura et al. 2010), the Serra do Urubu mountain range (46 species of amphibians and 42 reptiles; Roberto et al. 2017) and the *Reserva Biológica Guaribas* (34 species of amphibians and 72 reptiles; Mesquita et al. 2018). Thus, RVSMAA has a greater richness than that found in other important remnants within the state, such as the *Refúgio de Vida Silvestre Matas do Sistema Gurjaú* (24 species of amphibians and 43 of reptiles; Moura et al.2010) and the Dois Irmãos State Park (34 species of amphibians and 21 of reptiles; Melo et al. 2018).

The accumulation curves and richness estimate for RVSMAA revealed that our sampling is close to representing the total diversity of the herpetofauna (collected lizards and anurans) present in the area (N = 56; Chao 1 = 57.43 \pm 5.14; Figure 8), mainly regarding the amphibians (N = 41; Chao 1 = 41.20 \pm 3.56; Figure 8). The accumulation curve of lizards approaches but not reaches an asymptote (N = 15; Chao 1 = 15.99 \pm 2.87; Figure 8), what may be a result of the limiting factor of sampling efforts being divided into three different locations in the RVSMAA, two of which were visited only once and for short periods of time (9-25 days). Although we attempted to sample a wide area of RVSMAA, the great heterogeneity of habitats probably contributes to the occurrence of an even richer and more diverse herpetofauna that has



Figure 6. Reptiles of Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A - Phrynops geoffroanus (PMSN 634, adult female, SVL 309 mm); B - Dactyloa punctata (PMSN 401, adult male, SVL 77.2 mm); C - Norops fuscoauratus (PDM 49, adult male, SVL 49.2 mm); D - Diploglossus lessonae (PDM 136, adult male, SVL 56.11 mm); E - Iguana iguana (PMSN 632, juvenile male, SVL 171.53 mm); F - Enyalius aff. catenatus (unvouchered specimen); G - Gymnodactylus darwinii (PMSN 261, adult female, SVL 43.70 mm); H - Polychrus acutirostris (CHUFPE-R 0945, adult female, SVL 171.53 mm); I - P. marmoratus (CHUFPE-R 0937, adult female, SVL 136.22 mm); J - Coleodactylus meridionalis (PMSN 349, adult male, SVL 20.9 mm); K - Kentropyx calcarata (PDM 159, adult male, SVL 57.82 mm); L - Strobilurus (PMSN 415, adult female, SVL 45.52 mm); N - Corallus hortulana (PMSN 689, adult female, SVL 580 mm); O - Chironius flavolineatus (PMSN 474, adult male, SVL 940 mm).

not yet been assessed. Some species widely distributed across the state (Moura et al. 2010) and with probable occurrence in the area, have not been registered, such as amphisbaenids and crocodilians. This reinforces the need for new long-term studies in the region.

Most of the species registered are classified as Least Concern (LC) in the global, national and local conservation red lists. However, two species are categorized as being threatened at some level according to the IUCN (IUCN 2020), three according to the Brazilian national list (ICMBio 2018), and five according to local lists (SEMAS 2015, 2017) (Tables 2 and 3). All these threatened species were registered exclusively in the largest block of continuous forest (Mata de Xixá and Mata do Engenho Água Azul) and all were recorded as single individuals. The collection sites for all these species are highly conserved when compared to adjacent areas and present springs and/or streams that keep the area constantly and highly moist.

All the threatened species in the area are endemic to the Atlantic Forest and dependent on preserved forests for their survival. *Allobates olfersioides* (Table 2, Figure 3A), although widely distributed in the Atlantic Forest, is dependent on areas with clean water sources and moist soils and is usually found near watercourses (Verdade & Rodrigues 2007). This species has suffered severe population declines in many regions of Brazil (Eterovick et al. 2005, Silvano & Segalla 2005), with fragmentation and habitat loss,



Figure 7. Reptiles of Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. A - Dendrophidion atlantica (CHUFPE-R 393, adult male, SVL 840 mm); B - Oxybelis aeneus (PMSN 690, adult male, SVL 991 mm); C - Dipsas neuwiedi (PMSN 506, adult male, SVL 428 mm); D - Helicops angulatus (CHUFPE-R 602, adult female, SVL 515 mm); E - Imantodes cenchoa (unvouchered specimen); F - Oxyrhopus trigeminus (PMSN 400, adult female, SVL 330 mm); G - Pseudoboa nigra (PMSN 4273 mm); I - Xenodon rabdocephalus (CHUFPE-R 0590, adult female, SVL 271 mm); J - Amerotyphlops brongersmianus (CHUFPE-R 0962, adult female, SVL 301 mm); K - Bothrops leucurus (PMSN 1739, adult female, SVL 805 mm); L - Lachesis muta (PMSN 1757, adult male, SVL 515 mm).

resulting from deforestation, posing the greatest threats to this species (Campos et al. 2013). It has also been affected by chytridiomycosis caused by the keratinophilic fungus *Batrachochytrium dendrobatidis* (Bd) (Carnaval et al. 2006). In the RVSMAA, *Allobates olfersioides* was collected in the wet leaflitter next to a clean water stream inside the forest (near PF13). Another threatened species that was diagnosed with a Bd infection was *Hylomantis granulosa* (Table 2, Figure 5G) (Valencia-Aguilar et al. 2016). This species is restricted to the Atlantic Forest in Alagoas and Pernambuco states (Moura et al. 2010, Almeida et al. 2016), and occurs in isolated populations (ICMBio 2018). It is a forest species not commonly observed in degraded environments, whose occurrence is restricted to the interior and, occasionally, the edges of large forest remnants (Vilela et al. 2015). In RVSMAA, the specimen of *H. granulosa* was collected near WB7 and inside the forest about 600 meters from the nearest edge, close to PF13.

A similar situation was observed for *Chiasmocleis alagoana* (Table 2, Figure 5B), which is distributed across a few remnants of Atlantic Forest in Alagoas, Pernambuco and Paraíba states (Santana et al. 2004, Moura et al. 2010, Almeida et al. 2016). There are no studies indicating the presence of Bd in this species, and little is known about the conservation of this species (Nascimento & Skuk 2006, Nascimento et al. 2020). *Adelophyne baturitensis* (Table 2, Figure 3F), was considered endemic to two "brejos de altitude" (highland humid forest remnants scattered throughout the semiarid lowlands) in Ceará state (Loebmann & Haddad 2010). It was also posteriorly registered in a "brejo de altitude" in the state of Pernambuco (Loebmann et al. 2011),





Figure 8. Accumulation curves based on individuals for anurans, lizards and pooled herpetofauna to all combined campaigns in Refúgio de Vida Silvestre Matas de Água Azul, Serra do Mascarenhas, Pernambuco state, Brazil. Black line represents collected individuals and red line the estimated species richness based on Chao 1 estimator.

always above 600 meters of elevation. The record of *A. baturitensis* in Serra do Mascarenhas is the first finding of this species outside the "brejos de altitude", representing its record at the lowest altitude (\sim 300 m elevation) and expanding the known distribution of the species 95 km northeast of the nearest recorded locations in the municipality of Caruaru, state of Pernambuco (Loebmann et al. 2011).

Among the threatened reptiles, the "bushmaster" *Lachesis muta* (Table 3, Figure 7L) in RVSMAA was observed in dense and primary forests, as is usually the case for the species (Campbell & Lamar 2004). In this study, it was recorded in the largest forest fragment (Mata do Xixá). Records in disturbed forests are rare (Rodrigues et al. 2013a), revealing its fidelity to densely forested areas, making it highly

susceptible to habitat loss. In addition, the tropidurid lizard Strobilurus torquatus (Table 3, Figure 6L) also has a high association with forested habitats, with reports placing the species in the Atlantic Forest in nine Brazilian states (Rodrigues et al. 2013b) and in forest enclaves in Ceará state (Borges Nojosa & Caramaschi 2003). Additionally, four species are classified as Data Deficient (DD) in the local and/or national evaluation of threats (Tables 2 and 3). The lack of knowledge on their ecological and environmental needs, their geographical distribution and population sizes are some of the factors that complicate the assessment of their statuses (ICMBio 2018). The evaluation of these taxa is even more complicated because some are considered to be complexes of species, such as Pseudopaludicola mystacalis (Roberto et al. 2013). This species currently has a wide geographical distribution across Brazilian ecoregions (Frost 2020) but it is likely to harbor a great cryptic diversity not yet described and species that are possibly already threatened with extinction without our knowledge.

Some species have not been identified to a specific level, either because they escaped during collection, with no precise identification (Siphonops sp.), represent complexes of species with poorly defined lineage limits (e.g., Scinax gr. ruber) or can represent lineages not formally described or lacking clear diagnostic characters (Pristimantis sp.). In this last instance, according to Trevisan et al. (2020) three lineages of Pristimantis are currently found in the state of Pernambuco, two of which are not formally described (P. ramagii, "São Francisco River clade" and "Pseudoramagii clade). Although the type locality of P. ramagii (Boulenger, 1888) (municipality of Igarassu, state of Pernambuco) is 50 km from the study area, the lack of diagnostic characteristics in its original description, added to the high polymorphism present in the genus, makes it difficult to accurately identify the specimens sampled. Therefore, we preferred to keep the specimen identification restricted to the generic level until the other lineages of the genus are described and the diagnostic morphological characters are clearly proposed for them.

Our results indicate that the forest remnants of Serra do Mascarenhas house one of the greatest recorded diversities of amphibians and reptiles in the northern Atlantic Forest. The record of seven threatened species reinforces the relevance of the area for the conservation of herpetofauna in the BSR Pernambuco and northern Atlantic Forest. Similarly, the record of five Data Deficient species in this assessment lists provide important information to clarify the threatened statuses and distributions of these species. Finally, we expect that the diversity data we provide will be relevant to draw a more complete picture of local biodiversity and for conservation assessments, reinforcing the importance of this region as a significant biodiversity reservoir for the state and for northeastern Brazil.

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Conflicts of Interest

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

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In relation to the scientific paper "New records of ants (Hymenoptera: Formicidae) for Colombia", with DOI number: https://doi. org/10.1590/1676-0611-BN-2020-1088 published in Biota Neotropica, 2020;20(4) the authors are restoring to Dr. María Cristina Gallego the rights of possession of the information associated to three reported species: *Pheidole bruchi* Forel, 1914; *Pheidole kuna* Wilson, 2003, and *Leptogenys rasila* Lattke, 2011. Dr. Gallego and her working group at Universidad del Cauca, collected the specimens that were included in the aforementioned publication. Although there was collaboration between the authors of the article and the working group at Universidad del Cauca in the taxonomic identification of the specimens, at no time there was explicit authorization from the latter to use the information for publication.

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Florivory by lizards on *Tacinga inamoena* (K.Schum.) N.P.Taylor & Stuppy (Cactaceae) in the Brazilian Caatinga

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Abstract: Florivory can alter plant reproductive success by damaging sexual reproductive structures and disrupting plant-pollinator interactions through decreased flower attractiveness. Here, we report new records of the cactus *Tacinga inamoena* flower consumption by the *Tropidurus hispidus* lizard in the Brazilian Caatinga. We monitored 53 flowers from 11 *T. inamoena* individuals with camera traps over a 1-year period, totalling 450 camera-days of sample effort. We detected four florivory events. In three of these events, flowers were entirely consumed or had their reproductive structures severely damaged, leading to no fruit formation. Florivory events occurred in the morning, right after anthesis, in flowers near the ground, and lizards did not climb the cactus. Our results suggest that *T. hispidus* florivory on *T. inamoena* could have a negative impact on fruit set, since the consumed flowers were entirely destroyed. However, the long-term effects of florivory by lizards on *T. inamoena* reproductive success in the Caatinga still needs to be elucidated.

Keywords: Camera traps; Dry forest; Flower herbivory; Plant-animal interactions; Tropidurus hispidus.

Florivoria por lagartos em *Tacinga inamoena* (K.Schum.) N.P.Taylor & Stuppy (Cactaceae) na Caatinga brasileira

Resumo: A florivoria pode alterar o sucesso reprodutivo da planta através de danos às estruturas reprodutivas sexuais e interrupção das interações planta-polinizador por meio da diminuição da atratividade das flores. Aqui, relatamos novos registros de consumo de flores da cactácea opuntióide *Tacinga inamoena* pelo lagarto *Tropidurus hispidus* na Caatinga brasileira. Nós monitoramos 53 flores de 11 indivíduos de *T. inamoena* com armadilhas fotográficas por um período de um ano, totalizando 450 dias-câmera de esforço amostral. Detectamos quatro eventos de florivoria. Em três desses eventos, as flores foram totalmente consumidas ou tiveram suas estruturas reprodutivas severamente danificadas, não levando à formação de frutos. Os eventos de florivoria ocorreram pela manhã, logo após a antese, em flores próximas ao solo, e os lagartos não escalaram o cacto. Nossos resultados sugerem que a florivoria de *T. hispidus* em *T. inamoena* pode ter um impacto negativo na frutificação, uma vez que as flores consumidas foram totalmente destruídas. No entanto, os efeitos a longo prazo da florivoria por lagartos sobre o sucesso reprodutivo de *T. inamoena* na Caatinga ainda precisam ser elucidados.

Palavras-chave: Armadilhas fotográficas; Floresta seca; Herbivoria floral; Interações planta-animal; Tropidurus hispidus.

Introduction

Florivory refers to the consumption of floral structures by a wide variety of animal taxa (McCall & Irwin 2006). It can decrease plant reproductive success by damaging floral sexual structures or altering flower availability and attractiveness to pollinators (Cascante-Marín et al. 2009; Carper et al. 2016). Vertebrate florivory distinguishes from invertebrate florivory since entire flowers are frequently eaten in the former (Riba-Hernandez & Stoner 2005). Such is the case of florivory by lizards, as their feeding on flowers is often destructive (Olsson et al. 2000). Many lizard species feed on nectar and other flower parts because of their high nutritional and energetic values, potentially playing a significant positive role in pollen transfer (Pérez-Mellado & Casas 1997; Gomes et al. 2013; Hervías-Parejo et al. 2020).

Cactaceae Juss. is one of the most diverse plant families in the Neotropics, representing an important floristic element in arid and semiarid environments with extreme conditions (Mutke 2015). For this family, florivory has been found in drier areas, where cacti flowers stand out for their water content and nutritional value (Nobel 2002). In such environments, cacti flowers are partially or entirely consumed by invertebrates, e.g., Opuntia Mill. flowers eaten by Lepidoptera larvae in Chihuahuan Desert (Pinã et al. 2007, 2010), Ariocarpus Scheidw. flowers consumed by Coleoptera and Lepidoptera in Mexico (Cárdenas-Ramos & Mandujano 2019), destruction of flower parts (tepals, stamens, stigma) of Melocactus Link & Otto (Colaço et al. 2006) and Cereus Mill., Pilosocereus Byles & Rowley, Harrisia Britton, and Tacinga Britton & Rose (Rocha et al. 2020) by flower-damaging bees Trigona spinipes (Fabricius 1973), and vertebrates, e.g., Opuntia flowers eaten by lizards in Galápagos Island (Nobel 2002), Melocactus flowers eaten by Tropiduridae lizards in the Brazilian Caatinga (Gomes et al. 2013), and the short columnar cactus Echinopsis rhodotricha K.Schum. florivory by deer and peccaries in the Brazilian Chaco (Gomes et al. 2016).

Tacinga is a genus of the subfamily Opuntioideae, composed of eight species endemic from Brazil, restricted to the semiarid lands in the Eastern corner of the country (Zappi & Taylor 2020). Tacinga inamoena (K.Schum.) N.P.Taylor & Stuppy, commonly known as "cumbeba" or "quipá", is a subshrub cactus, with round to oblong pads and solitary subapical flowers at the top of the branches, $4-6 \times 3.5-4$ cm, with a green globose pericarpel, 10 mm and orange-red, acute, fleshy bract-scales subtending bristles (Taylor & Zappi 2004; Peixoto et al. 2016) (Figure 1a). Their flowers, which are the showiest of the genus, are large, shiny, have erect stamens forming a column surrounding the stigma, provide pollen and nectar as floral rewards, and are pollinated by hummingbirds (Lambert 2009). The flowering period starts at the end of the rainy season, continues throughout the dry season, peaking in September/October (Quirino 2006). Flower anthesis is diurnal, usually lasting one day, from eight to 11 hours in prickly pears, and sometimes opening on a second day (Pimienta-Barrios & del Castillo 2002).

Tropidurus hispidus (Spix, 1825) is a heliophilous lizard that can reach up to 35 cm in total length, the largest of the genus (Kolodiuk et al. 2010). It is widely distributed in South America, occurring in open landscapes such as Caatinga, and living in forest borders, rocky outcrops, and human-altered areas (Fialho et al. 2000). Its generalist diet comprises plant material, ants, insect larvae, and vertebrates. Plant material (e.g., leaves, non-identified flowers, and fruits) have been reported as one of the three most important food items for *T. hispidus* populations in Caatinga and in rocky outcrops areas, especially



Figure 1. a) *Tacinga inamoena* flower, (b-c) *Tropidurus hispidus* looking at an entire and partially eaten flower, respectively.

during the dry seasons (Van Sluys et al. 2004, Kolodiuk et al. 2010; Albuquerque et al. 2018), yet little is known about the identity of the plant species consumed (Koski et al. 2018).

During a study about Cactaceae frugivory by vertebrates in the Caatinga, a seasonally dry tropical forest in northeastern Brazil, we recorded florivory events by lizard *Tropidurus hispidus* on the cactus *Tacinga inamoena*. Herein, we quantified and described this flower herbivory interaction, which is new information for both species.

Material and Methods

Our study site was the Santo Antônio Ranch private property located in Lajes municipality (05°47'48"S, 36°14'23"W), Rio Grande do Norte state, northeastern Brazil (Brazilian Semiarid region). The region is characterized by low irregular rainfall (>300 mm/year), long periods of drought, and high average annual temperatures (30°C). We monitored 53 flowers from 11 *T. inamoena* individuals using camera traps (Bushnell Trophy CAM 8 MP HD) from September 2018 to July 2019. We had a total of 450 camera-days of sample effort distributed throughout the months (on average 45 camera-days per month). We placed the cameras one to two meters away from the flowering individuals and configured it to capture two photos followed by 10 second videos, in intervals of one minute. We then left the cameras in the field working 24 hours/day every month, recording the whole anthesis period of blooming flowers. We chose plants that were located at least 70 m from each other to ensure spatial heterogeneity.

We categorized the flower consumption records into four categories (from four to one) according to the Damage Intensity (D.I.) caused by the lizard on the flower. In the fourth category, the flower was entirely consumed (flower and pericarpel, which could be entirely consumed or detached from the plant); in the third category, the flower was entirely consumed and pericarpel was damaged; in the second category, the flower was entirely consumed without damaging the pericarpel, and in the first category, the flower was partially consumed, without damage to the pericarpel. Each record was classified into the four categories by analyzing the photos and videos. Day and time of consumption as well as the height of flowers consumed were also recorded.

Results

Tropidurus hispidus ate four *T. inamoena* flowers, which corresponded to 7.5% of the flowers monitored in the study period (N = 53). Three of the florivory events happened in the same cactus individual at the end of the 2018 dry season, and the other one during the 2019 rainy season (Table 1). All the florivory events occurred in the morning, right after anthesis, during single visits that, in some cases, lasted several minutes (Table 1). In the last florivory event we recorded, the flower started anthesis on one day and opened again on a second day, when it was then fully eaten by the lizard. However, we did not record flower visitors on this flower during its first day of anthesis.

Lizards approached the plant and started gradually eating the flowers located near the ground, eventually leaning on wooden sticks on the ground to avoid climbing the cactus, and began by tearing the petals until reaching the globose pericarpel (Table 1, Figure 1). In one of the events, the lizard jumped to finish consuming the globose pericarpel remains (<u>Video</u>).

In three florivory events, the flowers were either entirely consumed (N = 2, D.I. = 4), including petals, stamens, and stigma along with the globose pericarpel, or severely damaged (N = 1, D.I. = 3) (Table 1). No fruits developed in these damaged flowers. In only one of the events, the petals were not completely consumed and the pericarpel was not damaged (D.I. = 1), remaining attached to the plant for more than 34 days until we collected the camera. In this case, we cannot affirm whether this flower developed into a fruit, considering that *T. inamoena* fruits take an average of six months to ripen after fertilization (Quirino 2006). As we observed in the footage, *T. inamoena* buds took about 25 days to develop and start anthesis, remaining open for a day or sometimes opening a second day. Throughout the monitored months, other available structures (e.g. flower buds and ripe and/or unripe fruits) were not consumed.

Discussion

Florivory can greatly impact plant species, similar to or sometimes more than tissue herbivory (McCall & Irwin 2006). Flower herbivores can indirectly interfere in plant reproductive success by damaging the petals, making the flower unrecognizable or less attractive to pollinators (Krupnick & Weis 1999; Mothershead & Marquis 2000). In turn, this can affect the sexual display of the plant and disrupt plant-pollinator mutualistic interactions (Krupnick et al. 1999; Leavitt & Robertson 2006). In general, petal removal can indirectly decrease reproductive output (seed set) via decreased flower visits by pollinators (Leavitt & Robertson 2006; Carper et al. 2016; Tsuji & Ohgushi 2018).

In one of the florivory events recorded on T. inamoena, the petals were not entirely consumed by the lizard T. hispidus, and, although through the camera traps we could not determine whether the reproductive structures were damaged or not, we did not observe any flower visitors after consumption by the lizard. In the prickly pear Opuntia microdasys (Lehm.) Pfeiff., florivory by Lepidoptera larvae caused a substantial decrease in their reproductive success by destroying the plant reproductive structures (Pinã et al. 2010). Also, florivory by Lepidoptera larvae on O. microdasys floral buds caused a high percentage of abortion and, consequently, no fruits (Pinã et al. 2007). In E. rhodotricha, a short columnar cactus species from the Brazilian Chaco, flowers that were partially damaged by deer and peccaries did not set fruits (Gomes et al. 2016). In contrast, flowers of the globular cactus Melocactus ernestii Vaupel also had their petals consumed by Tropiduridae lizards. However, such florivory did not impact the plant reproductive success, probably because the flower reproductive structures of Melocactus species are protected inside a modified stem (cephalium) and M. ernestii is capable of self-pollination (Gomes et al. 2013).

It is expected that bigger animals such as vertebrates would remove the entire flower when feeding on this resource (Riba-Hernandez & Stoner 2005; Gomes et al. 2016). In this case, florivory can have a direct effect on the plant reproductive output because completely removing the flower takes away its chances of reproducing developing fruit (McCall & Irwin 2006; Cascante-Marín et al. 2009). This pattern has been observed in *E. rhodotricha* florivory by deer and peccaries (Gomes et al. 2016) and in this present study. In our records, in three of the four florivory events, *T. inamoena* flowers were severely damaged.

Lizards and cactus flowers appear to have an old relationship, especially Iguania lizards and prickly pear cacti on islands (Christian & Tracy 1982; Christian et al. 1984; Schluter 1984). A study about the diet of the Tropiduridae lizard *Microlophus pacificus* (Steindachner 1876) on Isla Pinta Galápagos found *Opuntia* flowers in its stomach and a positive correlation between lizard body size and herbivory (Schluter 1984), followed by a recent study that has observed *M. pacificus* consuming *Opuntia galapageia* Hensl. flowers in the field, possibly transporting its pollen and acting as a pollinator (Hervías-Parejo et al. 2020). *Opuntia* florivory by a non-insular lizard species of *Cnemidophorus* was first

Table 1. Florivory on *Tacinga inamoena* at a Caatinga site in Lajes municipality, Rio Grande do Norte state, northeastern Brazil. Day of flower anthesis, day of flower consumption by *Tropidurus hispidus* lizard, time of consumption (start and end time), and height of the flower consumed (m). Damage intensity of the florivory and geographic location of the cactus consumed.

Anthesis	Consumption	Start	End	Flower height	Damage intensity	Location
11/15/2018	11/15/2018	08:42	09:22	0.3	4	05°47'48.4"S,036°14'23.8"W
11/29/2018	11/29/2018	08:09	08:20	0.6	3	05°47'48.4"S,036°14'23.8"W
12/26/2018	12/26/2018	09:01	09:03	0.4	1	05°47'48.4"S,036°14'23.8"W
04/15/2019	04/16/2019	10:44	10:45	0.1	4	05°47'52.4"S,036°14'21.3"W

recorded at Paraguand Peninsula, Venezuela, where *C. lemniscatus* (Linnaeus 1758) was seen eating *Opuntia wentiana* (Britton & Rose) petals (Mijares-Urrutia et al. 1997). In this last case, flowers were located near the ground and the lizard did not have to climb the cactus.

Opuntioideae cacti offer a great amount of resources including cladodes, fruits, and flowers (Mellink & Riojas-López 2002). A common reproductive feature of cacti is asynchronous flower development, which is, many floral buds, flower at anthesis, and unripe fruits available at the same time (Pimienta-Barrios & Nobel 1995; Pimienta-Barrios & del Castillo 2002; Quirino 2006). Despite that, two-thirds of reptiles that consume platyopuntias on the Galápagos Islands specifically eat their flowers, probably because of the pollen and nectar of cacti, which are often rich in sucrose (Pimienta-Barrios & del Castillo 2002; Fleming et al. 1994). Similarly, when feeding on *T. inamoena* flowers in anthesis, *T. hispidus* lizards ignored flower buds and ripe or unripe fruits.

Tacinga flowers have erect stamens and showy colours, such as the orange-red *T. inamoena* flowers, which have evolved to be pollinated by hummingbirds (Lambert 2009). Lizards use their vision to search for food near the ground and some diurnal species may also have visual pigments and photoreceptors, similar to those in birds, which could help them detect food (Ellingson et al. 1995; Janzen & Brodie 1995). Such features may have favoured *T. inamoena* flower detection by Tropiduridae lizards, as these flowers are located near the ground (= 0.35 m in height, according to Table 1). Also, *T. inamoena* flower anthesis starts in the morning, overlapping with *T. hispidus* activity patterns, which is also when flowers are most conspicuous and provide copious amounts of nectar $22 \pm 1.8 \,\mu l$ [%] = $26 \pm 1.0 \,(N = 5 \,\text{flowers})$, corresponding to $6.3 \pm 0.5 \,\text{mg}$ sugar per flower (Galetto & Bernadello 2005).

Some lizards search for plant material mainly during dry periods in environments with high temperature and low water availability (Koski et al. 2018). Vitt (1995) reported the lizards *Tropidurus hispidus*, *Tropidurus semitaeniatus* (Spix 1825), and *Salvator merianae* (Duméril & Bibron 1839) feeding on plant material in the Caatinga. Other examples of plant consumption by lizards includes *Tropidurus pinima* (Rodrigues 1984) feeding on cactus fruits and seeds and nonidentified flowers (Xavier & Dias 2017), *Ameivula ocellifera* (Spix 1825) consumption of flowers of *Cassia* L. sp., *Tropidurus torquatus* (Wied-Neuwied 1820), which flowers comprise an important part of its diet (Rocha & Bergallo 1994), *Tropidurus psammonastes* Rodrigues, Kasahara and Yonenaga-Yassuda 1988 consumption of non-identified flowers (Lima & Rocha 2006), and *Liolaemus lutzae* Mertens 1938 consuming flowers of *Ipomoea litorallis* (L.) Boiss. (Rocha 1991).

According to Albuquerque et al. (2018) flower eating by *T. hispidus* was observed in populations of Caatinga and Cerrado, being higher during water scarcity periods in dryer areas. *Tropidurus hispidus* searches for floral food resources due to its need for water, as well as the high nutrient contents of flowers. The peak flowering of *T. inamoena* occurs in the dry season (Quirino 2006). Thereby, its flowers are an important resource for lizard energy requirements, especially in an environment as the Caatinga, where water and food resources are scarce in the dry months, which can last for a long part of the year. Despite this plant-animal interaction could bring negative impacts to the plant reproductive output, additional investigation is needed to evaluate the long-term effects of florivory by lizards on *T. inamoena* populations and whether it can influence its reproductive success by reducing the fruit and seed set.

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Author Contributions

Virgínia Helen Figueiredo Paixão: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Vanessa Gabrielle Nóbrega Gomes: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Eduardo Martins Venticinque: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation.

Conflicts of Interest

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

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Composition and distribution of fishes from the Perequê-Açu river basin, Paraty, Rio de Janeiro, Southeastern Brazil

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Abstract: The large number of less-known coastal drainages in the Atlantic Forest still represents an important gap in the knowledge of ichthyofauna. It is evaluated the taxonomic diversity, frequency and constancy of occurrence, and the distribution of species in the various segments of the Perequê-Açu river basin, state of Rio de Janeiro, Brazil. Twelve sampling campaigns resulted in 13,423 individuals, belonging to seven orders, 10 families, 21 genera, and 23 native species. Cyprinodontiformes and Siluriformes represented about 87.2% of the total abundance, mainly due to the contribution of *Phalloceros anisophallos* and *Schizolecis guntheri*, which account for 63.2% of the total sampled individuals. The analysis of constancy of occurrence reveals that 78.3% of the drainage species are residents, with approximately 93% of the residents of the Carrasquinho River sub-basin. The Sertões River sub-basin had the largest number of species in total: 18. The rarefaction curve did not reach an asymptote, although Chao 2 and bootstrap estimate the richness of 23.9 species and 23.7 species, respectively. The results of PERMANOVA, PCoA, and SIMPER indicate that the fish composition along the catchment is not homogeneous. The conservation status of species is also discussed, regionally, nationally, and globally.

Keywords: Atlantic Rainforest, Conservation, Freshwater fishes, Inventory, Serra da Bocaina National Park.

Composição e distribuição de peixes da bacia do rio Perequê-Açu, Paraty, Rio de Janeiro, Sudeste do Brasil

Resumo: O grande número de drenagens costeiras pouco estudadas na Mata Atlântica ainda representa uma lacuna importante no conhecimento da ictiofauna. Avalia-se aqui a diversidade taxonômica, frequência e constância de ocorrência e a distribuição das espécies nos vários segmentos da bacia do rio Perequê-Açu, estado do Rio de Janeiro, Brasil. Doze campanhas de amostragem resultaram em 13.423 indivíduos, pertencentes a sete ordens, 10 famílias, 21 gêneros e 23 espécies nativas. Cyprinodontiformes e Siluriformes representaram cerca de 87,2% da abundância total, devido sobretudo à contribuição de *Phalloceros anisophallos* e *Schizolecis guntheri*, que respondem por 63,2% do total de indivíduos amostrados. A análise de constância de ocorrência revela que 78,3% das espécies da drenagem são residentes, sendo cerca de 93% dos residentes da sub-bacia do rio Carrasquinho. A sub-bacia do rio Sertões apresentou o maior número de espécies no total: 18. A curva de rarefação não atingiu uma assíntota, embora Chao 2 e bootstrap estimem a riqueza de 23,9 espécies e 23,7 espécies, respectivamente. Os resultados de PERMANOVA, PCoA e SIMPER indicam que a composição dos peixes ao longo da bacia hidrográfica não é homogênea. Discute-se, ainda, o status de conservação das espécies, regional, nacional e globalmente.

Palavras-chave: Conservação, Inventário, Mata Atlântica, Parque Nacional da Serra da Bocaina, Peixes de água doce.

Introduction

The coastal area in the south of Rio de Janeiro state is characterized by presenting relatively narrow stretches of flat terrains located at the base of Serra do Mar (Guerra et al. 2013). The eastern slope of these mountains, the eastern edge of the Brazilian Plateau, is full of relatively short and fast rivers that generally run through a few dozen kilometers till the Atlantic Ocean (Andreata et al. 2002, Buckup 2011, Guerra et al. 2013). Ilha Grande Bay Hydrographic Region consists of independent river basins located in the municipalities of Angra dos Reis and Paraty. These areas are expected to exhibit relatively low diversity but high levels of endemism (Bizerril 1994, Ribeiro 2006, Abilhoa et al. 2011, Buckup 2011). Abell et al. (2008) called this as Ecoregion 325, Fluminense, with endemism rates of 29-39% (at least 28 endemic freshwater fish species). However, most studies approach marine environments (e.g. Andreata et al. 2002, Ferreira et al. 2007, Gaelzer et al. 2007, Amaral et al. 2018), while few studies about freshwater fishes are available (e.g. São-Thiago 1990).

Recent studies (Guerra et al. 2013) show the environmental fragility of this area. The first major impacts in this region were due to urban growth occurred after the construction of Rio-Santos (BR-101), a great scenic beauty road that connects the two biggest South American cities, São Paulo and Rio de Janeiro. Now, the newly paved stretch of Paraty-Cunha road (RJ-165) should increase the population density in this region, increasing negative impacts on watercourses, a fundamental resource both for the good quality of life in the region and for the maintenance of economic activities in that area of the state (Formiga-Johnsson 2015). Decree No. 5,092 / 2004 (Brasil 2004) recommended to a committee working in a Project for Conservation and Sustainable Use of Brazilian Biological Diversity (Probio), the evaluation of priority areas for conservation in the coastal and marine areas of Brazil; one of the extremely high priorities selected areas was exactly Ilha Grande Bay (Creed et al. 2007), although just its marine area. However, the lack of knowledge about the hydrographic basins of this region shows that we are not even able to assess the conservation status of these drainages. The most worrying aspect of this is that in addition to an important ecological role, much of the coastal region in the south of the state is supplied by these rivers, which play a fundamental role in the quality of life and economic activities developed in the region, including ecotourism and adventure tourism (Formiga-Johnsson et al. 2015). Deforestation and pollution caused by inadequate tourism infrastructure intensify the water demands, the uses of rivers and beaches, and is a specific environmental problem of this region (Gaelzer et al. 2007).

Ichthyofauna surveys are extremely important for management and conservation of freshwater fishes (Azevedo-Santos et al. 2019), serving as a first step as they provide the basis for future studies in other areas such as ecology, conservation, and systematics (Frota et al. 2019), as well as being the basis for the description of new species. The Neotropical region has the largest number of freshwater fish species on the planet, with circa of 5,000 species of fishes described, about 3,000 of them in Brazil (Reis et al. 2016). Buckup et al. (2007) pointed out that the number of descriptions of freshwater fish species has been increasing by over 20% annually, especially in the South and Southeast regions of Brazil. According to Langeani et al. (2009), such diversity reflects the complexity of habitat and the life history of freshwater fishes.

The evaluation presented here is a result of a wildlife monitoring project developed during the paving works of the Rio de Janeiro stretch of Paraty-Cunha road (RJ 165), which crosses Serra da Bocaina National Park. The purpose of the monitoring project was to identify specimens from fauna and flora and potential problems affecting them caused by the engineering work. Thus, the objective of this paper is to inventory the fish species of the Perequê-Açu river basin and its sub-basins, assess their conservation status, and identify the presence of resident, rare and accessory species. As the complexity of the community along the river is expected to increase from upstream to downstream (Vannote et al. 1980), we seek to determine whether the fauna of the high, medium and low stretches of each sub-basin are related to each other.

Material and Methods

1. Study area

The Perequê-Açu river basin (Figure 1) is part of the Ilha Grande Bay Hydrographic Region. Most of the headwaters of the basin are sheltered within Serra da Bocaina National Park (PNSB), the largest Brazilian coastal Park (104,000 hectares; IBAMA 2001), spread between the states of Rio de Janeiro and São Paulo. The Park presents dense ombrophilous forest, mixed ombrophilous forest, and subtropical highland grasslands, with altitudes that range from zero to 2,088 m, which confers to this conservation unit a great diversity and complexity of environments (IBAMA 2001). The climate in the region is temperate super humid, with annual precipitation of 1800 mm. The average temperature is around 16 °C, with high variation between altitude extremes: 38 °C in lowland areas and 0 °C in the highest areas (Guimarães et al. 2000).

The name by which this drainage is known, Perequê-Açu, refers only to the resulting river in the final stretch of this basin. For descriptive and analytic purposes, this drainage was thus separated into five sub-basins that represents its main tributaries, hereafter referred as Sertões river sub-basin (SBS), Pedra Branca river sub-basin (SBP), Muricana river sub-basin (SBM), Carrasquinho river sub-basin (SBC) and Perequê-Açu River itself (SBA).

2. Sampling and data analyses

Twelve samplings of fishes were performed from June 2013 to November 2016 under collecting permit 248/2013 (Ibama, Process 02001.003937/2008-18). Twenty-four georeferenced locations (Table 1) were sampled for 45 minutes each site, in distinct environments with altitudes ranging from 9 m to 1,044 m in stretches of variable length. We used hand nets, sieves, and two trawls: 1.5m long x 1m wide, with 5mm mesh, and 3m long x 2m trawl, with 5mm mesh. Most individuals were identified in the field, counted, and released. Individuals with taxonomic doubts were anesthetized in a 10% eugenol solution, immediately placed in a 10% formalin solution, and then permanently stored in 70% alcohol. Specimens for genetic analysis were immediately deposited in absolute ethanol. The material was deposited in the fish collection at Faculdade de Formação de Professores, Universidade do Estado do Rio de Janeiro (FFP/UERJ) and voucher material was deposited in the ichthyological collection of Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). Taxonomic identification followed specialized literature and classification adopted is summarized in Fricke et al. (2020).



Figure 1. Sampled sites in the Perequê-Açu river basin. SP = São Paulo; RJ = Rio de Janeiro.

A constancy of occurrence index (Dajoz 1983) was applied to analyze the total amount of species obtained in the 12 campaign samples and for the total obtained in each sub-basin to verify the frequency of species in the drainage and in each group of sites. According to this index, constant species are present in more than 50% of the samples, accessory species are present in 25 to 50% and rare species are present in less than 25%. The number of species collected was verified using a rarefaction curve, which is a plot of the number of species as a function of the number of samples, to standardize the species richness expected to be found in a certain number of samples (Melo 2008). We calculated the bootstrap and Chao 2 species estimators, species extrapolation methods to verify whether the maximum number of species has already been sampled in the basin (Efron 1979, Gotelli & Colwell 2011). Additionally, we applied four indices for each site to evaluate the structure of the ichthyofaunistic diversity: Shannon's diversity (H), Simpson's dominance (D), Pielou's equitability (J') and total richness (Simpson 1949, Pielou 1975, Krebs 1999, Magurran 2004).

Spatial variations on the ichthyofauna distribution among upper, middle, and lower sites of the basin followed Cavalheiro & Fialho (2020), where further explanations on these procedures can be found. Once the abundances of the species were logtransformed, we performed an a priori Permutational Multivariate Analysis of Variance (PERMANOVA), based on the Bray-Curtis dissimilarity index, to identify patterns of distribution of species, which if identified were plotted in a Principal Coordinates Analysis (PCoA) ordination graph, using the Bray-Curtis dissimilarity index (Legendre & Legendre 1998). In order to further assess species distribution patterns in the basin, we performed a Similarity Percentage (SIMPER) analysis (Clarke 1993), identifying species that most contributed to dissimilarities between groups of upstream, middlestream and downstream sites.

The rarefaction curve, diversity indices, and species estimators were calculated using the statistical software PAST v. 4.03 (Hammer et al. 2001). The PERMANOVA and SIMPER analyses were performed using the software PRIMER 6.1 with PERMANOVA+ (Clarke & Gorley 2006). The PCoA was performed using R Project for Statistical Computing v. 3.6.1 (R Core Team 2019), with the packages "ape" v. 5.4 (Paradis & Schliep 2019) and "adespatial" v. 0.3-8 (Dray et al. 2020), at a confidence level of 95% (p <0.05). The map of the basin was developed in the software QGis v. 3.12 (QGIS Development Team 2020).

Results

A total of 13,423 specimens were sampled, belonging to seven orders, 10 families, 21 genera, and 23 species (Table 2; Figures 2-3).

Sub-basin	Acronym	Site	Latitude (S)	Longitude (W)	Altitude (m)
	S1	Sertões river headwaters I	23° 12' 21.0"	44° 51' 30.8"	1044
	S2	Sertões river headwater II	23° 11' 29.2"	44° 50' 40.5"	932
CDC	S3	Estiva Preta stream	23° 12' 30.5"	44° 49' 45.8"	638
	S4	Middle Sertões river I	23° 12' 27.1"	44° 49' 32.0"	643
SBS	S5	Middle Sertões river II	23° 12' 32.4"	44° 49' 50.1"	647
	S6	Lower Sertões river	23° 12' 51.6"	44° 47' 48.5"	207
	S 7	Lavadeiras stream	23° 13' 43.0"	44° 47' 10.8"	150
	S 8	Unnamed Perequê-Açu river tributary	23° 13' 46.4"	44° 46' 24.6"	40
	S9	Sousa stream I	23° 12' 07.4"	44° 48' 19.9"	431
	S10	Sousa stream II	23° 12' 07.7"	44° 48' 15.8"	419
CDC	S11	Carrasquinho river I	23° 12' 35.4"	44° 47' 39.3"	191
SBC	S12	Carrasquinho river II	23° 12' 35.4"	44° 47' 39.3"	191
	S13	Carrasquinho river III	23° 12' 45.5"	44° 47' 31.7"	179
	S14	Carrasquinho river IV	23° 12' 46.0"	44° 47' 30.0"	175
CDM	S15	Upper Muricana stream	23° 12' 44.4"	44° 46' 09.2"	117
SBM	S16	Lower Muricana stream	23° 13' 04.3"	44° 45' 49.5"	40
	S17	Upper Pedra Branca river	23° 11' 00.9"	44° 46' 53.5"	442
GDD	S18	Middle Pedra Branca river	23° 11' 58.2"	44° 45' 52.2"	201
SBP	S19	Lower Pedra Branca river	23° 13' 02.9"	44° 45' 48.7"	42
	S20	Unnamed Pedra Branca river tributary	23° 13' 03.2"	44° 45' 47.9"	46
	S21	Upper Perequê-Açu river I	23° 13' 22.8"	44° 45' 30.3"	16
	S22	Upper Perequê-Açu river II	23° 13' 23.7"	44° 45' 29.9"	15
SBA	S23	Upper Perequê-Açu river III	Critical water II2.5112.5444050532Preta stream 23° 12' 30.5" 44° 49' 45.8"638Sertões river I 23° 12' 32.4" 44° 49' 32.0"643ertões river II 23° 12' 32.4" 44° 49' 50.1"647Sertões river 23° 12' 51.6" 44° 47' 48.5"207siras stream 23° 13' 43.0" 44° 47' 10.8"150sêrtões river 23° 13' 43.0" 44° 47' 10.8"150sêrtões river tributary 23° 13' 46.4" 44° 46' 24.6"40a stream I 23° 12' 07.4" 44° 48' 19.9"431a stream II 23° 12' 35.4" 44° 47' 39.3"191uinho river I 23° 12' 35.4" 44° 47' 39.3"191uinho river II 23° 12' 45.5" 44° 47' 30.0"175uricana stream 23° 12' 45.5" 44° 47' 30.0"175uricana stream 23° 13' 04.3" 44° 45' 49.5"40lra Branca river 23° 11' 00.9" 44° 45' 49.5"40lra Branca river 23° 13' 02.9" 44° 45' 29.9"15quê-Açu river II 23° 13' 22.8" <td< td=""><td>15</td></td<>	15	
	S24	Middle Perequê-Açu river	23° 13' 22.8"	44° 45' 14.7"	9

Table 1. Sampled localities in the Perequê-Açu river basin, Paraty, RJ. The sub-basin acronym, the acronym for each site, the name of the site, coordinates, and altitude are displayed. SBS = Sub-basin of Sertões river; SBC = Sub-basin of Carrasquinho river; SBL = Sub-basin of Laje river; SBP = Sub-basin of Pedra Branca river; SBA = Sub-basin of Perequê-Açu river.

The most representative orders in abundance were Cyprinodontiformes (46.8%) and Siluriformes (40.4%), with 11,700 individuals (about 87.2% of the total sampled, Figure 4), due mainly to the contribution of the two most abundant species in the basin: *Phalloceros anisophallos* (n = 5,853, Figure 3R-3S) and *Schizolecis guntheri* (n = 2,636, Figure 3L). These two species accounts for 63.2% of the sampled individuals. The order Siluriformes was the most taxonomically diverse, with 12 species, followed by Characiformes, with four species (17.4%). Gymnotiformes and Mugiliformes were the least representative orders, presenting only one species each (4.3%). Poeciliidae is the most representative of the families, contributing with 46.8% of individuals (n = 6,281), followed by Loricariidae (n = 4,725, 35.2%) and Characidae (n = 1,474, 11%; Figure 4).

The constancy of occurrence index revealed that most species in the basin are constant (Deuterodon hastatus, D. intermedius, Hollandichthys multifasciatus, Bryconamericus ornaticeps, Acentronicthhys leptos, Rhamdia quelen, Rhamdioglanis transfasciatus, Hemipsilichthys nimius, Kronichthys heylandi, Neoplecostomus paraty, Schizolecis guntheri, Trichogenes longipinnis, Trichomycterus sp., Geophagus brasiliensis, Awaous *tajasica, Eleotris pisonis, Phalloceros anisophallos*, and *P. harpagos*, Figure 5), with about 78.3% (n = 18) of frequency in the 12 sampling campaigns, followed by the accessory (13%, n = 3) and rare species (8.7%, n = 2). Carrasquinho river sub-basin proportionally presented the highest percentage of resident species, about 93% of frequency (n = 8), followed by Muricana stream (70%, n = 7) and Sertões river (55.5%, n = 10), the latter being also the sub-basin which presented the largest number of species in total: 18.

The site with the highest richness and abundance (S = 13, n = 1,520) was S23, Upper Perequê-Açu river III (Table 3). Sertões river headwater I (S1) presented the lowest richness (S = 2) and abundance (n = 34). Estiva Preta stream (S3) presented the lowest dominance rates of the basin (D = 0.197), as well as the highest values on diversity and evenness (H'= 1.675, J' = 0.935). A near site, Middle Sertões river I (S4), presented lower evenness (J' = 0.123), diversity (H' = 0.171), and high dominance values (D = 0.932). The rarefaction curve did not reach an asymptote (Figure 6), even in the 95% confidence interval. The Chao 2 and bootstrap richness estimators estimate the richness of 23.9 species (\pm 2.13) and 23.7 species, respectively. Information on the conservation status of the species can be seen in Table 2.

Table 2. Taxa sampled in the Perequê-Açu river basin, Paraty, RJ. The acronym used in analyses, relative frequency (%) of the species, their conservation status, the category of constancy, and the voucher of the ichthyological collection are presented. DD = Data deficient; LC = Least Concern; NT = Nearly threatened; VU = Vulnerable.

		E.	Conse	ervation Status	Constancy	X7 1	
Taxa	Acronym	Fr (%)	Rio de Janeiro Red List (2000)	Brazilian Red List (2014)	IUCN (2019)	of Occurrence	PX-FFP
Characiformes							
Characidae							
Deuterodon hastatus (Myers, 1928)	Dha	0.54		LC		Constant	1298
Deuterodon intermedius (Eigenmann, 1908)	Din	0.34		LC		Constant	1338
Hollandichthys multifasciatus (Eigenmann & Norris, 1900)	Hmu	1.43		DD		Constant	1189
Bryconamericus ornaticeps Bizerril & Perez- Neto, 1995	Bor	8.68		LC		Constant	1176
Siluriformes							
Heptapteridae							
Acentronichthys leptos Eigenmann & Eigenmann, 1889	Ale	0.69	VU	LC	LC	Constant	1194
Pimelodella lateristriga (Lichtenstein, 1823)	Pla	0.03		DD		Accessory	1156
Rhamdia quelen Quoy & Gaimard, 1824	Rqu	0.17		LC	LC	Constant	0849
Rhamdioglanis transfasciatus Miranda Ribeiro, 1908	Rtr	1.87		LC		Constant	0413
Loricariidae							
Ancistrus multispinis (Regan, 1912)	Amu	0.11		LC		Accessory	1361
Hemipsilichthys nimius Pereira, Reis, Souza & Lazzarotto, 2003	Hni	2.93		NT		Constant	0406
Kronichthys heylandi (Boulenger, 1900)	Khe	7.32	VU	LC		Constant	0424
Neoplecostomus paraty Cherobim, Lazzarotto & Langeani, 2016	Npa	5.01				Constant	0427
Pareiorhina rudolphi (Miranda Ribeiro, 1911)	Pru	0.19	VU	NT		Accessory	1250
Schizolecis guntheri Miranda Ribeiro, 1918	Sgu	19.64		LC		Constant	0408
Trichomycteridae	C						
Trichogenes longipinnis Britski & Ortega, 1983	Tlo	0.31		NT		Constant	1305
Trichomycterus sp.	Tsp	2.10				Constant	0414
Gymnotiformes							
Gymnotidae							
Gymnotus pantherinus (Steindachner, 1908)	Gpa	0.01		LC		Rare	1154
Cichliformes							
Cichlidae							
Geophagus brasiliensis Quoy & Gaimard, 1824	Gbr	0.94		LC		Constant	0815
Gobiiformes							
Gobiidae							
Awaous tajasica Lichtenstein, 1822	Ata	0.62		LC	LC	Constant	0832
Eleotridae							
Eleotris pisonis (Gmelin, 1789)	Epi	0.14		LC	LC	Constant	0475
Mugiliformes							
Mugilidae							
Mugil liza Valenciennes, 1836	Mli	0.15		NT	DD	Rare	0834
Cyprinodontiformes							
Poeciliidae							
Phalloceros anisophallos Lucinda, 2008	Pan	43.60		LC		Constant	0584
Phalloceros harpagos Lucinda, 2008	Pha	3.19		LC		Constant	1249
	Total	100					



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Figure 2. Species sampled in the Perequê-Açu river basin. (A) *Deuterodon* hastatus; (B) *Deuterodon intermedius*; (C) *Bryconamericus ornaticeps*; (D) *Hollandichthys multifasciatus*; (E) *Rhamdia quelen*; (F) *Rhamdioglanis* transfasciatus; (G) *Ancistrus multispinis*; (H) *Hemipsilichthys nimius*; (I) *Kronichthys heylandi*; (J) *Neoplecostomus paraty.*



Figure 3. Species sampled in the Perequê-Açu river basin. (K) *Pareiorhina rudolphi*; (L) *Schizolecis guntheri*; (M) *Trichogenes longipinnis*; (N) *Trichomyterus sp.*; (O) *Geophagus brasiliensis*; (P) *Awaous tajasica*; (Q) *Eleotris pisonis*; (R) female *Phalloceros harpagos*; (S) male *Phalloceros anisophallos*; (T) female *Phalloceros anisophallos*.



Figure 4. Representativeness graph of the sampled specimens regarding orders and families in the Perequê-Açu river basin.



Figure 5. Constancy of occurrence of the Perequê-Açu river basin fish species. SBS = Sertões river sub-basin; SBC = Carrasquinho river sub-basin; SBM = Muricana stream sub-basin; SBP = Pedra Branca river sub-basin; SBA = Perequê-Açu river sub-basin.

The PERMANOVA result indicated that the spatial distribution of the species is not homogenous along the longitudinal gradient of the drainage (Df = 2, Pseudo-F = 4.20, p = 0.0002), revealing differences in the composition of species among upstream, midstream, and

Site	n	S	D	Н'	J'
S1	34	2	0.701	0.451	0.651
S2	81	5	0.494	0.831	0.517
S3	169	6	0.197	1.675	0.935
S4	87	4	0.932	0.171	0.123
S5	486	7	0.451	1.009	0.518
S6	643	10	0.267	1.569	0.681
S7	150	5	0.339	1.165	0.724
S 8	217	9	0.458	1.109	0.505
S9	219	3	0.541	0.784	0.714
S10	707	7	0.449	1.170	0.601
S11	442	5	0.478	0.987	0.613
S12	448	8	0.348	1.282	0.616
S13	625	7	0.385	1.135	0.583
S14	419	8	0.329	1.342	0.645
S15	2337	6	0.516	0.913	0.510
S16	849	10	0.363	1.305	0.567
S17	161	5	0.348	1.244	0.773
S18	198	7	0.246	1.484	0.762
S19	460	10	0.291	1.494	0.649
S20	98	6	0.808	0.443	0.247
S21	1179	12	0.407	1.167	0.470
S22	384	10	0.298	1.383	0.601
S23	1520	13	0.507	0.953	0.371
S24	1510	12	0.324	1.383	0.557

Table 3. Diversity indexes of the sampling sites. n = total individuals. S =

total taxa. D = dominance index. H' = Shannon index. J' = Pielou index.



Figure 6. Sample rarefaction curve of the species in the Perequê-açu river basin. The middle line represents the number of sampled species. Dotted lines represent the 95% bootstrap interval.



Figure 7. Principal Coordinates Analysis ordination plot, evidencing the composition and distribution of species along upper (red), middle (green) and lower (blue) streams of the Perequê-Açu river basin. List of species abbreviations is presented in Table 2.

downstream sites. Species richness and abundance tended to increase towards downstream: 12 species in upper streams (n = 1,944), 15 in middle (n = 5,262) and 18 in lower streams (n = 6,217). The PCoA segregated groups of streams according to their faunistic composition (Figure 7). Neoplecostomus paraty, Phalloceros harpagos, and Pareorhina rudolphi were associated with the upper portions of the basin. Species associated with intermediate portions of the basin, such as Kronichthys heylandi, Rhamdioglanis transfasciatus, and Bryconamericus ornaticeps inhabit both upper and lower portions of this drainage. Schizolecis guntheri and Phalloceros anisophallos, very strongly associated with lower portions of the basin. The analysis also grouped species of low representativity and occurrence in the central part of the graph, such as Gymnotus pantherinus, Pimelodella lateristriga, Rhamdia quelen, and Ancistrus multispinis. The first axis of the graph (PCoA 1 = 20.31%) showed a pattern of decreasing representativeness of siluriform species in the downstream direction, and an increase in the occurrence of Characiformes and Cyprinodontiformes.

SIMPER shows that for the upper portion of the basin (average dissimilarity = 26.1%), *K. heylandi*, *N. paraty*, *Trichomycterus* sp., and *P. harpagos* are the species that most contribute to differentiate these locations from others; in middle streams (average dissimilarity = 48.4%) *P. anisophallos*, *S. guntheri*, *B. ornaticeps*, *Trichomycterus* sp., and *R. transfasciatus*; and in lower sites (average similarity: 56.3%) *P. anisophallos*, *S. guntheri*, *K. heylandi*, *Geophagus brasiliensis*, and *Awaous tajasica* (Table 4). The highest species composition dissimilarity among groups of locations was between upstream and downstream sites, with an average difference of 80.2%.

Spacios	Groups						
species	Upstream	Middlestream	Downstream				
Average similarity (%)	26.1	48.4	56.3				
Kronichthys heylandi	26.5		12.8				
Neoplecostomus paraty	23.8						
Trichomycterus sp.	20.7	13.7					
Phalloceros harpagos	17.8						
Phalloceros anisophallos	3.8	23.3	34.2				
Shizolecis guntheri		18.0	26.6				
Bryconamericus ornaticeps		14.4					
Rhamdioglanis transfasciatus		12.1					
Geophagus brasiliensis			6.2				
Awaous tajasica			6.2				
		Groups					
	Up x Middle	Up x Down	Middle x Down				
Average dissimilarity (%)	70.5	80.2	57.2				

Table 4. Contribution (%) of the most relevant species to dissimilarity in each portion of the basin according to the SIMPER analysis.

Discussion

It is already well known (e.g. Lowe-McConnell 1999) that the two largest native orders of Otophysi (Characiformes and Siluriformes) from South America are predominant in all Brazilian rivers and streams and, in this case, it was not different. This time, however, since the basin is constituted mostly by mountain streams, which form very steep rapids, the Siluriformes were represented by three times more species than Characiformes, since they fit well in this type of habitat. Loricariidae holds the highest taxonomic diversity of species in the drainage, as their members prefer rapids in bedsides or marginal vegetation in less sloping terrain, recurrent habitats in coastal drainages of the Atlantic Rainforest (Casatti 2005). Among the 12 species of this family, Kronichthys heylandi had the widest distribution in the basin, present in 20 of the 24 sampled locations, from the lowest to the highest site. This was also one of the most abundant species identified by Ferreira and collaborators (2019) in the Itanhaém river, although it was classified as a strongly rhithronic species, that is, more common in the high course of streams. Buck & Sazima (1995) report that the species is generalist, presenting a wide variety of eating habits and environments where they can live, clinging to rocks, submerged woods, plants and foraging algae, insect larvae, crustaceans and macrophytes throughout the day, which may explain the presence of this taxon at almost all sites of the drainage.

The most abundant species of the family Loricariidae and the order Siluriformes in the samplings was *Schizolecis guntheri*. This species is widely distributed in coastal basins from the north of Santa Catarina to Rio de Janeiro, adapting to several types of environment with shallow waters and abundant marginal vegetation, regardless of the speed of the water current (Buck & Sazima 1995, Bizerril & Primo 2001, Kusma 2013). The latter author reported an interesting type of sexual dimorphism that only occurs in the populations of *Schizolecis guntheri* in the hydrographic region of Ilha Grande Bay: in other locations females and males of *S. guntheri* have the same color and body size, but in Ilha Grande Bay the males have a bulkier body with many dark spots, as it could be attested in the specimens collected in the present study. Both *Kronichthys heylandi* and *Schizolecis guntheri* were also very abundant species in the area studied by Esteves & Lobón-Cerviá (2001), but the latter showed greater abundance in the rainy season; in Perequê, it was abundant throughout the year, mainly downstream, also differing from the findings of Ferreira et al. (2019). Also in this family, *Hemipsilichthys nimius* has a narrow distribution, as the other two species of this genus (Buckup 2011): it is endemic to this region, occurring only in the Perequê-Açu and the Taquari river basins, also in Paraty (Pereira et al. 2003, Pereira 2005), where they are relatively common in rapids in middle streams.

The constancy of occurrence analysis revealed that most species in the basin are resident and non-migratory, including when the analysis was applied for each sub-basin. Regarding the constancy of occurrence in the five sub-basins, the one with the highest percentage of rare species was Perequê-Açu, in its final stretch. This result has two reasons: first, it is due to the low representativeness of species that are normally found in the upper and middle reaches of the basin, such as Bryconamericus ornaticeps (Figure 2C), Rhamdioglanis transfasciatus (Figure 2F) and Rhamdia quelen (Figure 2E). Second, as it is the lowest part of the basin, closer to the ocean, it houses peripheral freshwater species like Awaous tajasica and Eleotris pisonis (Medeiros et al. 2019), rarer in our samples. Mugil liza, a marine species, also contributed to this highest rarity value, being found on only one occasion. Vieira (2000) remembers that this index does not consider the abundance of species, only the presence; so, the sampling effort should be distributed throughout all day long (Azevedo-Santos et al. 2019), and also seasonally, to ensure the capture of all species from a site. Alone or associated with the frequency of occurrence, this index can offer an indication of the distribution of species in a given location.

The rarefaction curve indicates that the maximum number of species in the basin may not have been reached, as the curve has not reached an asymptote. This result is in line with the values of species estimators, which also revealed that there is a possible variation in the richness of taxa in the basin. However, the results of both analyses estimate that we were close to the "real" number of species. Possibly, if we had collected in more stretches of the low course of the river, we would have captured species that are commonly obtained in these lowland areas. São-Thiago (1990), in an unpublished dissertation, mentions that she collected almost the same number of species that we collected in Perequê: 22, 13 of which apparently were also collected in the Perequê basin; to be sure, it would be necessary to re-examine the material collected by the author, to update some identifications.

Two species of *Phalloceros* occur in allopatry in this basin, with a clear preference of *P. harpagos* (Figure 3R) for headwaters and bedside environments, and *P. anisophallos* (Figure 3S, 3T), the most abundant species in the basin, which is a very tolerant species (Mazzoni et al. 2011), found in lowland environments with or without marginal vegetation. *P. harpagos* was one of the most abundant species in headwater streams from Paranapanema basin (Frota et al. 2020) or in São José dos Dourados and Turvo-Grande basins (Casatti et al. 2012) where, despite the large area collected, only one species of this genus was recorded.

The low representativeness of Gymnotiformes, otherwise, may happen mainly due to the habits of the species. Like other parts of the Brazilian Crystalline Complex, Serra do Mar has about 50% granitoids and 30% gneisses (Guerra et al. 2013); thus, the riverbed is sandy and shallow, with the bottom formed by large slabs of stones. This type of habitat does not favor the formation of marginal sites and, allied to the slope of the terrain, the water speed does not seem to be adequate to provide a good habitat for species like *Gymnotus pantherinus*. Nocturnal fishes, during the day they are used to shelter in cracks or holes in clayey or sandy ravines, under stones and submerged vegetation (Crampton & Albert 2006), places of low occurrence in this basin. The only specimen was found in a small, first-order stream on a flat stretch rich in silt; this stream was receiving remnants of construction works, which contributed to its silting up and possibly to the local extinction of the species.

Following the pattern presented in other drainages (e.g. Corrêa et al. 2015, Silva et al. 2016), the species richness increased in the upstreamdownstream direction, which is related to the increased diversity and complexity of microhabitats. The rise in richness values due to the increase in the types of environments is in line with what was expected by the River Continuum Concept (Vannote et al. 1980). According to Garutti (1988), as they have a smaller volume of water, the headwaters of the streams do not support a large number of individuals and do not favor the emergence of varied microhabitats, which influences species richness. Modifications in the fish assemblages following the longitudinal gradient of a river are expected, as a result of evolutionary adaptations of each species (Silva et al. 2016).

In this way, PERMANOVA, PCoA, and SIMPER results indicate that the composition of the fishes along the catchment is not homogenous. In the upper reaches of the basin, the substrate of rocks, fast-flowing water in rapids areas, and moderate riparian vegetation seem to be favorable to the greater abundances of Loricariidae species such as Neoplecostomus paraty, strongly associated with these environments (Cherobim et al. 2016). On the other hand, these slope headwater habitats limit the great majority of species, mainly Characiformes and Cyprinodontiformes. Middle and lower portions, exhibiting a greater availability of microhabitats, feeding resources, presence of sand and gravel, larger width and depth of watercourses, as well as the increase of marginal vegetation are capable of sustaining more species, especially those with greater abundance and versatility in the basin, such as P. anisophallos, B. ornaticeps, S. guntheri, and K. heylandi. The SIMPER result indicates that P. anisophallos and S. guntheri present an increasing contribution along the longitudinal gradient, being most prominent in the middle and lower reaches of the catchment, places with greater availability of detritus, allochthonous and autochthonous feeding resources (periphyton or animals; Buck & Sazima 1995, Mazzoni et al. 2010, Ceni & Barreto 2018).

The strong dissimilarity between upstream and downstream sites in the catchment is a result of the differences between their fauna and the contribution of each species within a group of streams. However, it is worth mentioning that while species such as *Hemipsilichthys nimius* and *Neoplecostomus paraty* are abundant in median portions of the basin, their frequencies of occurrence in these locations are lower than those of species such as *Bryconamericus ornaticeps* and *Rhamdioglanis transfasciatus*. Clarke (1993) then suggests that species that do not have a consistent abundance within a group are not good indicators for clusters of sites. Thus, drawing a parallel to the results of the analysis of constancy of occurrence, SIMPER deemed relevant for the dissimilarities only constant species, excluding accessory and rare species (even if they present higher abundance). For downstream sites, while *Awaous tajasica* showed lower abundance than other species in the same group of sites, it stands out because it occurs with more consistency than others.

All three works dealing with the conservation status of this fauna used the IUCN criteria (Mazzoni et al. 2000, ICMBio 2018 and IUCN 2019). Also, the data that is made available in the publication of ICMBio (2018) are the result of a large number of experts who analyzed each one of the Brazilian continental fish species, producing a detailed and comprehensive list. Most of the 23 species were considered as Least Concern (15); four were classified as are considered as nearly threatened (NT) and on two of them the researchers did not find enough data to apply the criteria (so, they were classified as Data Deficient); from the remaining two, one is recently described (Neoplecostomus paraty) and the last is not identified yet (Trichomycterus sp.). In the Rio de Janeiro survey (Mazzoni et al. 2000), Acentronichthys leptos, Kronichthys heylandi and Pareiorhina rudolphi are listed as Vulnerable (VU); in ICMBio (2018), these first two species are listed as Least Concern (LC). Globally (IUCN 2019), four species are categorized as Least Concern (LC, Acentronichthys leptos, Awaous tajasica, Eleotris pisonis, and Rhamdia quelen) and one as Data Deficient (DD, Mugil liza). The advice of ICMBio (2018) is that there should be an effort to expand research to include species about which we do not have sufficient knowledge. Although this seems like something that should be done immediately, in marine and freshwater fish there are 564 species listed as DD. It will take some time for each of these species to be included in a program that involves identifying populations for specimen collections and analysis of taxonomic, trophic and reproductive data, in addition to verifying how structural characters and physiological processes vary over time and in different spaces. There is no guarantee that no species will be lost in the process.

Only seven species are protected under the legislation of the National System of Conservation Units (SNUC), being found within the PNSB: Deuterodon intermedius, Kronichthys heylandi, Neoplecostomus paraty, Pareiohina rudolphi, Phalloceros harpagos, Trichogenes longipinnis, and Trichomycterus sp. Of these species, K. heylandi, N. paraty, and Trichomycterus sp. are also in the buffer zone of the park; the remaining 19 species are not included in any protected area. The river's banks in areas outside the park have already been or are being deforested now, giving way to urban advancement. This implies a loss of habitats outside PNSB and its buffer zone that is expected to simplify the structure of communities (Casatti et al. 2012, Miranda 2012, Teresa & Casatti 2012). This denotes the importance of studies that not only list species or seek to understand how the fish assemblages are organized, but address their conservation status for the improvement of future species lists and implementing practical management actions.

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Author Contributions

Felipe Vieira Guimarães: designed and wrote the manuscript, went to fieldwork, and discussed the results.

Rosana Souza-Lima: designed and wrote the manuscript, went to fieldwork and discussed the results.

Tiago Medeiros de Souza: went to fieldwork and discussed the results.

Richard Rangel Rodrigues: went to fieldwork and discussed the results.

Conflicts of Interest

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

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First record of *Moenkhausia costae* (Steindachner 1907) in the Paraíba do Norte basin after the São Francisco River diversion

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Abstract: Construction of water diversions in drylands is boosted by increasing demands for freshwater often due to prolonged droughts. Even though these mega-enterprises result in benefits to society, it also poses a threat to freshwater biodiversity. In Northeastern Brazil, for instance, the São Francisco River Integration Project already supplies water for millions of people, but over time it will also favor the introductions of multiple aquatic species in the river basins of the Northeastern Caatinga and Coastal Drainages ecoregion. These introductions can cause unprecedented impacts in the native ichthyofauna, such as homogenization of freshwater faunas, transmission of pathogens and loss of native species. This study compares the composition and relative frequency of fish species from Poções reservoir using data obtained by gillnetting and trawling before and after the São Francisco diversion in the dry and rainy seasons, and reports the first detection of *Moenkhausia costae* introduction in the Paraíba do Norte basin, through the São Francisco River channel. Our results show some evidences that *M. costae* may become dominant and invasive in Poções reservoir. The introduction of *M. costae* adds a new component of disruption for these freshwaters and may pose a serious threat to the endemic ichthyofauna in lentic and lotic systems from the Paraíba do Norte basin.

Keywords: drylands; reservoirs; water transfer; non-native species; water shortage.

Primeiro registro de *Moenkhausia costae* (Steindachner 1907) na bacia da Paraíba do Norte após a transposição do rio São Francisco

Resumo: Obras para transposição de rios em regiões secas do mundo têm sido impulsionadas pelo aumento da demanda por água doce, muitas vezes associadas às secas prolongadas que são intrínsecas a essas regiões. Embora tais megaempreendimentos possam trazer benefícios para a sociedade, também representam uma ameaça para a biodiversidade aquática. No Nordeste do Brasil, por exemplo, o Projeto de Integração do Rio São Francisco já fornece água para milhões de pessoas, mas com o tempo também contribuirá com a introdução de várias espécies aquáticas nas bacias hidrográficas da ecorregião Nordeste da Caatinga e Drenagem Costeira. Essas introduções podem causar impactos sem precedentes, tais como homogeneização da ictiofauna nativa, transmissão de patógenos e perda de espécies nativas. Este estudo compara a composição e frequência relativa de espécies de peixes do açude Poções usando dados coletados com redes de espera e de arrasto antes e depois da transposição do rio São Francisco, nos períodos seco e chuvoso, e relata o primeiro caso de introdução de *Moenkhausia costae* na Bacia do Rio Paraíba do Norte, através do canal do rio São Francisco. Nossos resultados mostram que *M. costae* poderá se tornar dominante e invasora no açude Poções. A introdução de *M. costae* adiciona um novo componente de perturbação para esse açude e pode representar uma séria ameaça à ictiofauna endêmica de sistemas lênticos e lóticos da bacia do rio Paraíba do Norte.

Palavras-chave: regiões secas; reservatórios; transposição; espécies exóticas; escassez de água.

Introduction

Moenkhausia costae (Steindachner 1907) is a small omnivorous characin fish (Characiformes: Characidae), popularly known as 'piaba' or 'lambari', and in aquarium trade this species is known as 'Tetra-fortuna', which naturally occurs in the São Francisco and Itapicuru basins as well as in some other basins in the Northeastern Caatinga and Coastal Drainages ecoregion (Eigenmann 1917, Reis et al. 2003, Buckup et al. 2007, Lima et al. 2017, Silva et al. 2020). *Moenkhausia costae* is morphologically distinguished by a diagonal black stripe going from the beginning of the anal fin to the tip of the upper lobe of the caudal fin (Britzke 2011). It is a sedentary species that breeds multi-annually through parceled spawning preferably in lentic habitats (Godinho et al. 2010, Martins et al. 2012). Despite not being currently recognized as an invasive species, *M. costae* has already been introduced into rivers in the Paraíba do Sul basin in southeastern Brazil probably through the trade by aquarists and fish farmers (Magalhães et al. 2019).

Globalization is enhancing species introductions worldwide due to increasing international trade, tourism and transportation of raw materials among countries and continents (Jenkins 1996, Perrings et al. 2005). The introduction of species is one of the main drivers of biodiversity loss in the world (Baskin 2002, Vázquez & Aragón 2002, GISP 2005, Bellard et al. 2016). Currently, many countries have faced complex and costly issues resulting from invasive species (Bradshaw et al. 2016) and the socio-ecological damages can surpass \$100 billion dollars in countries like the United States, United Kingdom, Australia, South Africa and Brazil (CBD 2006).

Water shortage combined with rising water consumption and climate change have led worldwide governments to adopt increasingly radical solutions, such as mega-enterprises like river dammings and diversions that can affect the ecological services provided by these aquatic ecosystems (Marengo 2008). For example, in Northeastern Brazil, the São Francisco River Integration Project (SFRIP) has been developed to ease the effects of prolonged droughts (i.e. historically frequent in the region) and meet the water supply needs of part of the Brazilian semi-arid region (Lima 2005, Brazil 2004, Pittock et al. 2009, Andrade et al. 2011, Silva et al. 2020).

The SFRIP aims to divert water from the main and largest river of Northeastern Brazil (640 thousand km²) and the third largest in the country (Rosa et al. 2003) to the main river basins in the Northeastern Caatinga and Coastal Drainages (NCCD) ecoregion (Abell et al. 2018). However, this enterprise can affect the native biodiversity and speed up the process of biotic homogenization among basins (Daga et 2020). For instance, the SFRIP will initially supply intermittent basins of four important rivers in the Brazilian semi-arid such as Jaguaribe, Piranhas-Açu, Apodi-Mossoró and Paraíba do Norte (all from the NCCD ecoregion) (Ramos et al. 2018). However, a potential massive introduction of exotic species (e.g. parasites, invertebrates, algae and fish) can reach the NCCD ecoregion. In this context, on March 8 of 2017, the Paraíba do Norte river basin was the first to receive the waters from the São Francisco River. This paper reports the first case of Moenkhausia costae (Figure 1) introduction in the Paraíba do Norte basin, in Poções reservoir, through the São Francisco River East Axis channel. Moreover, this study compares the fish species assemblages before and after the São Francisco diversion into Poções reservoir, in a dry and in a rainy season, using data obtained by gillnetting and trawling, to assess the potential of M. costae becoming dominant and invasive in the reservoir,



Figure 1. Moenkhausia costae (UFPB 12069 - 50,55 mm SL).

reflecting what might happen in habitats that will indirectly receive the São Francisco waters from the Paraíba do Norte basin.

Material and Methods

1. Study Site

The study was carried out in Poções reservoir (Figure 2), located in the upper Paraíba do Norte River basin (7°53'38"S and 37°0'30"W) in the Monteiro municipality, Paraíba State, Northeastern Brazil. The reservoir has a maximum storage capacity of 29,861,562 m³ and is used for irrigation, public supply, leisure and fishing. The Paraíba do Norte River basin is the second largest in the Paraíba State, comprises 20,071.83 km², and it currently houses 52% of the state's population (AESA 2006, PARAÍBA 2007). The climate is semi-arid (according to the Köppen-Geiger climate classification) with a dry season that can last 10 months (AESA 2010). The annual mean temperature and annual mean precipitation in the Monteiro municipality are 23°C and 600 mm year, respectively (Fick & Hijmans, 2017). The reservoir margins are mostly vegetated by shrubs (26%) and grasslands (73%) and its littoral zones are generally vegetated by amphibian macrophytes from the genus Polygonum (visually dominant). In our study, the sediment in the littoral zones was mostly comprised by organic matter and clastic material (i.e., 44% of silt, 35% of sand and 19% of clay).

2. Sampling and analysis

Fish samplings were performed in a dry and in a rainy season before and after the São Francisco River diversion (Figure 3). Before the river diversion, samplings were carried out in July (rainy season) and in November 2016 (dry season). After the river diversion, samplings were carried out in July 2018 (rainy season) and in January 2020 (dry season).

Fishes were captured through standardized methods, applying two different fishing gears: (i) multi mesh gillnets and (ii) trawl nets. Two sets of gillnets (30 m long and 20, 25, 40, 50, 60, 100 and 120 mm meshes), were randomly placed at the littoral zone for at least four hours. The trawl nets (10 m long and 12 mm mesh) were dragged twice in three different regions of the littoral zone. Fish trawling and gillnetting were carried out in a littoral zone near the Poções reservoir dam (Figure 3). The sampled site was chosen based on sampling reports performed before the transfer of water from the São Francisco River to Poções reservoir.



Figure 2. Map of South America highlighting the Paraíba do Norte River Basin in Northeastern Brazil, the East Axis channel from the São Francisco River (in red), and the Poções reservoir (A). The white circle in "A" indicates the entrance of East Axis channel into Poções reservoir, whereas the black circle indicates the sampling site.



Figure 3. Sampled site in Poções reservoir in the (A) rainy and (B) dry seasons (Coordinates: 7°53'18"S and 37°59'58"W).

All specimens were anesthetized in the field with eugenol solution (Lucena et al. 2013) and then their morphological features were quantified. Next, the specimens were preserved in a 10% formaldehyde solution. At the laboratory, fishes were sorted, labeled and preserved in 75° GL alcohol according to Malabarba & Reis (1987). Specimens were taxonomically identified and were added to the Ichthyological Collection of the Department of Systematic and Ecology of Federal University of Paraíba (*Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba*) (CIUFPB), Brazil. Samplings were performed under the collection permit (N°56416-4/2019) from Chico Mendes Institute for Biodiversity and Conservation/Biodiversity Authorization and Information System (*Instituto Chico Mendes de Conservação da Biodiversidade/Sistema de Autorização e Informação em Biodiversidade – ICMBio/SISBIO*).

Meristic and morphometric data were gathered according to Hubbs & Lagler (2006), using caliper rule and stereomicroscope. Specimens were taxonomically identified following Ramos et al. (2018). *Moenkhausia costae* was identified according to Britzki (2011) genus key.

The relative frequency of species were calculated by using the following formula:

$$\%Spi = n\left(\frac{100}{N}\right)$$

Where %*Spi* is the relative frequency of the species, **N** is the total number of individuals of the species, and *n* is the total number of individuals of all species sampled. The calculations were separately performed for gillnets and trawl nets.

Results

1. Fish community structure before the river diversion

A total of 905 individuals and 15 species were caught before the river diversion in Poções reservoir. Of these, 805 individuals were caught by trawl nets and 100 individuals by gillnets. Five species were caught by trawl nets alone and two species, *Crenicichla brasiliensis* and *Poecilia vivipara*, were only caught by this method (Figure 4). Eight species were captured by gillnets and five species, *Astyanax bimaculatus*, *Hypostomus pusarum*, *Plagioscion squamosissimus*, *Coptodon rendalli* and *Cichlasoma orientale* were only caught by this method. Three non-native species, *P. squamosissimus*, *Oreochromis niloticus* and *C. rendalli*, were captured. However, only *O. niloticus* was captured by both trawl and gill nets (Figure 4).

Species composition was slightly different between seasons, but the differences observed were driven by three low abundant species, *Leporinus piau*, *Cichlasoma orientale* and *Crenicichla brasiliensis*, that were only captured in the dry period (Figure 4). When comparing the catches by gillnets and trawl nets, *O. niloticus, A. bimaculatus, P. vivipara, P. squamosissimus* and *H. pusarum* were the most common species in the reservoir and together they comprised more than 85% of the catches by numbers (Figure 4; Tables 1 and 2). *Oreochromis niloticus* was the first most common species captured by trawl nets in both dry and rainy periods and comprised more than 75% of all individuals captured. In the rainy season, *O. niloticus* was also the first most common species captured by gillnets, but in the dry season *A. bimaculatus* was the first most common species caught by the same method and comprised about 30% of all individuals (Figure 4).

2. Fish community structure after the river diversion

A total of 231 individuals and 11 species were caught after the river diversion in Poções reservoir. Of these, 138 individuals were caught by trawling and 93 individuals by gillnetting. Nine species were caught by trawl nets alone and two species (*P. vivipara* and *Hemigrammus* cf. *marginatus*) were only captured by this method (Figure 4). Nine species were also caught by gillnets and *Prochilodus brevis* and *C. brasiliensis* were only captured by this method. Two non-native species, *O. niloticus* and *M. costae*, were captured and both caught by trawl and gill nets, but only *O. niloticus* was captured before the river diversion

Species dominance changed substantially after the river diversion and between seasons. When comparing the catches by gillnets and trawl nets, *Steindachnerina* cf. *notonota*, *A. bimaculatus*, *M. costae* and *P. brevis* were the most common species in the reservoir and together they comprised more than 90% of the catches by numbers (Figure 4; Tables 1



Figure 4. Frequency distribution of the fish species found in Poções reservoir sampled with gill and trawl nets before and after the São Francisco River diversion. The non-native species *Moenkhausia costae* is highlighted in red bold italic.

Table 1. List of fish species found in Poções reservoir in the rainy and dry seasons, before (A) and after (B) the São Francisco River diversion, and sampled with gill nets. For each species it is shown the numbers of specimens captured (N) and its relative abundance (RA). The asterisk before the species names denotes they are non-native.

	(A) Before			(B) After				
	Rainy		Ι	Dry	Rainy]	Dry
	Ν	RA(%)	Ν	RA(%)	Ν	RA(%)	Ν	RA(%)
CHARACIFORMES								
Family Characidae								
Astyanax bimaculatus (Linnaeus, 1758)	0	0	0	0	8	10	26	45
Psalidodon fasciatus (Cuvier, 1819)	0	0	3	1	0	0	0	0
Hemigrammus cf. marginatus (Ellis, 1911)	0	0	0	0	0	0	1	1
*Moenkhausia costae (Steindachner, 1907)	0	0	0	0	2	3	17	29
Leporinus piau Fowler, 1941	0	0	5	9	4	14	0	0
Family Erythrinidae	-		-		-		-	
Hoplias malabaricus (Bloch, 1794)	3	7	4	7	0	0	1	1
CICHLIFORMES								
Family Cichlidae	-		-		-		-	
Cichlasoma orientale Kullander, 1983	0	0	1	1	0	0	1	1
Crenicichla brasiliensis (Ploeg, 1991)	0	0	0	0	0	0	4	6
*Oreochromis niloticus (Linnaeus, 1758)	17	40	7	12	1	3	0	0
*Coptodon rendalli (Boulenger, 1897)	2	5	9	16	0	0	0	0
SILURIFORMES								
Family Loricariidae	-		-		-		-	
Hypostomus pusarum (Starks, 1913)	7	16	14	25	0	0	0	0
PERCIFORMES								
Family Sciaenidae	-		-		-		-	
*Plagioscion squamosissimus (Heckel, 1840)	13	30	0	0	0	0	0	0
TOTAL	43		57		29		64	

and 2). In the rainy season, *S.* cf. *notonota* (45%), *A. bimaculatus* (24%) and *M. costae* (14%) were the three most common species captured by gillnets. A similar pattern was observed for trawl nets, but *M. costae* (3%) was the forth most common species caught (Figure 4; Tables 1 and 2). In the dry period, *P. brevis* (21%) and *M. costae* (19%) were the most common species captured by gillnets. For trawl nets, however, *A. bimaculatus* and *M. costae* were the most common species and together they comprised 74% of all individuals caught (Figure 4; Tables 1 and 2). *Prochilodus brevis, C. orientale, C. brasiliensis* and *Hemigrammus* cf. *marginatus* were only captured in the dry period and their relative frequencies were very low (Figure 4). Despite the non-native *M. costae* was only captured after the river diversion, it was interestingly among the top three dominant species in Poções reservoir irrespective of the sampling method used (Figure 4).

Discussion

This study reports the first detection of *Moenkhausia costae* in the Paraíba do Norte basin after the São Francisco River diversion. Furthermore, our results show evidence that this species might become dominant and with potential of dominance and invasiveness in other river basins from the Northeastern Caatinga and Coastal Drainages (NCCD) ecoregion. Moreover, we also showed that a single method used to capture fish species does not provide a representative of the whole-lake assemblage, as both gillnetting and trawling might miss important species that other methods capture.

Before the São Francisco River diversion, Ramos et al. (2018) investigated the ichthyofauna in the Paraíba do Norte River and reported 47 fish species corresponding to 38 genera, 20 families and six orders. The authors aimed to gather data before the river diversion to allow later detections of putative fish introductions from the São Francisco basin. Following this line, Costa et al. (2017) surveyed the ichthyofauna of two reservoirs from the same river basin. The authors registered 17 fish species, corresponding to 16 genera, 10 families and three orders. Conversely, M. costae was not found in none of the previous studies. However, one year later, the São Francisco waters reached Poções reservoirs and M. costae was already established. Moenkhausia costae and M. intermedia (Eigenman 1908) have already been reported in the NCCD ecoregion in the Apodi-Mossoró, Choró and Jaguaribe Rivers basins (Lima et al. 2017, Berbel-Filho et al. 2018). However, no individual from this genus had ever been registered in the Paraíba do Norte River basin before the São Francisco River diversion (Costa et al. 2017, Ramos et al. 2018).

Table 2. List of fish species found in Poções reservoir in the rainy and dry seasons, before (A) and after (B) the São Francisco River diversion, and sampled with trawl nets. For each species, it is shown the numbers of specimens captured (N) and its relative abundance (RA). The asterisk before the species names denotes they are non-native.

	(A) Before				(B) A	fter		
	Rainy		Ι	Dry	R	ainy	I	Dry
	Ν	RA(%)	Ν	RA(%)	Ν	RA(%)	Ν	RA(%)
CHARACIFORMES								
Family Characidae								
Astyanax bimaculatus (Linnaeus, 1758)	0	0	0	0	8	10	26	45
Psalidodon fasciatus (Cuvier, 1819)	0	0	3	1	0	0	0	0
Hemigrammus cf. marginatus (Ellis, 1911)	0	0	0	0	0	0	1	1
*Moenkhausia costae (Steindachner, 1907)	0	0	0	0	2	3	17	29
Family Curimatidae	-		-		-		-	
Steindachnerina cf. notonota (Miranda Ribeiro,	0	0	0	0	65	81	0	0
1937)								
Family Anostomidae	-		-		-		-	
Leporinus piau Fowler, 1941	0	0	5	1	4	5	0	0
Family Erythrinidae	-		-		-		-	
Hoplias malabaricus (Bloch, 1794)	0	0	4	1	1	1	0	0
CICHLIFORMES								
Family Cichlidae	-		-		-		-	
Cichlasoma orientale Kullander, 1983	0	0	0	0	0	0	5	9
Crenicichla brasiliensis (Ploeg, 1991)	0	0	1	0	0	0	0	0
*Oreochromis niloticus (Linnaeus, 1758)	153	81	457	73	0	0	4	7
CYPRINODONTIFORMES								
Family Poeciliidae	-		-		-		-	
Poecilia vivipara Bloch & Schneider 1801	37	19	145	24	0	0	5	9
TOTAL	190		615		80		58	

Environmental disturbances driven by hydrological changes may affect the structure of ecological systems and alter species composition and dominance (Daga et al. 2020, Yang et al. 2020). For instance, Astyanax bimaculatus is an endemic species in Central and South America that is usually very abundant both in brackish and freshwaters (Lima et al. 2003, Buckup et al. 2007). Costa et al. (2017) reported that A. bimaculatus was dominant in Acauã e Boqueirão reservoirs, both inserted in the Paraíba do Norte basin. In our study, conversely, O. niloticus and P. vivipara were two of the three most abundant species before the São Francisco diversion, both in the dry and the rainy seasons. Their abundances dropped drastically after the river diversion, but this pattern was stronger for O. niloticus (Figure 4). This drastic reduction in O. niloticus abundances may be related to the fact that all aquaculture tanks for tilapia farming were removed from Poções reservoir just before the river diversion. Morever, the number of individuals of A. bimaculatus captured in our study was lower than that of M. costae (Figure 4 and Table 1). Da Luz & Okada (1999) draw the attention to the fact that Moenkausia and Astyanax species usually share resources and coexist, since they both have the same feeding strategy (omnivores with a tendency to insectivory). A similar pattern of coexistence between

these two species was observed in our study, but further studies are needed to assess how these species interact in freshwater ecosystems.

Our first sampling in Poções reservoir, after the river diversion, resulted in relatively low abundances of *M. costae*, with only six specimens captured representing 5.1% of the samples. After one and half year, M. costae had already become the top three most abundant species recorded in the reservoir and overcame O. niloticus and P. vivipara reaching about 19% and 29% of the total number of individuals captured by gill and trawl nets, respectively. These results show that M. costae has managed to establish in the new habitat, which suggests that this species can become invasive with potential to affect other systems from the NCCD ecoregion that will also receive the waters from the São Francisco River. Furthermore, among the three cascading reservoirs (Poções > Camalaú > Boqueirão) that received the waters from the São Francisco River, M. costae was only reported so far in Poções reservoir. Camalaú and Boqueirão reservoirs are inserted in the Paraíba do Norte basin and are located downstream the East Axis channel, so they are the most likely routes of dispersion of *M. costae* within this basin.

In the Paraíba do Norte basin, *Apareiodon davisi* Fowler 1941 and *Parotocinclus spilosoma* (Fowler 1941) might be the main native species affected by limnological changes and by the introduction of non-native species such as *M. costae. Apareiodon davisi* is endemic to the NCCD ecoregion and is classified as endangered according to the Official National List of Threatened Faunistic Species - Fishes and Aquatic Invertebrates (Brasil 2014) and *Parotocinclus spilosoma* is endemic to the Paraíba do Norte River basin (Ramos et al. 2018). Despite the lack of studies on life history and behaviour of these two species, it is important to draw attention to them given the degree of threat they have been historically facing in the basin and now with the São Francisco River diversion. Thus, these threatened species are under the risk of having their populations reduced or even eliminated due to the presence of non-native species coming from the São Francisco River and continuous environmental changes in the basin.

Most ecoregions involved in the São Francisco River Integration Project are located in the Caatinga biome. The freshwater ecosystems from this biome have been suffering with multiple and chronicle impacts mostly driven by anthropogenic activities, such as riparian deforestation, erosion, eutrophication, introduction of exotic species, salinization, water abstraction and rainfall reduction (Jeppesen et al. 2015, Menezes et al. 2018) and this biome is currently estimated to be 50% degraded (Leal et al. 2005, Lima et al. 2017). This scenario is commonly seen in a large number of river basins in the Caatinga biome, so the introduction of *M. costae* adds a new component of disruption for these freshwaters and poses a threat to the native ichthyofauna in the Paraíba do Norte River basin. Furthermore, the natural condition of low biodiversity and high degree of endemism of Caatinga, highlight the need for attention to the freshwater ichthyofauna and a better system of monitoring and control of the aquatic species that may establish in the Northeastern Caatinga and Coastal Drainages ecoregion.

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Author Contributions

Telton Pedro Anselmo Ramos: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; data collection contribution to manuscript preparation;

Silvia Yasmin Lustosa Costa: Substantial contribution in the concept and design of the study; data collection; contribution to critical revision.

Railla Maria Oliveira Lima: Contribution to data analysis and interpretation; data collection

José Etham de Lucena Barbosa: Contribution to data analysis and interpretation; substantial contribution in the design of the study;

Rosemberg Fernandes de Menezes: Contribution to data analysis and interpretation; substantial contribution in the design of the study; manuscript preparation; data collection; contribution to critical revision, adding intellectual content. The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethics

We was complied with the guidelines established by the ethics committees.

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Severe coral bleaching of *Siderastrea stellata* at the only atoll in the South Atlantic driven by sequential Marine Heatwaves

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Abstract: Threatened by global warming and extreme climatic events, such as El Niño Southern Oscillation (ENSO) and Marine Heatwaves (MHW), coral reefs worldwide faced the worst bleaching and mortality event between 2014 and 2017, induced by the 2015/2016 ENSO. We evaluated the impacts of ENSO and MHW episodes on bleaching and mortality frequencies of *Siderastrea stellata* at Rocas Atoll, Southwestern Atlantic, using visual censuses conducted in 2016, 2017 and 2019. Bleaching rate varied significantly along the sampling period (11.71% in 2016, 1.52% in 2017, and 88% in 2019), but mortality was always less than 4%. Bleaching events in Atlantic reefs have been constantly associated with ENSO, until these recent events of the last two years. We suggest that MHW were probably the primary driver of the observed bleaching, especially in 2019, when much higher bleaching rates were observed than in ENSO periods. Although Southwestern Atlantic massive corals are considered more resistant to thermal stress than reefs corals worldwide, the strong events registered since 2019 highlight the need for continuous monitoring to better understand coral bleaching dynamics and improve predictions on the effects of global change in the region. *Keywords: Climate change; coral mortality; El Niño Southern Oscillation (ENSO); Brazilian reefs; Rocas Atoll.*

Branqueamento severo de *Siderastrea stellata* no único atol do Atlântico Sul impulsionado por ondas de calor marinhas sequenciais

Resumo: Ameaçados pelo aquecimento global e eventos climáticos extremos, como El Niño Oscilação Sul (ENSO) e Ondas de Calor Marinhas (MHW), os recifes de coral em todo o mundo enfrentaram o pior evento de branqueamento e mortalidade entre 2014 e 2017, induzido pelo ENSO 2015/2016. Nesse estudo, avaliamos os impactos dos episódios de ENSO e MHW nas frequências de branqueamento e mortalidade de *Siderastrea stellata* no Atol de Rocas, Atlântico Sudoeste, a partir de censos visuais realizados em 2016, 2017 e 2019. O branqueamento variou significativamente ao longo do período de amostragem (11,71% em 2016, 1,52% em 2017, e 88% em 2019), mas a mortalidade não, sendo sempre inferior a 4%. Eventos de branqueamento em recifes do Atlântico têm sido constantemente associados ao ENSO, até os eventos recentes dos últimos dois anos. Nós sugerimos que as MHW foram provavelmente o principal impulsionador do branqueamento observado, especialmente em 2019, quando as taxas de branqueamento observadas foram maiores do que nos períodos de ENSO. Embora os corais massivos do Atlântico Sudoeste sejam considerados mais resistentes ao estresse térmico quando comparados com corais recifais de outros oceanos, os fortes eventos registrados desde 2019 destacam a necessidade de monitoramento contínuo para entender melhor a dinâmica do branqueamento de corais e melhorar as previsões sobre os efeitos das mudanças globais na região.

Palavras-chave: Mudanças climáticas; Mortalidade de corais; El Niño (ENSO); Recifes brasileiros; Atol das Rocas.

Introduction

Coral reefs are among the most diverse and economically important environments on the planet, as they harbor most of the marine biodiversity and provide a large number of ecosystem services (Graham & Nash 2013). Consequently, these systems face a number of local and global anthropogenic impacts, such as overfishing, pollution, habitat destruction, introduction of exotic species, and ocean acidification (Hughes et al. 2003). However, global warming is undisputedly the biggest threat to coral reefs worldwide, especially taking into account that global climate models predict an average increase in sea surface temperature (SST) of 0.027 °C per year from 1990 to 2090 (Bopp et al. 2013). The major negative effect caused by the increase in SST on coral reefs is the disruption of the symbiotic relationships between corals and dinoflagellates of the family Symbiodiniaceae, in a phenomenon known as "coral bleaching" (Glynn 1993). In this relationship, microalgae can provide most of the energy requirements of the coral host (Muscatine et al. 1984), and its disruption damages the physiology and energy budget of the coral (Lesser 2011). If prolonged, this disruption may lead to high levels of coral mortality (Berkelmans et al. 2004, Oliver et al. 2009, Eakin et al. 2019). Since the twentieth century, coral bleaching has been reported in scattered localities and was related to small-scale stressors such as sedimentation, freshwater supply, and colder or hotter than normal weather (Vaughan 1914, Glynn 1993, Hughes et al. 2003). However, after the 1980s coral bleaching became more frequent worldwide (Hughes et al. 2017), with three major global scale coral bleaching events: 1997-1998, 2010 and 2014-2017 (Eakin et al. 2019).

The average annual global temperatures of the ocean and atmosphere of 2015, 2016, and 2017 were the highest ever recorded since the 1800s (Hughes et al. 2017). During 2015/2016 a strong and prolonged ENSO (El Niño Southern Oscillation) raised the global mean SST by 0.5 °C, and remarkably, 2017 was the warmest non-El Niño year ever registered (Eakin et al. 2019). This severe warm period sparked the most intense massive bleaching of corals worldwide and became known as the third global coral bleaching event (GCBE) (Hughes et al. 2018, Eakin et al. 2019). During this event, the highest incidence of coral bleaching and mortality in the Atlantic was recorded in the Caribbean (Hughes et al. 2017). In Brazil, some reefs showed up to 73% bleaching for some coral species, but mortality was overall lower than 3% (Teixeira et al. 2019). In addition, Southwestern Atlantic reefs faced a record-breaking heatwave event in 2019, which, together with the ENSO positive phase, increased SST and triggered mass coral bleaching (Banha et al. 2019, Duarte et al. 2020). These bleaching episodes have been reported in the Southwestern Atlantic reefs since 1993 (Migotto 1997), but while most of them have been related to ENSO (Kelmo & Atrill 2013, Ferreira et al. 2013, Miranda et al. 2013, Dias & Gondim 2016, Leão et al. 2016, Lisboa et al. 2018), there is still no consensus about the main drivers generating recent thermal anomalies that trigger coral bleaching in the region (Soares et. al. 2019).

Besides ENSO, another climatic factor that has impacted the Brazilian marine ecosystem are the Marine Heatwaves – MHW – (Rodrigues et al. 2019), which probability of occurrence has already increased more than 20 times due to global warming (Laufkötter et al. 2020). Defined as a period of at least five days wherein the temperature is above the 90th percentile of the values historically observed for a given location and at a certain time of the year, the MHW are extreme climatic events in oceanic systems (Hobday et al. 2016). The impacts of MHW on marine ecosystems reported so far include, for example,

mass mortality of seabirds in the Northeast Pacific (Jones et al. 2018), biomass decrease, and shifts in the distribution of fish stocks (Cheung & Frölicher 2020). However, the relationship between MHW and coral bleaching has been only recently reported (Fordyce et al. 2019, Smale et al. 2019), with MHW promoting immediate mortality and microbial biofilm formation over dead coral skeletons (Leggat et al. 2019). In view of all the impacts to coral reefs, studies that evaluate the effects of thermal anomalies on coral communities, whether by ENSO or MHW, are key to understand the dynamics of coral bleaching and to predict these impacts (Hughes et al. 2017). This is especially critical in the Southwestern Atlantic, where bleaching studies are still scarce, endemism in reef corals is high (Leão et al. 2016) and unprecedented thermal stresses have been reported (Rodrigues et al. 2019). Thus, we assessed the frequency of bleaching and mortality of the coral Siderastrea stellata Verrill, 1868 at the Rocas Atoll from 2016 to 2019, and evaluated its relation with the 2015/2016 ENSO and other climatic events, such as MHW. Rocas is potentially one of the most "pristine" areas of the Southwest Atlantic, and figures as a natural laboratory to understand the effect of thermal stress on corals, since it is not directly exposed to other anthropogenic impacts, such as pollution, urbanization and fishing (Longo et al. 2015).

Material and Methods

1. Study area

Located 230 km off the northeastern coast of Brazil, Rocas Atoll (03°50'S, 33°49'W, Figure 1a) is the only atoll in the South Atlantic, and is the first no-entry marine protected area in Brazil, created in 1979. The biological reserve encompasses about 360 km² of strictly protected area managed for research, where fishing activities and tourism are not allowed, leading to low human impact (Kikuchi & Leão 1997, Soares 2018). The reef substrate is mainly formed by coralline algae, vermetid gastropods and encrusting foraminifera (Kikuchi & Leão 1997), but seven species of scleractinian corals and one hydrocoral occur there (Leão et al. 2016). The atoll is subjected to intense wave action in comparison to coastal systems and is characterized by a semi-diurnal and mesotidal regime (Kikuchi & Leão 1997). During the low tide, two main habitats can be distinguished, open pools that constantly communicate with the exterior of the atoll and are more exposed to wave action than closed pools, which remain completely isolated from the exterior area of the atoll during low tides (Figure 1b). The benthic community in Rocas is dominated by the abundant reef-building coral Siderastrea stellata (Echeverria et al. 1997, Longo et al. 2015). This species is highly tolerant to thermal stress and widely distributed and common on Brazilian coastal reefs (Leão et al. 2016, Garcia et al. 2017).

2. Data collection

We conducted the sampling in four tide pools (Cemitério, Tartarugas, Âncoras and Falsa Barreta; Figure 1b) during low tides in May 2016 and 2017, and in June 2019. In each pool, we performed five visual censuses along 20 x 1 m ($20m^2$) belt transects, at depths up to 5 m, to estimate the frequency of bleaching and dead corals, according to ReBentos Protocol (Leão et al. 2015). All colonies of *S. stellata* with more than 3 cm in diameter within the transect area were visually counted (Nmin = 1533; Nmax = 1791) and classified into three categories: (1) no bleaching = with no sign of affected color, (2) bleached



Figure 1. A) Geographic location of the Rocas Atoll and B) Position of study sites, at the tide pools: Falsa Barreta, Cemitério, Salão, Tartarugas, and Âncoras. Adapted from Longo et al. (2015)

= lighter in color than normal, with different degrees of discoloration, including patches of bleaching and whiteness, and (3) recently dead
= bare skeleton without living tissue and minimal algal overgrowth.

3. Abiotic factors

To examine the effect of thermal stress events on S. stellata bleaching and mortality, we compiled a set of ocean temperature data during the sampling period at Rocas Atoll, and analyzed the coral's response considering these thermal stresses in the four months prior to the surveys. This 4 month-period was previously indicated as the time of response seen in Rocas Atoll corals (Ferreira et al. 2013). We collected the daily mean SST between 2016 and 2019 from the US National Oceanic and Atmospheric Administration -Advanced Very High Resolution Radiometer (NOAA - AVHRR) (http://www. esrl.noaa.gov/psd/). Then, we obtained the Monthly Multivariate ENSO Index (MEI) values (Wolter & Timlin 1993) from NOAA's Earth System Research Laboratory (esrl.noaa.gov/psd/enso/mei) from January 2015 to December 2019 to evaluate the effect of ENSO phases on the bleaching observed at Rocas Atoll. Considering the SST and MHW, we built an event curve between 2016 and 2019 with the Marine Heatwave Tracker, which uses the daily Optimally Interpolated Sea Surface Temperature (OISST) from the NOAA, available at http://www.marineheatwaves.org/tracker.html, (Schlegel 2018). To assess cumulative intensity, we obtained the maximum degree heating week (DHW) value during each time interval of 4 months before sampling, from NOAA Coral Reef Watch (CRW's), available at https://coralreefwatch. noaa.gov/product/vs/gauges/fernando de noronha.php. These values, based on CRW's Regional Virtual Station time series data, reflect the observed and forecasted bleaching alert level surrounding Rocas Atoll.

4. Data analysis

To examine whether the bleaching and mortality frequencies varied among 2016, 2017 and 2019, we performed two Kruskal-Wallis tests. We transformed the bleaching and mortality frequencies using the arcsine square root transformation before the analyses. We used this approach because bleaching and mortality data are percentages with a non-normal distribution, thus precluding the use of parametric tests. Additionally, we used Dunn tests for testing the difference observed in Kruskal-Wallis tests. All the analyses were performed with the R software (R Core Team 2018).

Results and Discussion

We observed a significant difference in the bleaching frequency among years (Kruskal-Wallis test: $X^2 = 47.7$, df = 2, p-value < 0.01), in contrast to the mortality frequency (Kruskal-Wallis test: $X^2 = 0.51$, df = 2, p-value = 0.77; Figure 2). During all sampled years, bleaching episodes were underway, which may underestimate mortality. In 2016, average bleaching (11.71% ± SD 13.81) and mortality (0.97% ± SD 2.39) frequencies at Rocas Atoll (Figure 2b and 2c, respectively) were small compared to the severe global trend observed during the 2015/2016 ENSO (Hughes et al. 2017). This pattern was also observed in another less abundant coral species (e.g. *Favia gravida, Montastraea cavernosa* and *Porites astreoides*,), which presented few signs of bleaching during this year (authors pers. obs.). In 2017, average bleaching frequency (1.52% ± SD 1.89) was almost eight times smaller than 2016 (Dunn test: z = 2.30, p-value < 0.01; Figure 2b), but the frequency of dead colonies was three times higher (3.2% ± SD 6.6), despite no significant difference (Figure 2c).

Average bleaching frequency in 2019 was 88.01% (\pm SD 8.97), almost sixty times higher than 2016 (Dunn test: z = -4.68, p-value < 0.01) and 2017 (Dunn test: z = -6.82, p-value < 0.01) (Figure 2b). Unlike the upward trend in bleaching frequency, the number of dead colonies has halved (1.31% \pm SD 4.12) when comparing 2017 to 2019, despite the lack of statistically significant difference (Figure 2c). Although 2019 was a non-El Niño year, the most severe bleaching event to date at the Southwestern Atlantic was recorded at that time, with high bleaching but low coral mortality (Banha et al. 2019, Duarte et al. 2020, Mies et al. 2020), similar to Rocas Atoll. The bleaching history at Rocas Atoll coincides with the ENSO years, such as the 2003 event that caused bleaching in less than 4% of the colonies studied by Ferreira et al. 2006, while during ENSO 2010, less than 20% of the colonies bleached, but up to 60% showed signs of disease (Ferreira et al. 2013).

In 2016, thermal stress events in the four months preceding the survey reached a maximum SST of 29.5 °C and a high frequency of positive thermal anomalies that did not exceed 1 °C (Figure 3a), with a maximum DHW value less than 1 °C-week. In the same period, there were three moderate MHW (Figure 3b) with an average duration of 6.6 days and average intensities, maximum and cumulative averages of 0.85 °C, 1.06 °C and 5.67 °C, respectively. Therefore, coral bleaching at Rocas Atoll in 2016 may not have been triggered only by ENSO (Figure 3c), but also by MHW that occurred before the sampling period. Soares et al. (2019) highlighted a bleaching event in the Southwestern Atlantic triggered by anomalous SST in 2010 that was not correlated with the ENSO, but with lower wind speeds and water turbidity.

In 2017, the daily average SST during the four months prior to the survey was similar to that observed in 2016, with maximum DHW value less than 1 ° C-week, and a maximum SST of 29.5 °C (Figure 3a), characterizing a hot year even without ENSO (Figure 3c). However, during this period a lower frequency of positive thermal anomalies was observed, with two episodes reaching 1 °C (Figure 3a). Two moderate MHW occurred before sampling in 2017, with an average duration of 12 days, mean accumulated intensity of 10.57 °C, average intensity of 0.89 °C, and maximum average of 1.04 °C. The MHW events during the pre-sampling period in 2017 were less frequent and more interspaced, despite being longer, than the pre-sampling period in 2016 (Figure 3b), which may have influenced the low bleaching frequency in 2017.

In 2019, SST reached a maximum of 30.0 °C, and presented higher frequency and intensity (1.5 °C) of positive anomalies than in 2016, with more thermal anomalies than 2017 (Figure 3a). The maximum DHW value in the period was higher than previous years, reaching 7.5 ° C-week. Two MHW were registered in 2019, with an average duration of 31.5 days and



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Figure 2. A) Coral communities at Rocas Atoll over sampling period (2016, 2017 and 2019), at the sampled areas. Note a few bleaching colonies of *Siderastrea stellata* in 2016, and several bleached colonies in 2019, while 2017, colonies were mostly healthy. B) Bleaching frequency of *Siderastrea stellata* at Rocas Atoll, as a function of sampling years: 2016, 2017 and 2019. Box plot center = median, outerlimit = 75% percentile, and range is shown by the whiskers. In each case, data from N=20 belt transects are shown. C) Mortality frequency of *Siderastrea stellata* at Rocas Atoll, as a function of sampling years: 2016, 2017 and 2019. Box plot center = median, outerlimit = 75% percentile, and range is shown by the whiskers. In each case, data from N=20 belt transects are shown. C) Mortality frequency of *Siderastrea stellata* at Rocas Atoll, as a function of sampling years: 2016, 2017 and 2019. Box plot center = median, outerlimit = 75% percentile, and range is shown by the whiskers. In each case, data from N=20 belt center = median, outerlimit = 75% percentile, and range is shown by the whiskers. In each case, data from N=20 belt center = median, outerlimit = 75% percentile, and range is shown by the whiskers. In each case, data from N=20 belt transects are shown

a cumulative intensity of 31.51 °C, both three times higher than in previous years (Figure 3b). The intensity and maximum averages of MHW were similar to those of previous years: 1.02 °C and 1.46 °C, respectively. A much lower ENSO index was detected in 2019 compared to 2016 (Figure 3c). Regarding the three sampling years, 2019 reached the highest SST, presented larger and more frequent thermal anomalies, as well as more intense MHW, which may explain the severe bleaching frequency (i.e. >50% of bleached colonies, according to Donner et al. 2017) in 2019 (Figure 2a and 2b).

Bleaching events may have distinct outcomes, from full recovery to mass mortality, depending on the intensity and duration of the environmental stress (Glynn 1996, Baker et al. 2008), as well as the overall health state of the ecosystem (Hoegh-Guldberg 1999). Thermal stress in the South Atlantic has historically been lower than in other regions such as the Caribbean and the Indo-Pacific (Skirving et al. 2019), and its reefs have escaped multiple thermal stress events which have plagued reefs elsewhere. Despite the massive coral loss observed around the world, Southwestern Atlantic reefs have remained relatively stable in terms of mortality (Perry et al. 2013, Banha et al. 2019, Teixeira et al. 2019). Between 2014 and 2017, when the most severe, widespread, and longest-lasting global-scale coral bleaching event was recorded (Eakin et al. 2017, 2019), Abrolhos reef, in Brazil, suffered less than 3% of coral cover loss due to bleaching and mortality (Teixeira et al. 2019).

Coral species from the Southwestern Atlantic, including *S. stellata*, have been considered highly resistant to thermal stress, which is possibly related to associations with thermotolerant endosymbionts (Marshall &

nts which have
observed around1997). In addition, Rocas Atoll is isolated from the mainland and is the most
effective marine protected area in Brazil, lacking local stressors (Brandão
et al. 2017) that could act in concert with global drivers that promote coral
bleaching. On the other hand, Rocas Atoll is a shallow and non-turbid reef,
therefore more susceptible to bleaching due to thermal stress than coastal
ones (Glynn 1996, Takahashi et al. 2004).widespread, and
recorded (Eakin
ess than 3% of
ra et al. 2019).This scenario may have started to change from 2019, when the incidence
of severe MHW in some reefs, such as at Rocas Atoll, Abrolhos coral reefs
and São Paulo rocky reefs, triggered the highest bleaching events registered
so far in the Southwestern Atlantic (Banha et al. 2019, Duarte et al. 2020).Impacts of MHW on coral reefs have been reported in Australia (Le Nohaïc

Baird 2000, Loya et al. 2001, Costa et al. 2004, 2008). Colonies of S. stellata

from the Northeastern Brazilian reefs harbors mainly symbionts from the

genus Cladocopium (Costa et al. 2008, Monteiro et al. 2013), a genus

whose lineages most frequently found on corals are thermotolerant (Swain

et al. 2017). Coral colony morphology is also often related to bleaching

susceptibility, with massive forms less susceptible to bleaching (Brown et

al. 1990, Gleason 1993). Most Brazilian coral species, such as S. stellata,

have a massive growth form (Leão et al. 2003), that is also associated with a higher thermal stress tolerance (Loya et al. 2001, Schlöder & D'Croz 2004) in

comparison to branching corals (Brown et al. 1990, Glynn 1996, Smith et al.

2014). These traits of Brazilian coral may drive the higher resistance during

thermal stress events registered so far, especially for S. stellata at Rocas Atoll,

due to its massive morphology, possible thermal tolerant symbiont diversity

(Costa et al. 2008) inhabiting shallow and warm tide pools (Echeverria et al.



Figure 3. Temporal thermal series. A) Daily mean values of sea surface temperature (red line) and thermal anomaly (blue line) reported for Rocas Atoll. Numbers (1-3) within a small square indicate the sampling moment in each year and the big dashed square shows the four months prior to the survey, when thermal stresses were analyzed. The x-axis represents time, with monthly intervals, from January 2016 to June 2019. The main y-axis represents the SST (°C) and the secondary the anomaly (°C). Data extracted from the satellite (AVHRR) and made available by NOAA (http://www.esrl.noaa.gov/psd/). B) Marine Heatwaves at Rocas Atoll, between 01/01/2016 and 05/31/2019. The red dotted line is the threshold value for each location for each day of the year and is defined based on the 90th percentile value. A thermal stress event that is at least five days or more above this threshold value represents a MHW (orange areas). The lightest orange areas represent moderate MHW, and the darkest strong events. The dashed blue line represents the climatological mean. Numbers (1-3) within a small square indicate the sampling moment in each year. The x-axis represents time, with monthly intervals, and the y-axis represents the SST (°C). Available at http://www.marineheatwaves.org/tracker.html. C) Multivariate ENSO indices (y-axis) from 1979 to 2020 (x-axis). El Niño (positive phase) in red; La Niña (negative phase) in blue. Numbers (1-3) within a small white square next to the arrows indicate the sampling years (1 = 2016; 2 = 2017; 3 = 2019). Data source: (esrl.noaa.gov/psd/enso/mei)

et al. 2017, Clarke et al. 2019) and some Pacific islands, with different coral species being affected (Rubio-Portillo et al. 2016, Falter et al. 2016, Couch et al. 2017). Unlike large spatial and temporal scale ENSO events, MHW represents the most extreme and "rare" incidences of thermal stress relative to a seasonally dependent historical baseline (Hobday et al. 2016). However, similarly to ENSO, the duration and frequency of MHW has increased significantly since the early twentieth century (Oliver et al. 2018, Laufkötter et al. 2020). It is worth mentioning that the effects of MHW on coral reefs are distinct from how coral bleaching has been understood to date, resulting

in an immediate heat-induced coral mortality, rapid dissolution of the coral skeleton, and loss of the three-dimensional reef structure (Leggat et al. 2019).

This work highlights the need to re-think our understanding of coral bleaching events in Southwestern Atlantic reefs, its drivers and the immediate impact on corals, especially in Brazilian reefs whose responses to thermal stress are still poorly understood (Mies et al. 2020). Maintaining time series for monitoring coral population parameters and environmental drivers is crucial for understanding coral bleaching phenomena in the Southwestern Atlantic and better addressing the coral reef crisis. Rocas Atoll is one of the most effective marine protected areas in Brazil, with minimal local anthropogenic impacts and may be used as a natural model system for evaluating global impacts on its reef community. Indeed, a better understanding of what are the main drivers of coral bleaching in Southwestern Atlantic can be useful to improve predictions and anticipate impacts on Brazilian reefs related to global change.

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Author Contributions

Tainá Gaspar: substantial contribution to the concept and design of the study; contribution to the data collection; contribution to the data analysis and interpretation; contribution to manuscript preparation.

Juan Quimbayo: substantial contribution to the concept and design of the study; contribution to the data collection; contribution to the data analysis and interpretation; contribution to manuscript preparation.

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Conflicts of Interest

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

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Composition and diversity of phytophilous cladocerans of oxbow lakes of Southwest Amazonian, Acre state, Brazil

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Abstract: The objective of this study was to inventory the community of cladocerans associated with macrophytes in oxbow lakes of the Chandless River, located within the Chandless State Park, during the local dry season. In three lakes covered with macrophytes, 90 sample units were collected, arranged over nine transects. Twenty species of cladocerans were found, which 16 are new records for the state of Acre. Mascote Lake had the largest abundance and richness, while São João Lake had the lowest indexes. The Shannon-Wiener index points to an average diversity within the work area, probably due to the difference in richness and abundance between the lakes. *Keywords: Cladocera; Freshwater; Macrophytes; Species Inventory*.

Composição e diversidade de cladóceros fitófilos de lagos de meandro do sudoeste da Amazônia, estado do Acre, Brasil

Resumo: O objetivo desse estudo foi inventariar a comunidade de cladóceros associados às macrófitas aquáticas em três lagos de meandro do rio Chandless, dentro do Parque Estadual Chandless, no período da estação seca local. Em três lagos com cobertura de macrófitas foram amostradas noventa unidades amostrais, dispostas ao longo de nove transectos. Foram encontradas vinte espécies de cladóceros, sendo que dessas dezesseis são novos registros para o estado do Acre. O lago Mascote foi o que apresentou maior abundância e riqueza, enquanto o lago São João foi o que apresentou as menores. O índice de Shannon-Wiener aponta para uma diversidade dentro de valores locais conhecidos, provavelmente por conta da diferença da riqueza e da abundância entre os lagos. **Palavras-chave:** Cladocera; Água Doce; Macrófitas; Inventário De Espécies.

Introdution

Tropical varzea Amazon ecosystems undergo regular changes in water level, whose fluctuation can exceed 10 meters (Correa & Winemiller 2014, Junk et al. 2014). These flood pulses are reflected in all local fauna and flora, even those of oxbow lakes, which are formed due to the process of sedimentation and erosion of the river beds (Junk et al. 2014, Esteves, 1998). To aquatic plants, the flood pulse promotes an homogenizer effect (Thomaz et al. 2007). These local flood pulses are extremely important for the local diversity of cladocerans (Guntzel et al. 2010), including those that interact with aquatic plants, the phytophilous cladocerans.

Aquatic plants confer important habitat heterogeneity in aquatic environments, and the unique spatial structure of plants is important for the growth and survival of the other aquatic organisms (Dibble & Thomaz 2006, Suçuarana et al. 2016), such as phytophilous cladocerans. The high ecological plasticity of the macrophytes make these plants potential colonizers of a huge variety of aquatic ecosystems (Pompêo 2008). In contrast, several taxa of cladocerans are specialized in colonizing these environments, presenting evolutionary adaptations for this way of life (Fryer 1995).

The scarcity of data on phytophilous cladocerans makes it difficult to understand the relationship of this group with macrophytes, although more than two-thirds of the known cladocerans are associated with this group of plants (Elmoor-Loureiro 2000). However, Forró (2008) estimated that the fauna of freshwater cladocerans is two to four times richer than what is currently known, which indicates that this number may be even higher. The author also pointed out that half of the most common species in Brazil are from typically coastal families. In general, the cladocerans reach their largest diversity in lentic environments (Rocha et al. 2011), because they present better conditions for this type of organism, specially when we consider that most of the species are not effective swimmers (Bolduc et al. 2020).

Another contribution to the scarcity of phytophilous cladocerans data is the frequency of most studies in the limnetic zone. Ghidini et al. (2017) focused his study on the cladocerans associated with *Eichhornia azurea* (Pontederiaceae) in the central Amazon and already warned about the scarcity of similar studies in the region. Despite this, there is a recent effort to increase knowledge about the group (eg Guntzel et al. 2010, Souza et al. 2013, Ghidini et al. 2017). However, the group continues to be scarcely sampled in the Amazon.

When analyzing the species of cladocerans registered for Acre state, we found only 17 records. These records were made in studies that covered zooplankton as a whole group (Sendacz & Melo-Costa 1991, Keppeler & Hardy 2002, Keppeler 2003a, Keppeler 2003b, Keppeler & Hardy 2004, Oliveira et al. 2010, Silva et al. 2012, Santos et al. 2013, Silva et al. 2014, Nascimento & Keppeler 2017a, Nascimento & Keppeler 2017b), but most of them were focused on rotifers. One species of the Eurycercidae family, one species of Ilyocryptidae, two species of Bosminidae, two species of Moinidae, three species of Sididae, four species of Chydoridae and four species of Daphnidae are registered. However, it is worth pointing out that almost all of these records came from studies carried out in the limnetic zone, which probably resulted in a species composition quite different from what could be collected in the coastal zone.

Studying phytophilous cladocerans becomes relevant, especially because of their great bioindicator potential, and a greater understanding of this group should help in understanding their contribution to the stability and maintenance of aquatic systems (De Eyto et al. 2002). In addition to the aforementioned little knowledge of the group, the importance of these studies is reinforced when it is considered that the diversity of cladocerans increases as the available vegetation abounds to be used as their ecological niches (Bolduc et al. 2020). Therefore, the objective of this study was to evaluate the composition and abundance of cladocerans associated with macrophyte beds in oxbow lakes in the southwestern Amazon and make new records for the group in the state of Acre. We theorize that local diversity would be high, given the seemingly favorable conditions for this.

Material and Methods

1. Study area

This study was carried out in three oxbow lakes from the Chandless River, located in the municipality of Manoel Urbano-AC, within the geographical limits of the Chandless State Park. Chandless State Park (Parque Estadual Chandless- PEC) is an Integral Protection Conservation Unit (IPCU) and it was created by decree 10.670, of September 2, 2004, with 695,303 ha, which also cover part of the municipalities of Sena Madureira and Santa Rosa do Purus (Acre 2010).

Located in the center-south of the state of Acre (Figure 1), bordering the Brazilian border with Peru, the Chandless State Park is the largest conservation unit in the state, with an open rainforest as dominant vegetation, in addition to the dominance of bamboos (Mielke et al. 2010), which is typical of the region. The local climate at PEC is hot and humid, with an average temperature above 18°C (Acre 2010). A monsoon climate is a main characteristic of the region, with a moderate dry season and an average monthly rainfall of less than 60 mm (SEMA 2010). In this part of the state, the dry season runs from June to September, considering the previous and subsequent months as transition months (Acre 2012).



Figure 1. Map indicating the location of Chandless State Park (Parque Estadual Chandless- PEC) and the lakes available for the study (Buião lake, Mascote lake and São João lake).

According to the Sioli classification (1984), the lakes, created by the river as well, have white waters, that are subjected to the annual flood pulse of the river, which carries nutrients into the lakes. They vary in length, but all are at least 1,000 meters long. The study was carried out only in
the dry season (July 2019), due to the reduction of lake area that occurs at that time, increasing the chances of finding individuals in abundance.

The region has lakes along the Chandless River, at the north end of the park. Of these lakes, only three were able to carry out the study, due to the size of the macrophyte beds. Still, the beds had different successional stages on the lakes.

2. Data collection and analysis

In each lake, three transects of 200 m were sampled, two of them at the lakes ends and the third at the center of the lake. Within each transect, ten sample units were made, with a distance of 20 m between them. In each sample unit, 50 cm X 50 cm, two samples of cladocerans were collected, a quantitative sample and a qualitative sample. The quantitative samples were taken with the aid of bucket and filtered through a 100 μ m mesh zooplankton conical net, 100 L of water in total was filtered to sample the macrophytes. For qualitative samples, macrophytes were collected inside the sample unit with the D grid, also with 100 μ m mesh. The macrophytes and collected water were poured into a polyethylene container, where the macrophytes were washed and returned. The resulting water was also filtered through the 100 μ m conical zooplankton mesh.

All samples were labeled and stored in 100 mL bottles. The collected micro-crustaceans were anesthetized with carbonated water (25 mL) and subsequently fixed in alcohol (final concentration at 70%), according to the methodology of Pinto-Coelho (2004). Analyses of the samples of phytophilous cladocerans were set at the Limnology Laboratory of the Federal University of Acre - UFAC, with the aid of an optical microscope. Due to the low number of organisms found in the pilot collection, the samples were analyzed in their entirety. The identification keys of Smirnov (1971), Smirnov (1976), Korovchinsky (1992) and Orlova-Bienkowskaja (2001) were used.

A multiparameter probe (model Akron AK87) estimated the temperature, pH, dissolved oxygen and electrical conductivity, while turbidity was determined with an Instrutherm turbidimeter and depth and transparency were measured using a Secchi disk. These measurements were carried out close to the macrophyte beds of the transect to be collected in at a standardized time, totaling nine collections of physicalchemical parameters. In addition, we checked the macrophyte species composition to provide an improved differentiate of the lakes.

The importance of limnological variables for cladoceran community occurrence and abundance was quantified through a Redundancy Analysis (RDA), preceded by standardization (Legendre & Legendre 2012). Limnological variables were standardized (method: standardization) with mean = 0 and standard deviation = 1. The abundance data were also transformed by Hellinger (Legendre & Gallagher 2001) before the analysis, as this procedure produces precise estimates of the percentage of variation explained by the predictor variables (Peres-Neto et al. 2006), reproducing a species matrix (Hellinger) as a function of abiotic variables. The significance of the influence was obtained from 999 permutations, defining the significance for the axes together (Legendre & Legendre 2012). To determine whether the variables were significantly correlated with each other, Pearson's correlation was performed. If there was a correlation between them, one of the correlated variables would be discarded.

The frequency of occurrence and the Dajoz Index (Dajoz 1973) was used to classify the cladocerans species as accidental (occurrence below 25%), accessory (occurrence between 25 to 50%) and constant (occurrence in more than 50% of the samples), calculated from the

formula $c = p \ge 100/P$, where p is the number of samples containing the species and P is the total number of samples. For the analysis of diversity of phytophilous cladocerans, we used the Shannon-Wiener index, whose formula is where pi is the relative abundance of each species and Inpi is the Neperian logarithm of relative abundance.

The analysis were performed with the aid of the Vegan (Oksanen et al. 2013) and Psych (Revelle 2020) packages in the RStudio software (R Core Team, 2020), considering the 5% significance level.

Results

Regarding macrophytes, the three lakes showed dominance of different species, despite having the common characteristic of the abundance of macrophytes. At Buião Lake, which had well-established beds all through its surface, in addition to some loose beds, the species Oxycaryum cubense (Poepp. & Kunth) Palla and Salvinia minima Baker predominated. In Mascote Lake, the predominant species were S. minima and Ricciocarpus natans (L.) Corda, which are pioneer species in the colonization of macrophytes beds; moreover, the beds were small and always on the margins of the lake. Finally, at São João Lake there was no longer any surface water free of macrophytes. The dominant species were Pistia stratiotes L and Utricularia gibba L. The water lettuce (P. stratiotes) organisms almost completely cover the water surface, and the few vacant spaces are filled by U. gibba individuals, who intertwine among the roots of water lettuce, forming a continuous network, making navigation within the lake difficult, which shows an advanced stage of colonization of macrophytes in the lake.

Besides the difference in the composition of the species of macrophytes in the lakes, their dissimilarity in limnological variables was also notable (see Table 1). Buião Lake is the deepest ($\overline{X} = 1.65$ m deep in the vicinity of the macrophyte beds), the least turbid and with the largest transparency (\overline{X} = 7.08 UNT and 55 cm, respectively) and the most alkaline, evidenced by means of DO and pH (\overline{X} DO = 9.93 and \overline{X} pH = 7.28, respectively). Mascote Lake is shallower ($\overline{X} = 0.58$ m) and most acidic, with an average of 26.3°C, 4.63 DO mg L⁻¹, 66.3 µS cm⁻¹ of electrical conductivity and a pH of 6.75. São João Lake also showed a tendency toward acidity, despite presenting the lowest average temperature ($\overline{X} = 24.2$ °C), which can be explained by the total vegetation cover of the lake.

During the investigation we found 2,529 individuals of cladocerans among the macrophytes, among them were 20 species, 16 genera and six families (Chydoridae, Daphnidae, Ilyocryptidae, Macrothricidae, Moinidae and Sididae). The Chydoridae family was the most represented in variety (12 species) and abundance (1,641 individuals). The species *Ephemeroporus hybridus* Daday, 1905 was the most abundant species, with 1,109 individuals, while the most frequent species was *Oxyurella ciliata* Bergamin, 1939, which occurred in 44 of the 90 sample units. Among all the registered species, only four of them (*Alonella dadayi*, *Diaphanosoma spinulosum, Ovalona glabra* and *Simocephalus latirostris*) had previous records for the state of Acre, thus 15 new records were established for the state (Table 2).

The Macrothricidae family was the second most representative, with three species, followed by the Sididae family, with two species. The families Daphnidae, Ilyocryptidae and Moinidae registered only one species and that was in the Buião Lake. In addition to *D. spinulosum*, two other taxa occurred strictly in one lake. The species *Leydigiopsis*

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Lakes	Depth (m)	Tran (m)	Temp (°C)	DO (mg L ⁻¹)	EC (μS cm ⁻¹)	pН	Turb (UNT)
Buião T1	1.20	0.60	25.8	13.6	133.1	7.3	6.67
Buião T2	1.97	0.45	25.3	6.7	137.8	7.3	6.28
Buião T3	1.80	0.60	25.3	9.5	137.1	7.3	8.29
Mascote T1	0.55	0.15	27.6	6.0	104.8	6.7	13.03
Mascote T2	0.45	0.15	25.6	2.3	48.3	6.8	17.00
Mascote T3	0.75	0.45	25.6	5.6	45.7	6.8	16.04
São João T1	0.50	0.30	24.1	5.8	139.7	6.7	19.31
São João T2	1.50	0.50	24.1	1.2	132.8	6.8	17.55
São João T3	0.15	0.15	24.3	7.3	146.8	6.8	16.55

Table 1. Limnological variables measured in the lakes. T1 (Transect one); T2 (Transect two); T3 (Transect three); Dep (Depth); Tran (Transparency); Temp (Temperature); DO (Dissolved oxygen); EC (Electrical conductivity); Turb (Turbidity).

Table 2. Taxon registered in each studied oxbow lakes with their abundance and species richness. Species with (*) are new records for the Acre state

	Lakes			
Taxon	Buião	Mascote	São João	
Chydoridae				
*Acroperus tupinamba Sinev & Elmoor-Loureiro, 2010	16	82	4	
Alonella dadayi Birge, 1910	2	12	1	
*Anthalona verrucosa (Sars, 1901)	6	33	4	
*Chydorus eurynotus Sars, 1901		43	3	
*Chydorus pubescens Sars, 1901	1	3		
*Coronatella cf. monacantha (Sars, 1901)	3	10		
*Ephemeroporus hybridus(Sars,1901)		1106	3	
Kurzia polyspina Hudec, 2000	44	9		
*Leydigiopsis curvirostris Sars, 1901		6		
*Leydigiopsis ornata Daday, 1905	14	61	22	
Ovalona glabra (Sars, 1901)		32		
*Oxyurella ciliata Bergamin, 1939	48	69	4	
Daphnidae				
Simocephalus latirostris Stingelin,1906	7	26		
Ilyocryptidae				
Ilyocryptus spinifer Herrick, 1882	16	176	1	
Macrothricidae				
*Macrothrix elegans Sars, 1901		5	5	
*Macrothrix paulensis (Sars, 1900)		15	4	
*Streblocerus pigmaeus Sars, 1901	2	53	14	
Moinidae				
*Moina micrura Kurz, 1874		9	7	
Sididae				
*Diaphanosoma birgei Korineck, 1981	32	510	5	
Diaphanosoma spinulosum Herbst, 1967	1			
Total	192	2260	77	

curvirostris and *O. glabra* occurred exclusively in Mascote Lake. São João Lake did not present an exclusive record.

Analyzing the rates of occurrence according to the Dajoz Index (Table 3), it is evident that Mascote Lake showed a higher frequency of occurrence in general. In this lake, four species were classified as accessory and four as constant, compared to three accessory and one constant in Buião Lake and only one constant in São João Lake, which did not present accessory species.

As for the influence of limnological variables, it was found that those that did not show correlation were conductivity, dissolved oxygen, temperature and turbidity. The variation in the community composition was significant (p = 0.024; $R^2 = 0.32$). The RDA ordered the collection sites according to the environmental variables, which allowed us to verify how the variables were related to the species composition (Figure 2), according to the direction of the axis.

The RDA results showed that approximately 66% of this observed variation can be explained by the environmental variables selected for the model. The first axis showed that 30.34% of the variation in total abundance is related to electrical conductivity, dissolved oxygen and turbidity, while the second axis indicates that temperature explains 20.41% of the variation in total species abundance.

The Buião lake transects have the largest amplitude of dissolved oxygen than the other lakes. The turbidity variable showed the largest amplitude in the São João lake transects. The species *Leydigiopsis ornata* presented a relationship with the electrical conductivity variable. The species *Oxyurella ciliata* and *Kurzia polyspina*, however, were related to dissolved oxygen. All species of the Macrothricidae family showed correlation with turbidity.

The Shannon-Wiener index pointed to a diversity of 1.89 for phytophilous cladocerans.

Discussion

The limnological variables of the lakes were within the average fluctuation described by Salimon et al. (2013) for the Purus basin. Despite the strong differences between the lentic environment of the lake and the lotic of the river, the averages found were very similar to that described for the dry season. Ríos-Villamizar et al. (2011) corroborates by adding data for the lakes, where the physical-chemical characteristics were compatible with those found, except for the average transparency (120 cm).

The abundance of phytophilous cladocerans was much larger in Mascote Lake than in the other two lakes. Therefore, it is deducible that this community prefers shallow, turbid waters with a tendency to acidity. When addressing the phytophilous zooplankton of the Paraná River, Chaparro et al. (2016) also found a tendency toward larger abundance in the community when the waters were warmer and with low electrical

Table 3. Absolute frequency and frequency of occurrence (F.O.) of species of cladocerans per lake. The frequencies of occurrence were classified according to the Dajoz Occurrence Index as accidental (until 25% occurrence, in white), accessory (from 25 to 50% occurrence, in light gray) and constant (more than 50% occurrence, in dark gray).

Family	Species	Buião	F.O.	Mascote	F.O.	São João	F.O.	Total
	*Acroperus tupinamba	7	23.3	6	20	3	10	16
	Alonella dadayi	1	3.3	8	26.6	1	3.3	10
	*Anthalona verrucosa	5	16.6	7	23.3	4	13.3	16
	*Chydorus eurynotus	0	0	10	33.3	2	6.6	12
	*Chydorus pubescens	1	3.3	3	10	0	0	4
Chydoridae	*Coronatella cf. monocantha	3	10	5	16.6	0	0	8
	*Ephemeroporus hybridus	0	0	20	66.6	2	6.6	22
	*Kurzia polyspina	11	36.6	4	13.3	0	0	15
	*Leydigiopsis curvirostris	0	0	4	13.3	0	0	4
	*Leydigiopsis ornata	9	30	16	53.3	18	60	43
	Ovalona glabra	0	0	7	23.3	0	0	7
	*Oxyurella ciliata	19	63.3	21	70	4	13.3	44
Daphnidae	Simocephalus latirostris	7	23.3	9	30	0	0	16
Ilyocryptidae	*Ilyocryptus spinifer	5	16.6	19	63.3	1	3.3	25
	*Macrothrix elegans	0	0	2	6.6	3	10	5
Macrothricidae	*Macrothrix paulensis	0	0	8	26.6	1	3.3	9
	*Streblocerus pigmaeus	2	6.6	9	30	5	16.6	16
Moinidae	*Moina micrura	7	23.3	0	0	3	10	10
Sididae	*Diaphanosoma birgei	13	43.3	24	80	3	10	40
	Diaphanosoma spinulosum	1	3.3	0	0	0	0	1



Figure 2. Biplot of the RDA showing the collection sites (LBU corresponding to Buião Lake, LMA corresponding to Mascote Lake, LSJ corresponding to São João Lake) and the relationship of the variables with the species.

conductivity, characteristics that tend to be similar to those found in Mascote Lake. Although the Buião Lake had a higher correlation with temperature by the RDA, the lake with the highest average temperature was Mascote (Buião \overline{X} = 25.5°C; Mascote \overline{X} = 26.3°C). Choedchim et al. (2017), when working on a lake, noticed that the shallower regions concentrated a larger abundance of phytophilous cladocerans, which were also the most acidic waters, thus corroborating with our theory.

Melão (1999) pointed to temperature as one of the two main factors for the development and reproduction of cladocerans (food being the second factor). According to the author, waters with higher temperatures positively influence the metabolism of cladocerans. These data describe an environment more similar to that found at Mascote Lake. Another factor that may also justify the predilection of cladocerans in this lake is the low biomass produced by the most common macrophyte species in the lake (*S. minima* and *R. natans*), which relates less with the reduction of oxygen levels by decomposition (Bianchini Jr. et al. 2008), since oxygenation is also an important factor for the greater abundance of phytophilous cladocerans (Takeda et al. 2003).

The RDA showed a preference of the Sididae, as well as the species *Simocephalus latirostris*, for higher temperatures. This trend had already been described by Zhao et al. (2020). Macrothricidae, however, seem to have a correlation with low levels of turbidity, which can complement the data of Guntzel et al. (2002), who found individuals of this family in abundance in high transparency lakes. The species *Moina minuta*, on the other hand, appears to have a correlation with lower levels of dissolved oxygen (DO), since it appeared at the base of the arrow of this variable in the RDA.

The Chydoridae family, being the largest in number of species in the study, did not present a common pattern of distribution. *Kurzia polyspina* and *Oxyurella ciliata* showed positive correlation with DO; in contrast, the species *Acroperus tupinamba* and *Coronatella* cf. *monacantha* was correlated with lower DO rates. Similarly, the species *Leydigiopsis ornata* showed a correlation with higher rates of electrical conductivity, in contrast to the species *Chydorus pubescens*, which demonstrated to prefer areas with low electrical conductivity.

The low number of species previously registered for the state of Acre corroborates the idea that the species composition differs largely from the limnetic zone to the littoral zone, even more if the collection in the latter is carried out among the aquatic vegetation, although the majority of the species found are not considered constant by the Dajoz Index. Furthermore, even if the other studies in the state have all been carried out in the limnetic zone, the sampling effort for cladocerans is still considerably low, since in a space of 26 years, only 11 studies on zooplankton (including cladocerans) have been published.

Despite being considered a benthic species, *Ilyocryptus spinifer* Herick, 1882 was a constant species in Mascote Lake. This probably happened because it is the shallowest of the three lakes with an average depth of 0.58 m in the transects. Another factor that may have contributed to the incidence of this species in the study is the abundance of the large grass *Panicum aquaticum* Poir., which was sometimes fixed or very close to being fixed to the substrate, which may have contributed to this high constancy. The same can be considered for the registration of species of the genus *Chydorus*, which are also considered benthic.

The Chydoridae, Macrothricidae and the species *Simocephalus latirostris* Stingelin, 1906 frequently appear in association with macrophytes (e.g., Guntzel et al. 2010 and Sousa et al. 2013) and are referred to as typically limnetic. However, in our study they were mostly found at an accessory frequency (except for *Ephemeroporus hybridus* and *Leydigiopsis ornata*). It is likely that these cladocerans were in the process of colonizing the macrophytes of the sampled lakes.

The species *Diaphanosoma birgei* Korineck, 1981 appeared as an accessory in Buião Lake and as a constant in Mascote Lake. This species is considered common (Santos-Wisniewski et al. 2011, Maia-Barbosa et al. 2014) and appears frequently among phytophilous cladocerans records (Sipaúba-Tavares & Dias 2014, Souza et al. 2017), possibly because of foraging among the macrophytes, since it is considered limnetic.

Although 14 of the 20 species are new records for the state of Acre, all of them have previous records for the Amazon. Santos et al. (2014) reports the occurrence of three species on the list for the Madeira River basin, in Rondônia. These are *Chydorus pubescens*, *D. birgei* and *I. spinifer*. More recently, Souza et al. (2019) included the species *Anthalona verrucosa*, *Chydorus eurynotus* and *Macrothrix elegans* among the records for the Madeira basin. Thus, the records of these species closest to the state of Acre were more than 700 km away.

For the other species considered new records, the closest records are from the state of Amazonas. Rocha et al. (2017) recorded the species *Oxyurella ciliata* and *L. ornata* in the Amazon river Basin, more than 1,200 km from the points collected in this study. The other taxa were previously registered in the Negro River basin, with Brandorff et al. (1982) those responsible for the registration of *Macrothrix paulensis*, Ghidini & Santos-Silva (2011) those responsible for the registration of *Streblocerus pigmaeus*, Ghidini et al. (2017) those responsible for the registration of *Acroperus tupinamba*, and finally Carvalho-Pereira et al. (2015) confirmed the presence of *Coronatella monacantha*, *E. hybridus*, *Kurzia polyspina*, *Leydigiopsis curvirostris* and *Moina micrura* in this basin.

The Shannon-Wiener Index pointed to an intermediate diversity (1.89), being higher than that found by Neves (2003), which was a diversity of 1.62. It was also shown to be higher than what was found by Zanatta et al. (2010), who found rates below 1.85. However, it is still lower than most values registered by Guntzel et al. (2010) and Simões & Sonoda (2009). It is worth noting that the work of Zanatta et al. (2010) and Simões & Sonoda (2009) are related to samplings in the limnetic zone. A possible explanation for this number is the advanced stage of colonization of the macrophytes of São João Lake, which had inferior richness and abundance than the other lakes. Lakes with an advanced stage of ecological succession of macrophytes, to the point that they

completely cover the water surface, hinder local oxygenation, which contributes to the low local diversity of cladocerans (Coutinho et al. 2017, Takeda et al. 2003).

In summary, the lakes vary in their physical-chemical characteristics and in the dominance of macrophyte species, which is sufficient to cause large differences in the abundance and composition of phytophilous cladoceran species, corroborating the idea of an environmental heterogeneity from one lake to the other. The RDA showed a preference of the Macrothricidae family for points of largest turbidity. The value found by the Shannon-Wiener diversity index is similar to that found in Brazilian vegetated aquatic environments, but below the value we had theorized. Finally, the maintenance of the preservation of the lakes in Chandless State Park is indicated in this study, so the largest diversity can be protected.

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Author Contrubutions

Guilherme Sampaio Cabral: Contribution in the concept of the study; data collection; data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

Ronaldo Souza da Silva: Contribution in data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

Ronnilda Maria Gonçalves Araujo: Contribution in data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

André Ricardo Ghidini: Contribution in the concept of the study; data collection; data analysis and interpretation; 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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Ecological and growth patterns of the longsnout seahorse *Hippocampus reidi* inferred by mark-recapture techniques in a tropical estuary

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Abstract: The population structure of the longsnout seahorse *Hippocampus reidi* (Ginsburg 1933) was evaluated in an estuarine system in northeastern Brazil. A total of 425 captures were performed in the Vaza-Barris estuary and 232 individuals were marked with Visible Implant Fluorescent Elastomers. The population was estimated to be composed of 428 fish. The von Bertalanffy growth curve shows a steeper growth during the first months of life, up to about 17 months, and stabilization after 16 cm and 27 months of age. The hereby study indicates that the Vaza-Barris estuary is home to resident populations of longsnout seahorses. Moreover, these populations use mangrove roots as the main substrate for attachment and present well-defined ecological characteristics, such as habitat fidelity and highly structured social organization.

Keywords: Fishes; population dynamics; life history; fluorescent elastomer.

Padrões ecológicos e de crescimento do cavalo-marinho *Hippocampus reidi* inferidos por técnicas de recaptura em um estuário tropical

Resumo: A estrutura populacional do cavalo-marinho *Hippocampus reidi* (Ginsburg 1933) foi avaliada em um sistema estuarino no nordeste do Brasil. Um total de 425 capturas foram realizadas no estuário de Vaza-Barris e 232 indivíduos foram marcados com Elastômeros Fluorescentes de Implante Visível. A população foi estimada em 428 peixes. A curva de crescimento de von Bertalanffy mostra um crescimento mais acentuado durante os primeiros meses de vida, até cerca de 17 meses, e estabilização após os 16 cm e 27 meses de idade. O presente estudo indica que o estuário Vaza-Barris é o lar de populações residentes de cavalos-marinhos de focinho comprido. Além disso, essas populações utilizam raízes de mangue como principal substrato de fixação e apresentam características ecológicas bem definidas, como fidelidade de habitat e organização social altamente estruturada. *Palavras-chave: Peixes; dinâmica populacional; historia de vida; elastômero fluorescente.*

Introduction

Human activity in the marine environment has caused a decline in the number of species worldwide. Overfishing, pollution, bioinvasions, climate change, and habitat loss pose serious threats to marine species worldwide (Lotze et al. 2006; Worm et al. 2006; Hughes et al. 2009). According to the International Union for Conservation of Nature red list (IUCN), from 17,228 fish species evaluated in 2019, a total of 2,341 are considered vulnerable, including 11 species of the family Syngnathidae (IUCN 2019). The three species of the genus Hippocampus in Brazil (H. erectus, H. patagonicus, and H. reidi) are also considered vulnerable in the list of Brazilian fauna species threatened with extinction (Portarias MMA nº 444/2014 e nº 445/2014). Hippocampus reidi (Ginsburg 1933), has been included in Appendix II of the National List of Species of Aquatic Invertebrates and Fish Overexploited or Threatened by Overexploitation since 2004, in accordance with Normative Instruction 05 (dated May 21, 2004) of the Brazilian Ministry of the Environment. Currently, this species is classified as near-threatened by the IUCN (Oliveira & Pollom 2017).

Hippocampus species are usually monogamous and form couples in nature (Lourie et al. 1999), with all having vital parental care (Foster & Vincent 2004). In addition, studies have shown that seahorses have high site fidelity (Perante et al. 2002), highly structured social behavior (Vincent & Sadler 1995), sparse distribution, low mobility, and low fecundity (Foster & Vincent 2004).

Despite being highly exploited, seahorses are not included in the official records of fisheries statistics in Brazil, and there are few papers aimed at understanding seahorse populations in their natural habitats (Rosa et al. 2007, Curtis et al. 2017, Siqueira et al. 2017) which ultimately helps to assess their conservation status. The seahorse Hippocampus reidi is directly exposed to anthropogenic threats along the Brazilian coast, but the species still figures as Data Deficient (DD). The lack of information has made it difficult to predict the real susceptibility of populations. On the other hand, some methodological tools have been recently developed and improved so that more reliable population monitoring data are generated. Within the scope of tools, the marking procedure with Visible Implant Fluorescent Elastomer (VIE) is noteworthy. It was developed to provide externally visible internal marks for fish and other aquatic animals. In view of the short application time biocompatible composition of the merker this type of marking is suitable for use on seahorses (Curtis 2006). As demonstrated by Woods & Martin-Smith (2004), implanting elastomer tags in seahorses does not alter their behaviour or growth, and eventhough these tags are considered permanent, they are unlikely to affect crypsis, as animals havebeen observed alive 4 years after initial tagging (Harasti et al. 2012). As these fish have low mobility, such markings with elastomer implants provide good responses from an ecological point of view, making it possible to monitor marked individuals in their natural environment.

The present study was developed to demonstrate the applicability of this marking-recapture method to determine the population structure and growth patterns of seahorses and to estimate population parameters in order to assess their conservation status.

Materials and Methods

The study region comprises the Vaza-Barris estuary, which is located to the south of the Aracaju city Sergipe, Brazil, within the Tropical Western Atlantic (Figure 1). This estuary is surrounded by several cities, including São Cristóvão, Pedreira, Colégio, Itaporanga d'Ajuda, and Aracaju. It is a tourist place under high real estate pressure due to the expansion of the cities. In this estuary, there are also several sources of anthropogenic contamination, such as the discharge of untreated urban sewage and effluents from agricultural, livestock, and shrimp farming activities (Vasco et al. 2010; Lima & Silva 2014). The samples were conducted at three sites, known as Viral Stream (11°07'33.9" S, 37°09'20.7" W), Caruara Stream (11°07'05.7" S, 37°09'16.6" W), and Baleia Stream (11°07'23.9" S, 37°10'22.3" W). Samples were collected monthly between September 2013 and May 2015, comprising twenty-one field observations. The number of transects varied among the sampling sites because the extent of the vegetated margin was different between them. So, three transects were carried out monthly in the Baleia Stream (62 transects in total), one transect in the Viral Stream (21 transects in total), and four transects in the Caruara Stream (84 transects in total). Transects of 50 m length were previously marked with the help of a measuring tape positioned near the bank. For monitoring purposes, the sampling sites were georeferenced with a GPS and transects were always carried out in the same places. To determine the geographical coordinates of each fish, a standard GPS was used.



Figure 1. Lower portion of the Vaza-Barris estuary, Sergipe State, Brazil, indicating collection sites.

Markings started in January 2014 after team training. The training was carried out in the Laboratório de Ictiologia Estuarina e Marinha (Universidade Federal de Sergipe) using captive seahorses, for safe and effective application of the method in field (Capture and Handling Permition Nr. 38844-2 – SISBIO/ICMBio/MMA).

At each sampling site, an observer in the water visually searched for seahorses near the margins, especially among vegetation roots, mostly of *Rhizophora mangle* (red mangrove). The average depth at the margins was approximately 1.5 meters and the width of the transect was approximately 1 meter. With visibility covering the entire water column, it was possible to view the fish even when they were fixed close to the bottom, with no need for snorkel or diving equipment for underwater viewing. The fish were caught manually, without the need for collection equipment.

Samples were preferably performed at low spring tides, when mangrove roots allowing better detection of the fish.

For each individual, the following data were recorded in the field form: location (GPS coordinates); sex; fish height (stretching the tail), tail and head length (in cm); width (greater lateral size, from the abdomen to the back of the fish, in cm); attachment substrate; and behavior (standing or swimming). The sizes were measured using a caliper, and measurements accuracy was 1 mm (Figure 2). Also, the formation and quantification of groups and probable couples were recorded. Regarding sex, were considered the following categories: (I) Undetermined, individuals with no visible sexual differentiation; (M) Male, presence of brood pouch, prominent or not; and (F) Female, absence of brood pouch and presence of evident egg-laying organ (Lourie et al. 2004).



Figure 2. Measures taken in H. reidi captured specimens.

In order to mark individuals, the biocompatible Fluorescent Elastomer (Northwest Marine Technology Inc.) implant was preferably injected into translucent fish tissues between the bony rings of the tail. Before proceeding with the in-situ marking, five specimens were held captive and monitored in the laboratory for the detection of possible responses to the implant (especially susceptibility to infection after application), thereby ensuring the effectiveness of the method. An alphanumeric coding was created, which, together with the specific color of the elastomer, allowed a rapid detection of the code related to the marked individual. For example, the code 7r8y9 indicates that the recaptured individual has a red marking between the seventh and eighth tail rings and a yellow marking between the eighth and ninth tail rings (Figure 3). The bone rings were counted starting from the anus of the fish, taking care (particularly in males) to not reach the brood pouch, thus avoiding lesions to this structure. In these cases, the implant was placed after the seventh or eighth bone ring of the tail. Considering the markings made in distinct body places and with different colors, the identification codes of the specimens were created and recorded in the field form. In this study, the smallest marked specimen had a body height of 6 cm. Individuals smaller than 6 cm were not marked in order to avoid injuries to the tail bone structure and physical damage to the animal due to their small size and fragility.



Figure 3. Seahorse marked using the visible elastomer implant method. Recaptured fish with green and pink markings on the tail.

The injections containing the markers were prepared in the field at the time of collection and preserved in ice to avoid curing and consequent hardening of the marker. A combination of two colors per field phase was used. Individuals monitored in the field were quickly returned to their habitat, taking the utmost care during handling. In the case of recapture, the data were compared with previous observations to determine patterns of occurrence, group formation, growth, and reproduction.

The obtained data were processed in Excel and Statistica 10 (Stat Soft®). Excel was used for the preliminary organization of the data and for the descriptive analysis of growth and fidelity to the partner. Statistica 10 (StatSoft, Corp.) was used to perform descriptive statistical analyses, frequency tables, graphs, and analytical procedures of data management, as well as analyzing variables. Results are presented as mean and SD.

For density calculation for each transect, the observations per fifty-meter transect performed at each collection site were used. In order to estimate population density, the Capture Calendar, which was popularized by Krebs (1966) as the Minimum Number Known to be Alive (NMA) and considers the catch history (1st catch + recaptures) to evaluate the population density, was used. According to this postulate, the history of catches of each individual is analyzed over a series of successive events (i) of capture. The estimate of Ni (in this case N represents the total number of individuals in the population) is given by the number of individuals captured at i plus the number of individuals not seen at i but captured before and after i. There are a number of methods for estimating Ni in a population closed by removal, of which Hayne's (modified from Leslie's) is one of the best currently known (Brower & Zar 1984, Le Cheminant 2000). In this analysis, the number of previously caught and marked animals is virtually removed, and a linear regression is performed between the number of animals captured each day (y-axis) and the number of previously captured animals (x-axis). The point where the line intercepts the abscissa axis provides the Ni, and it can be estimated by regression analysis without the need to remove all the individuals. The assumptions for Hayne's postulate, which are the condition of closed population and equal catchability among individuals, have been shown to be applicable to this study as it was observed that the populations of seahorses behave this way due to their ecological characteristics.

Applying this premise to the data, considering the Y-axis composed of the number of new individuals captured at each field phase and the X-axis corresponding to the cumulative total of individuals was observed. Thus, the linear regression equation is obtained, and when the value 0 is applied to Y, the projection of X is determined. According to Hayne's postulate, this value corresponds to the estimated total number of seahorses for each area studied. The estimation of the population size, generated from the method of calculation of density of Capture-Mark-Recapture, estimated the number of seahorses for each area studied.

The size-at-time data were also adjusted to the Von Bertalanffy growth model (VBGM), which expresses the height (H) as a function of the age (t) of the fish, through the equation: $H(t) = H_{\infty}^{*} [1 - \exp(t)]$ $(K^{*}(t-t0))$]. The asymptotic height (H₂) was calculated by the observed maximum height (H_{max}) following the Taylor (1958) equation, where $H_{\infty} = H_{max} / 0.95$. After the calculation of H_{∞} , the growth constant K was calculated by the method of Munro (1982), in which $K = [log (H_{\infty} - Hi)]$ $-\log (H_{\infty} - Hf)/(tf - ti)]$, being Hi the fish height observed at the time of its capture and Hf the height of the same at the time of its recapture. The variable tf - ti represents the temporal variation between the two capture moments. For VBGM, considering that t_0 is the theoretical age (years) at which the height is zero, a value of $t_0 = -0.04$ was used. This value represents the period of incubation of the brood that, despite being born with an average height of 0.6 cm, remain in the pouch for about 12 to 20 days (Silveira & Fontoura 2010), with an average incubation time of 16 days, or 0.04 years ($t_0 = -0.04$).

For spatial distribution analyses, the areas and sampling sites for fish habitat characterization were georeferenced and plotted on Google Maps.

Results

4

1. Abundance

A total of 425 observations were made, including counts and recounts, of which 232 were marked. With the gradual increase in the number of fish collected, and increase in the number of new markings, the proportion of new individuals collected relative to recaptured individuals, tended to gradually decrease. After the sixth sampling, the proportion between new individuals and individuals already marked was relatively balanced, remaining very similar in almost all of the following samplings, with recaptured fish representing on average 44.6% of the total observations (Figure 4).

From the analysis of the Capture Calendar, the site with the highest occurrence of *H. reidi* specimens was the Baleia Stream, presenting an estimated density of about 294 specimens. For Viral, it was obtained



Figure 4. Capture and recaptured longsnout seahorse individuals caught by month from September 2014 to May 2015 in the Vaza-Barris estuary.

an estimate of 69 *H. reidi*, while for Caruara, the total population density estimate was 65 specimens. Thus, it is estimated that the three sampling areas should house approximately 430 individuals of longsnout seahorses. Therefore, it is estimated that 55% of the seahorse population was marked during this study.

The density of *H. reidi* at the Viral stream ranged from 0 to 0.32 fish/m²; with an average of 0.113 fish/m² (\pm 0.088). At the Caruara stream, density ranged from 0 to 0.095 fish/m², with an average of 0.035 fish/m² (\pm 0.034). At the Riacho da Baleia stream, density ranged from 0 to 0.233 ind./m², with an average of 0.103 fish/m² (\pm 0.066).

2. Height and sex

In total, 251 fish were males, 195 were females, and 27 were very young individuals considered of undetermined sex. Of these, 279 (135 males, 123 females, and 21 undetermined) were found in the Baleia Stream, 105 (64 males, 38 females, and 3 undetermined) in the Viral Stream, and 89 (52 males, 34 females, and 3 undetermined) were found in the Caruara Stream. The samplings with the highest number of observations were October 2013 (53 individuals), September 2013 (45 individuals), April 2014 (35 individuals), and July 2014 (32 individuals).

Measurements on total fish height varied from 2.1 cm to 19.2 cm, with a mean 14.4 \pm 2.6 cm. The mean size of the females and males was very close (14.5 cm and 15 cm, respectively), and the largest difference was observed in the smallest individuals (7 cm and 10.2 cm, respectively, Table 1).

Table 1. Total number of captured fish (n), mean, minimum (Min), and maximum (Max) total length (cm) by sex of the longsnout seahorse *Hippocampus reidi* in the Vaza-Barris estuary.

Sex	n	Mean	Min	Max
Undetermined	27	6.9 ± 2.4	2.1	10.1
Females	195	14.5 ± 1.9	7	19
Males	251	15 ± 1.8	10.2	19.2

3. Habitat use

Regarding the 425 fish observed, 64% of individuals were found alone, 25% of individuals were found in pairs, and 11% were found in groups of three or more individuals. Most observations (94%), of the longsnout seahorses were standing or attached to some substrate, while only 6% were swimming. Of those found standing, almost 90% were attached to roots of *R. mangle*, while 10% were attached to branches of mangrove trees (6%), oysters (1%), or supported on the mud in the margins (4%).

4. Reproduction

For 85% ot individuals it was not possible to define the stage of the reproductive cycle when observed, while 7.4% were mature females, 7% were males at an advanced stage of incubation, and 0.6% were males that had recently released the brood, with one of these males being observed at the final moment of release of the brood. Mature individuals were found in almost all sampling months, with reproduction peaks occurring in February, July, and December 2014, when the proportions between fish in reproductive activity/total catch remained above 30%, while in the other months this ratio averaged 12%. In the months of November 2013, March and April 2015, no *H. reidi* were observed in reproduction activity.

Throughout the sampling period, the overall sex ratio (M:F) was 1.3. Regarding the sampling sites, the ratio was 1.7 in Viral, 1.5 in Caruara, and 1.1 in the Baleia Stream.

5. Growth patterns

The VGBM parameters obtained for 42 individuals (sex combined) were K = 0.78431 and L_{∞} = 20.21 cm. It is possible to observe in the curve that the largest individuals observed are ~48 months of age (Figure 5). The smallest individuals observed in reproduction were a 12 cm male and a female of 13.1 cm, indicating that in the study area the species starts to reproduce only after 15 months of age. The Von Bertalanffy growth curve (Figure 5) shows a more pronounced growth of the monitored seahorses during the first months of life, until about 17 months, and more stability after 16 cm and 27 months of age.



Figure 5. Von Bertalanffy growth model appied to *Hippocampus reidi* (sex combined) in the Vaza-Barris estuary.

6. Structured Social Behavior

From the beginning of the markings, in January 2014, 26 pairs of males and females, 11 pairs of males, three pairs of females, two pairs of males with juvenile individuals, and nine groups with three or more seahorses were observed together. Among the couples found together, eight were in the hatching period (full pouch) when observed. Over time, five couples, two pairs of males with juveniles, and a pair of males stayed together. The information on the recaptures of couples and other groups during the sampling period is illustrated in Figure 6. The observed couples that remained together the most were those formed

by individuals 131 and 138, found in October 2014 and later on May 2015, and individuals 3 and 4, found together in January and July 2014.

Once the recaptures of grouped individuals were observed, whether couples or distinct groups, the joint displacement of these individuals was monitored over time, by georeferencing the recaptures. The displacement patterns of couples 17 and 54, 31 and 108, and pair 4 and 42 are described below. The pair of males represented by individuals I4 and I42 in the Baleia Stream, moved together a distance of 150 m from April to July 2014 (from 11°07'05.0" S 37°09'12.2" W to 11°07'14.8" S 37°10'20" W). The pair formed by individuals 17 and 54, resident in the Baleia Stream, moved together between April and July 2014 a distance of 11 m (from 11°07'23.4" S, 37°10'21.4" W to 11°07'23.0" S, 37°10'21.7" W) and was recaptured (together) in August 2014, about 10 m away from the site of its previous capture (at 11°07'23.3" S, 37°10'21.8" W). In turn, the couple formed by individuals I31 and I108, also resident in the Baleia Stream, moved together a distance of 65 m during the period from October 2014 to January 2015 (from 11°07'23.1" S, 37°10'21.7" W to 11°07'21.3" S, 37°10'20.2" W), when it was recaptured.

7. Long Term Movement

All fish remained in the areas where they were first caught, which indicates that they did not change streams. The monitored fish were found between 0 and 290 m away from the first capture site, with an average displacement of 49 m. In general it was observed that short displacements occurred between two or more observations. More than 55% of the recaptured fish had displacements of less than 15 meters between two or more collections. Considering the accuracy of the GPS equipment, it is possible to infer that these fish made small displacements or have not moved between capture events (Figure 7). A fish was initially recaptured 237 meters away from the point of first capture and later recaptured 5 meters away from it. This indicates a initial distance and a later return to the original site (Fish 28, Figure 7).

Discussion

The present study makes a broad ecological characterization of *H. reidi* in the Vaza-Barris estuary and provides concrete behavioral ecological information based on the mark-recapture method. In relation to the maximum total length of the species, Mai & Rosa (2009), studying these fish in the Camurupim/Cardoso and Timonha/Ubatuba estuaries, state of Piauí, NE Brazil indicated that the total height for the species is 20 cm, a value very close to the estimation (L_{∞}) in hereby study (20.21 cm). The mean size of the females and males had the largest difference in the smallest individuals. This result is probably related to the late development of the brood pouch and the identification of male individuals in the juvenile phase being more difficult than females, which results in several male juveniles being classified as undetermined sex.

Rosa et al. (2007), using underwater visual sighting data (50 x 2m transect) gathered along the NE, SE and S portions of the Brazilian coast indicated that individuals of this species are usually found allone or in small groups of up to seven individuals, as also observed herein. The reproduction of the species occurred throughout the year, with breeding peaks occurring in February, July, and October 2014. This result is similar to that published by Rosa et al. (2007), who identified reproductive peaks from October to February along the Brazilian coast.



Figure 6. Recaptures of couples and other groups during the sampling period.



Figure 7. Displacement pattern of H. reidi between capture events.

In the present study, few juvenile individuals were collected (less than 6% of total). This low catch of juveniles is probably explained by some hypotheses related to the sampling method and the species ecology, namely the difficulty of visualizing the smaller fish during the collection procedure, and the fact that seahorses at the initial stages of development have a planktonic life habit, not yet grasping the roots of the mangrove or other substrates analyzed. Also the young stages may be occupying another habitat of the mangrove ecosystem as a way of avoiding direct competition with the adults of the species. Onthogenetic differences in the use of habitat were demonstrated by Morgan & Vincent (2007) for the tropical tiger tail seahorrse in a coral reef eocosystem and also by Harasti et al. (2014) for the species *Hippocampus whitei* in Port Stephens, New South Wales, Australia. Therefore, it is important that future biological and ecological studies investigate the early stages of development.

Regarding the growth pattern, it was observed that the younger individuals show higher growth rates, and that, as expected, this rate slows with aging. The growth constant in this study (K = 0.784310) presented a lower value for this species than previous studies in northeastern Brazil (K = 1.195; Mai & Velasco 2012) most likely because few juveniles were sampled in the present study. Considering the relationship between growth and life expectancy, it is possible to infer from the VBGC, that individuals from the monitored population live on average 48 months, a longevity much longer than the 30 months pointed out by Mai & Rosa (2009). It is worth mentioning that the low frequency of capture and also the low number of young individuals in this study influenced the growth parameter of the curve.

The average height at first maturity of *H. reidi* in natural environments was estimated to be between 10 cm (Mai & Velasco 2012) and 12.3 cm (Silveira 2005) in northeastern Brazil, while the average height at first maturity was estimated at 12.4 cm (Mai 2008). In the present study, the smallest individuals observed in reproduction were

a male of 12 cm and a female of 13.1 cm. Considering these sizes, and considering the projection of VBGC, it is possible to infer that *H. reidi* reproduces in the studied area only after 15 months of life.

The distribution of individuals in the environment was analyzed through the fish marking and monitoring performed in this study. No *H. reidi* moved between any of the three streams, with tagged fish being resighted only at the site at which they were initially tagged. So, recapture data indicate that the population of seahorses in the area is resident and present fidelity to the habitat what corroborate the published literature on issues related to the theme for *H. reidi* and other seahorse species (Moreau & Vincent 2004, Curtis & Vincent 2006, Rosa 2007, Caldwell & Vincent 2013, Gristina et al. 2014, Harasti et al. 2014). However, some fish were found far from the initial observation site. This pattern of movement probably occurred owing to tidal currents, which are used by seahorses for their movement.

In an experiment that analyzed the displacement of *H. guttulattus*, it was possible to identify that marked individuals were able to travel great distances in a short period of time (150 m in 8 days) with a single animal moving 60 m in a single day (Caldwell & Vincent, 2013). Although this study indicates that *H. reidi* are closely associated with the monitored sites, individual fish can still move great distances, as shown by a seahorse that moved 273 m and then returned to his previous home range centers. Harasti et al. (2014) demonstrated that even though some individual *H. whitei* were observed moving around their site, they displayed strong site fidelity, as evident through repeated observations of the same individuals existing at the same sites and even on the same holdfasts for long durations in this study. Small displacements were also significantly observed through the recaptures in this study, with more than 55% of the recaptured fish moving less than 15 meters between recaptures.

It must be considered that, according to Acosta & Toloza (2012), a standard market receiver GPS has an accuracy of 10-15 meters, in 95% of times. In order to achieve a sub-metric level of precision, to more accurately recognize movement patterns, it is necessary to apply additional techniques.

The data related to the grouping of seahorses indicates that there is fidelity to the partner, as previously mentioned by other authors (Lourie et al. 1999, Mai & Rosa 2009). Moreover, the pairs of males, females, and adults with juveniles found together exemplify the highly structured social behavior of seahorses, as previously reported in the literature (Vincent & Sadler 1995, Rosa et al. 2007). In this study it was observed a predominance of pairs of individuals, with higher occurrences of couples, pairs of males and pairs of females, respectively. The same observation was made by Perante et al. (2002) when analyzing social associations of Hippocampus comes in the central Phillipines, where seahorses were most commonly sighted in pairs (60% of 516 observations). These autors considered that the higher number of sightings per pair-wise combination of seahorses suggested that these were probably more stable than the quartets. They demonstrated that social groups of more than two animals were fluid: one often sighted trio evolved after 4 weeks into a quartet of two males and two females, and one set of trio sightings represented a quartet which one individual was sometimes missing (Perante et al 2002).

By exhibiting a patchy distribution pattern, *Hippocampus* species tends to present low population densities (Lourie et al. 2004, Foster & Vincent 2004, Siqueira et al. 2017). Rosa et al. (2007) point out that the population densities registered for *H. reidi* in Brazil vary from 0.0023 to 0.066 fishes/m². On the coast of Piauí, in the Camurupim Cardoso estuary, a high population density (0.04 fishes/m²) was recorded (Mai & Rosa 2009). Freret-Meurer & Andreata (2008), at Araçatiba beach, Rio de Janeiro, found an average density of 0.18 ± 0.089 fishes/m², ranging from 0 to 0.4 m².

Our findings, especially in the Viral and Baleia streams, show that *H. reidi* densities are almost twice bigger tham those observed in other studies performed in tropical northeast of the Brazilian coast, where densities are usually higher than of subtropical populations near the southern limit of distribution of this species (Siqueira et al. 2017). This demonstrate the importance of the study area for the conservation of the species.

The biocompatible elastomer implant marking tool proved to be an efficient method for Capture-Mark-Recapture (CMR) studies, allowing greater reliability in the evaluation of population size estimation data when compared to probabilistic estimators applied to punctual capture data. The use of a marking method capable of providing reliable responses to fish population studies is currently a major challenge for researchers around the world, especially considering the nektonic character of fish, which results in a large capacity for displacement and distribution. An increasing literature documents the utility of VIE in very small aquatic animals, like fish and invertebrates (Godin et al. 1996, Frederick 1997, Malone et al. 1999, Brennan et al. 2005, Curtis 2006, Imbert et al. 2007).

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Authors' Contributions

Roberto Schwarz 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.

Ana Cristina Novelino Penna Franco: Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Adauto de Souza Ribeiro: Contribution to data collection.

Marcel Andrade Martins: Contribution to data collection.

Marcelo Soeth: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Olímpio Rafael Cardoso: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Henry Louis Spach: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of Interest

We reiterate have no conflicts of interest to disclose.

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Chemical, diversity and biotechnological potential of endophytic fungi isolated from Brazilian Cerrado plants

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Abstract: This is a systematic review of scientific articles reporting the biodiversity of endophytic fungi isolated from endemic Cerrado plants in Brazil and exposes the potential applications of these microorganisms in the production of known and new metabolites. The "Portal de Periódicos" (CAPES), Science Direct, PubMed, Scielo, and Biblioteca Virtual em Saúde (BVS) databases were used. A total of 418 articles were found using the following sets of keywords: *Endophytic, fungi, and Cerrado; endophytic, fungi, rupestrian, and grass lands; endophytic, fungi, Brazilian, and savanna*; among these, 14 articles were selected. The articles were grouped into studies on the biodiversity of Cerrado endophytic fungi, chemical studies, and other subjects. All the articles were found to be related to the diversity present in the Cerrado of the southeastern and midwestern regions, they show high fungal biodiversity with *Diaporthe* genus predominant in almost all plants studied. In all the studies on the chemical diversity of endophytic fungi, new compounds or new natural products were found associated with a range of bioactivity, especially antifungal. It shows the great potential of endophytes isolated from endemic plant species. The review shows that rare studies performed in regions where the Cerrado biome are predominant, including the central, north, and northeast regions, representing an important niche that is still unexplored. *Keywords: Endophytic fungi; Cerrado; Biotechnology; Natural products; Biodiversity*.

Química, diversidade e potencial biotecnológico de fungos endofíticos isolados de plantas do Cerrado Brasileiro

Resumo: Esta é uma revisão sistemática de artigos científicos que relatam a biodiversidade de fungos endofíticos isolados de plantas endêmicas do Cerrado no Brasil, e expõe as possíveis aplicações desses microrganismos na produção de metabólitos novos e conhecidos. Foram utilizadas as bases de dados "Portal de Periódicos" (CAPES), Science Direct, PubMed, Scielo e Biblioteca Virtual em Saúde (BVS). Foram encontrados 418 artigos, usando os seguintes conjuntos de palavras-chave: *Endophytic, fungi, and Cerrado; endophytic, fungi, rupestrian, and grasslands; endophytic, fungi, Brazilian, and savanna*. Destes, 14 artigos foram selecionados. Os artigos foram agrupados em estudos sobre a biodiversidade de fungos endofíticos do Cerrado, estudos químicos e outros estudos. Todos os artigos foram relacionados à diversidade presente no Cerrado das regiões sudeste e centro-oeste, estes apresentam alta biodiversidade fúngica, com o gênero *Diaporthe* predominando em quase todas as plantas estudadas. Em todos os estudos sobre a diversidade química de fungos endofíticos, novos compostos ou novos produtos naturais foram identificados, associados a uma série de bioatividades, especialmente antifúngica. Isso mostra o grande potencial dos endófitos na produção de metabólitos secundários novos e bioativos, bem como a diversidade química inexplorada de endófitos isolados de espécies de plantas endêmicas. Esta revisão mostra que raros estudos foram realizados em regiões onde o bioma Cerrado é predominante, incluindo as regiões central, norte e nordeste, que representam um nicho importante ainda inexplorado.

Palavras-chave: Fungos endofíticos; Cerrado; Biotecnologia; Produtos naturais; Biodiversidade.

Introduction

Endophytic fungi are a highly diverse group of microorganisms that asymptomatically inhabit the interior of healthy plant tissues (Arnold 2007, Huang et al. 2001). Research on endophytic microorganisms is aimed at understanding the ecology and evolution of these microorganisms, their impact on plant communities, and the natural bioactive compounds that these microorganisms produce (Aly et al. 2011). Considering some endophytes have specific hosts, the wide diversity of biomes and endemic plants found in Brazil represent a potential source of unknown endophytic species (Savi et al. 2019).

The Cerrado, also known as the Brazilian savanna, is the second largest biome in Brazil and is located mainly in the central part of the country (Figure 1). In this biome, there is a rich chemical and biological diversity associated with plant species and microorganisms that can withstand extreme environments, such as high temperature and poor water availability over a long period of the year (Rodrigues et al. 2016). The Cerrado has ~7,000 plant species and it is estimated that ~4,400 of these are endemic, potentially edible, medicinal, wood suppliers, and other industrial raw materials (ICMBio 2018, Silva et al. 2015).





According to Silva et al. (2015) the Cerrado is one of the most threatened biomes owing to increased agricultural activity. It is among the regions of the world with the richest and most endangered biodiversity (ICMBio 2018). Therefore, studies have been focused on bioprospecting its plant species and associated microbiota to identify and ensure the existence of this rich biodiversity.

Therefore, this review brings together publications reporting the endophytic fungal diversity residing in the endemic plants of the Cerrado, as well as the diversity of the metabolites they produced. In addition, we have included important chemical studies carried out to understand the relationship between the incidence, biodiversity, and production of the metabolites of endophytic fungi isolated from this biome.

Material and Methods

To identify the articles for this review, firstly we searched the "Portal de Periódicos" in CAPES, a Brazilian federal agency, which gives access to several databases. We also individually searched the Biblioteca Virtual em Saúde (BVS), PubMed, Science Direct, and Scielo databases between August and September 2019. The search strategy consisted of using the following keywords: Endophytic, fungi, and Cerrado; endophytic, fungi, rupestrian, and grasslands; endophytic, fungi, Brazilian, and savanna. The choice of these keywords was made based on the other names used in regard to the Cerrado biome, which is often reported as the rupestrian fields or Brazilian savanna.

After searching the databases and applying the inclusion and exclusion criteria, studies identified as duplicates (that is, containing the same title, author and year of publication) were identified and excluded. The inclusion criteria were: Original and research articles reporting the biodiversity of Cerrado endophytic fungi covering chemical, biological and other studies, in all languages. The excluded articles were grouped into: repeated, irrelevant (consider not connected with the subject studied), review, and other publication formats (book chapter, public notice, short communications, reports, perspectives, and letters). Manual searches were also performed using bibliographic references of the articles included in the review. There was no loss of studies by the exclusion criteria chosen.

The data obtained from the selected articles and their subsequent analysis resulted in two tables containing information obtained from these studies.

Results

After removing the repeated articles obtained using the various search databases, the exclusion criteria were applied as shown in Figure 2. Of the remaining 418 articles, 14 original research articles on endophytic fungi isolated from the Cerrado were evaluated.

The data obtained from the selected articles and their subsequent analysis resulted in two tables: Table 1 contains information regarding the authors, year of publication, study site, host plant, and isolated endophytic fungi. Table 2 lists the articles grouped according to the type of study performed, including articles on the diversity and biological activities of Cerrado endophytic fungi, chemical studies on Cerrado endophytic fungi, and other subjects.

Discussion

1. Diversity of the endophytic fungi isolated from the Cerrado

Endophytic fungi inhabit the interior of plant species without causing any immediate negative effects to their hosts (Chapla et al. 2014). In



Figure 2. Identification and article selection flowchart

general, endophytic microorganisms enter the interior of plants through natural openings and wounds. Greater access for endophytes is obtained through the plant roots, but can be caused by natural openings, such as stomata and hydatodes, openings caused by insects and pathogenic fungal structures (Azevedo 1998). These microorganisms have stimulated the interest of the scientific community owing to their invaluable biological and chemical diversity, and have been considered as potential sources for the production of metabolites of economic interest, including those related to the host plants (Oliveira et al. 2013, Gonçalves et al. 2017).

Although rich and valuable, endophytic biodiversity is still poorly understood and exploited. According to Esposito & Azevedo (2010) majority of this biodiversity is found in tropical regions, such as the Cerrado, which have immense chemical and biological diversity associated with their microorganisms and endemic plant species. In this sense, studies have been directed toward bioprospecting the endophytic communities associated with the plant species found in this biome.

Noriler et al. (2018) evaluated the biodiversity of endophytic fungal communities associated with the medicinal plants, *Stryphnodendron adstringens* (from Cerrado) and *Vochysia divergens* (from Pantanal). Of the 1,146 endophytic fungi, 339 were isolated from *S. adstrigens*. Molecular analyzes revealed Ascomycota as the predominant phylum among the isolates and Diaporthe was identified as the main genus. The extracts of *Diaporthe* cf. inhibit the mycelial growth of plant pathogens, also presenting antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*, and represent an alternative in the biological control of these pathogens.

Diaporthe has also been identified as the dominant genus in *S. adstringen* by Carvalho et al. (2012), along with Guignardia and Preussia. 320 endophytes were isolated, which were classified into 66 phylotypes and 25 different genera. The endophytic community presents high richness, uniformity, and diversity based on the Margalef, Simpson, and Fisher indices, respectively. Biological evaluation of the fungal

isolates showed that *Diaporthe phaseolorum* and *Xylaria* sp. exhibited anticancer activity, and the *Nigrospora oryzae* extract showed antifungal activity against *Candida albicans* and *Cladosporium sphaerospermum*.

A similar study was conducted on the *Baccharis trimera* plant, where Vieira et al. (2014) analyzed the diversity and antimicrobial activity of their endophytic fungi communities. 179 endophytic fungi were isolated and identified in 25 taxons. The most abundant species were also closely related to *D. phaseolorume, Preussia pseudo minima* and *Pestalotiopsis* sp. Although it presents a lower abundance of fungal isolates when compared to other plants, the richness and diversity were consistent with other studies on medicinal plant endophytes. After biological evaluation of the fungal extracts, 23 showed antimicrobial activity against at least one target microorganism.

Ferreira et al. (2017) investigated the microbial diversity associated with *Vellozia gigantea*, an endemic, ancient, and endangered plant species that is only found in the Cerrado grasslands. Herein, the authors isolated 285 fungi associated with the leaves and roots of the plant, which were identified in 87 taxa and 27 different genera. Diaporthe was also the most abundant genus among the isolates. Xylaria, Nigrospora, Colletotrichum and Trichoderma were also reported, which are genera often associated with tropical species. Based on the same indexes used by Carvalho et al. (2012), *V. gigantea* also showed high diversity indices, displaying a complex and rich microbiota with rare species not yet reported as endophytes. The authors suggested that this microbial diversity may be an important biological component that contributes to the millenary age of this plant in the natural environment.

In a later study, Ferreira et al. (2017), evaluated the antibacterial and antimalarial activities of 285 endophytes isolated from *V. gigantea*. The fungal extracts were obtained via solid medium fermentation and submitted to biological analysis, chromatographic fractionation and ¹H NMR analysis. Among the extracts analyzed, five presented antimicrobial and antimalarial activities. The *Diaporthe miriciae* extracts showed antifungal, antibacterial and antimalarial activities, as well as presenting highly functionalized secondary metabolites, such as epoxycytochalasin H, which displays potent antimalarial activity against *Plasmodium falciparum. Trichoderma effusum* and three Penicillium species exhibited antibacterial activity.

The endophytic diversity changes with the host plant species (Azevedo et al. 2000), age (Arnold & Herre 2003, Arnold et al. 2003), plant tissue type (Rodrigues 1994), climatic factors (Carroll 1988, Rodrigues 1994), and geographic distribution of the host plant (Arnold & Herre 2003). In general, a few species are dominant in the endophytic communities of a given host and there is a certain degree of endophytic-host specificity (Azevedo 1998). In addition, diversity varies with the seasons. Several studies have shown that the diversity displayed by endophytic fungi is greater during the rainy season, when the spore dispersion is higher (Faeth & Hammon 1997, Collado et al. 1999). The variety in terms of the fungal diversity according to the host sex (male and female) has also been studied (Fernandes et al. 2018) and can be attributed to the chemical differences between the two genera, as reported by Ferracini (1995) and Verdi et al. (2005).

2. Chemical studies on endophytic fungi isolated from the Cerrado

Endophyte-plant interactions are not yet clearly understood, however, are believed to be mutualistic, neutral or antagonistic (Souza

	Place	Plant	Endophytic species
FERNANDES et al. (2018)	Belo Horizonte, Minas Gerais, Brazil	Baccharis dracunculifolia	Xylaria sp., X. venulosa, X. apiculata, Cladosporium halotolerans, Coriolopsis rigida, Myceli asterilia, Preussia sp., P. africana
NORILER et al. (2018)	Miranda, Mato Grosso do Sul, Brazil	Stryphnodendron adstringens	Acrocaly medicaginis, Bjerkandera sp., Colletotrichum siamense, C. gloeosporioides sensulato, C. siamense, C. boninense, Corynespora cambrensis, Curvularia sp., Diaporthe sp., D. schini, D. cf. heveae, D. ocoteae, Didymella sp., Epicoccum sp., Fusarium sp., Hypoxylon sp., Lasiodiplodia sp., Neofusicoccum brasiliense, Nigrospora hainanensis, Paraphaeosphaeria sp., Pestalotiopsis sp., Phaeophleospora sp., Phyllosticta sp., Pleomassariaceae sp., Pseudofusicoccum sp., P. stromaticcum, Xylariaceae sp.
CHAPLA et al. (2014)	Araraquara, São Paulo, Brazil	Sennas pectabilis (Fabaceae)	Phomopsis sp.
VITORINO & SILVA (2013)	Rio Verde, Goiás, Brazil	Hyptis marrubioides Epling	Trichoderma sp., Papulaspora sp., Fusarium sp.
FERREIRA et al. (2017)	Serra do Cipó, Minas Gerais, Brazil	Vellozia gigantea	Xylaria berteri, Diaporthe sp., Nigrospora oryzae, Muscodor sp., Colletotrichum aeschynomene e Trichoderma viride
FERREIRA et al. (2017)	Serra do Cipó, Minas Gerais, Brazil	Vellozia gigantea	Diaporthe miriciae, Trichoderma effusum, Penicillium sp.
PINEDO-RIVILLA et al. (2009)	São Paulo, Brazil	Casearia sylvestris	Colletotrichum crassipes e Xylaria sp.
SILVA et al. (2006)	Araraquara, São Paulo, Brazil	Cassia spectabilis	Phomopsis cassiae
TELES et al. (2006)	Araraquara, São Paulo, Brazil	Xylopia aromatica	Periconia atropurpurea
TELES et al. (2005)	Araraquara, São Paulo, Brazil	Ocotea corymbosa	<i>Curvularia</i> sp.
CARVALHO et al. (2012)	Serra do Cipó e Serra de São José, Minas Gerais, Brazil	Stryphnodendron adstringens	Diaporthe sp., D. phaseolorum, Guignardia camelliae, G. mangiferae, Preussia sp., Phomopsis sp., P. theicola, Pseudofusicoccum stromaticum, Cytospora sp., C. rhizophorae, Nigrospora oryzae, Neofusicoccum ribis, Colletotrichum boninense, C. gloeosporioides, Xylariaceae sp., Pestalotiopsis clavispora, P. microspora, P. cocculi, Sporormiella sp., Alternaria alternata, Cladosporium cladosporioides, Penicillium glabrum, P. minioluteum, Sordaria fimicola, S. tomento-alba, Aspergillus ustus, A. flavipes, Arthrobotrys foliicola, Botryosphaeria dothidea, Coniochaeta discoidea, Muscodor vitigenus, Paraconiothyrium brasiliense, Sarcosomataceous sp., Trichoderma sp., Xylaria sp.
DE OLIVEIRA et al. (2018)	Belo Horizonte, Minas Gerais, Brazil	Eugenia bimarginata	<i>Mycosphaerella</i> sp.
VIEIRA et al. (2014)	Ouro Branco, Minas Gerais, Brazil	Baccharis trimera	Diaporthe sp., D. phaseolorum, Pestalotiopsis sp., Cochliobolus lunatus, Epicoccum nigrum, Guignardia sp., Preussia sp., P. africana, P. pseudominima, Xylaria sp., Chaetomium sp., Phoma sp., Alternaria sp., Sporormiella sp., Nigrospora sp., Podospora sp.

Table 1. Endophytic fungi isolated from Cerrado biome

et al. 2004). In mutualistic interactions, fungi can perform functions relevant to plant health, producing substances that can protect plants against pests and pathogens, increase their tolerance to abiotic stress, and produce chemical compounds, such as hormones and antibiotics (Azevedo 1998, Souza et al. 2004).

Owing to their interaction with the host plant, many endophytes are also capable of producing the same substance synthesized by their host or bioactive analogs, which display various activities of interest, such as antibacterial, antifungal, anticancer, and immunosuppressive properties (Lacava et al. 2004, Gonçalves et al. 2017). Thus, chemical studies have been performed using endophytes mainly associated with medicinal plants in an attempt to increase the number of new biotechnological discoveries.

	Reference	Subjects		
	VIEIRA et al. (2014)	Diversity and antimicrobial activity.		
Diversity and	CARVALHO et al. (2012)	Diversity, antimicrobial and anticancer activities.		
	NORILER et al. (2018)	Biodiversity and structure of endophytic communities; antimicrobial activity.		
biological activity	FERREIRA et al. (2017)	Taxonomy and diversity.		
	FERREIRA et al. (2017)	Antimicrobial and antimalarial activities.		
	DE OLIVEIRA et al. (2018)	Structural elucidation of aromatic compounds produced by endophytes.		
Chemical studies	DA SILVA et al. (2006)	Cadinan sesquiterpene derivatives and antifungal activity.		
	TELES et al. (2006)	Aromatic compounds and biological activities against carcinoma in rats.*		
	TELES et al. (2005)	Structural analysis of compounds; antifungal and biological activities.*		
	CHAPLA et al. (2014)	Isolation and identification of natural products.		
	FERNANDES et al. (2018)	Multitrophic interaction between bees, plants, and endophytes.		
	LISBOA et al. (2013)	Enzymatic activity of esterases produced by endophytic fungi.		
Other subjects	VITORINO & SILVA (2013)	In vitro microplant response to inoculation with endophytic isolates.		
	PINEDO-RIVILLA et al. (2009)	Microbial biocatalysts for ketone reduction.		

Table 2. Research developed on endophytic fungi obtained from the Cerrado

* Biological assays were performed on the crude extracts produced by the studied endophytic fungi.

A chemical study on the extract of *Mycospharella* sp., an endophytic fungus associated with the endemic plant of the Cerrado *Eugenia bimarginata*, was performed by De Oliveira et al. (2018). The authors isolated two new usnic acid derivatives: mycousfuranine (1) and mycousnicdiol (2) (Figure 3). The two new compounds were isolated from the ethyl acetate fraction of the extract using chromatographic techniques. Compounds 1 and 2 exhibited moderate antifungal activity against *Cryptococcus neoformans* and *Cryptococcus gattii*.

Silva et al. (2006) isolated new cadinan sesquiterpene derivatives from the crude extract of *Phomopsis cassiae*, an endophytic fungus isolated from *Cassia spectabilis*, a plant species found in the Cerrado. The fungus was cultivated in a liquid medium and extracted with ethyl acetate. Bioassay-guided fractionation was used to isolate the compounds 3,9,12-trihydroxycalamenenes (3 and 4), 3,12-dihydroxycalamenene (5), 3,12-dihydroxycadalene (6), and 3,11,12-trihydroxycadalene (7) (Figure 3). Compounds 4 and 7 showed antifungal activity when evaluated against *Cladosporium sphaerospermum* and *C. cladosporioides*.

A similar study was conducted by Chapla et al. (2014), which evaluated the secondary metabolites produced by *Phomopsis* sp., an endophyte associated with the *Senna spectabilis* plant. A novel natural product, 2-hydroxy-alternariol (8), together with known compounds, cytochalasins J and H, 5'-epialtenuene, and the mycotoxins, alternariol monomethyl ether (AME), alternariol (AOH), and cytosporone C, were obtained using chromatographic techniques. Cytochalasin H inhibits acetylcholinesterase (AChE) enzyme *in vitro* and exhibits antifungal activity. In addition, cytochalasins J and H, and alternariol showed potent inhibitory effects on reactive oxygen species (ROS) produced by human neutrophils and may be promising targets for the development of anti-inflammatory agents.

Studying the metabolites produced by *Periconia atropurpurea*, a fungus isolated as an endophyte from the *Xylopia aromatica* plant, Teles et al. (2006) isolated several new aromatic compounds exhibiting biological activity. 6,8-Dimethoxy-3-(2'-oxo-propyl)-coumarin (9) and 2,4-dihydroxy-6-[(1'E,3'E)-penta-1',3'-dienyl]-benzaldehyde (10), in addition to the known compound, periconicin B (11), were isolated from the ethyl acetate fraction of the extract. Biological analyzes showed that compound 10 exhibits strong antifungal activity against

C. sphaerospermum and *C. cladosporioides*, and compound 11 showed potent cytotoxic activity against the two cell lines studied (human cervical carcinoma (HeLa) and Chinese hamster ovary (CHO) cells).

In another chemical study, Teles et al. (2005) isolated a new benzopyran from the extract of *Curvularia* sp. The fungus was isolated as an endophyte of the leaves of *Ocotea corymbosa*, a native plant found in the Brazilian Cerrado. Chromatographic analysis led to the isolation of two new benzopyran derivatives, (2'S)-2-(propan-2'-ol)-5-hydroxy-benzopyran-4-one (12) and 2,3-dihydro-2-methyl benzopyran-4,5-diol (13), and two known benzopyrans, 2-methyl-5-methoxy-benzopyran-4-one (14) and (2R)-2,3-dihydro-2-methyl-5-methoxy-benzopyran-4-one (15). Compounds 12 and 14 showed antifungal activity against *C. sphaerospermum* and *C. Cladosporium*. In addition, compound 12



Figure 3. Chemical structures of the compounds isolated from endophytic fungi

was able to induce cell proliferation (70% in HeLa cells and 25% in CHO cells).

In all the studies on the chemical diversity of endophytic fungi, new compounds or new natural products exhibiting a range of bioactivities were isolated, which shows the great potential of endophytes in the production of new and bioactive secondary metabolites, as well as the unexplored chemical diversity of endophytes isolated from the Cerrado.

3. Other Cerrado endophyte fungal studies

In addition to the huge potential for the discovery of new pharmaceutical substances from this wide variety of plant species and secondary metabolites (Gonçalves et al. 2017), endophytic fungi are also considered for application in other areas, such as the production of enzymes, flavors and flavors of value in the food/cosmetics industry, and hydrocarbons and fatty acids for use in energy production (Corrêa et al. 2014, Souza et al. 2004). Owing to this biotechnological potential, these microorganisms have been the target of various chemical and biological studies.

Lisboa et al. (2013) evaluated a new method for the detection of esterase producing microorganisms via the *in vitro* detection of enzymatic activity using a pH indicator (bromothymol blue). This method evaluated the production of esterases using endophytic fungi isolated from Cerrado and Atlantic Forest plants. The results showed that the method was efficient, fast, and cheap with low reagent consumption and easy development. The authors also highlighted the potential of endophytic fungi isolated from Cerrado and Atlantic Forest plant species as successful producers of esterases.

In order to detect monooxygenase and alcohol dehydrogenase activity, Pinedo-Rivilla et al. (2009) studied the biotransformation of pro-chiral ketones (4-ethylcyclohexanone and acetophenone) by filamentous fungi and endophytic fungi isolated from *Casearia sylvestris*. The analyses showed the biocatalytic potential for obtaining biotransformation compounds with high enantiomeric purity. The main reaction pathways involved reduction and hydroxylation in various positions. In addition, trans-4-ethyl-1-(1S-hydroxyethyl)cyclohexanol was reported for the first time, which was obtained via an atypical acyl reaction and indicated the biocatalytic potential of the endophytic fungus *Eutypa lata*.

Vitorino & Silva (2013) evaluated the metabolic response of microplants *in vitro* to inoculation with endophytic bacteria and fungi isolated from *Hypti smarrubioides* Epling. The results of the study indicated that the endophyte–plant interaction led to qualitative and quantitative changes in the host phytochemical profile because some of the compounds evaluated in the host plant were produced only after inoculation with the endophytes. According to the authors, this technique may be an important strategy for the production of bioactive compounds from medicinal plants.

Studying the interactions between microorganisms and plant species also has a significant biotechnological relevance because in addition to contributing to understanding the chemical and ecological processes, it can result in the discovery of new bioactive substances (Dos Santos & Varavallo 2011).

Fernandes et al. (2018) studied the multitrophic interaction between endophytic fungi, bees, and *Baccharia dracunculifolia* plant, a plant species found in the Cerrado, to evaluate whether there was any relationship between the resin collected by bees and fungal endophytes, considering the sex of the host plant. The nine identified taxa were found exclusively in damaged leaves. The endophyte richness and proportion of leaf shoot damage did not change between the plant sexes, however, the composition of endophytes changed with gender. These results reinforced the importance of physical damage to plant tissues as a gateway to endophyte infection, as well as the selective potential of chemical differences in the host plant sex against invasion by microorganisms.

Conclusions

This survey conducted a systematic review of the literature and resulted in 418 articles using selected keywords and platforms. After critical analysis, 14 articles on endophytic fungi isolated from the Cerrado were selected. The analysis of these articles showed the potential of Cerrado plants to act as a rich and diversified repository of endophytic fungi, which have proven to be a potential source of novel and bioactive substances, and have applications in several areas of interest.

The endophytic production of secondary metabolites varies according to the biology and growth conditions of the microorganism and are produced as an adaptation to specific functions in nature. They play vital roles *in vivo* owing to the numerous metabolic interactions observed between endophytes and their hosts, such as signaling, defense, and regulation of symbiosis (De Andrade et al. 2018). In view of this, the search for new bioactive molecules should focus on organisms that inhabit new ecosystems or niches that are still unexplored, because natural products are adapted to a specific function in nature (Azevedo et al. 2000, Nisa et al. 2015).

Although the Cerrado is considered as one of the largest areas of biodiversity in the world containing ~7,000 plant species, there are few studies on endophytic fungi associated with these species. Consequently, the development of chemical and biological studies on the microbiota associated with Brazilian Cerrado plant species, particularly in the central, northern, and northeastern regions of Brazil are recommended, given that most of these studies are related to plant species. The Southeast and Midwest regions contain rich and valuable biodiversity that are yet to be explored.

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Author Contributions

Sara Bruna Souza Dantas: substantial contribution in the concept and design of the study, to data collection and to data analysis and interpretation; wrote, added intellectual content and review the final version of the manuscript; provided significant contribution to manuscript preparation.

Flavia Alessandra Mota Alves: substantial contribution in the concept and design of the study, to data collection and to data analysis and interpretation; wrote, added intellectual content and review the final version of the manuscript; provided significant contribution to manuscript preparation.

Vanessa Mara Chapla: substantial contribution in the concept and design of the study, data analysis and interpretation; added intellectual

content and review the final version of the manuscript; provided significant contribution to manuscript preparation.

Conflicts of Interest

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

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Gastrotrichs and tardigrades in a remnant of Atlantic Forest (Serra do Japi, SP, Brazil)

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Abstract: Serra do Japi, located in the southeast of São Paulo State, is considered a priority area for conservation, as it houses original Atlantic Forest cover remains. Despite the significant number of studies about vertebrates and invertebrates that were carried out in this region, the meiofauna biodiversity is completely unknown. Thus, the present study aimed to investigate for the first time freshwater Gastrotricha and limnoterrestrial Tardigrada in Serra do Japi Biological Reserve. Samples of sediments, periphyton and floating vegetation in reservoirs and natural lagoons, and mosses growing on native and non-native tree trunks were collected in May 2019. At least five gastrotrichs morphotypes were identified and three of them were formally described: Chaetonotus acanthocephalus, C. dadayi (first record in Brazil), and Heterolepidoderma mariae (first record outside the type locality). In regards to tardigrades, twelve morphotypes were identified and four of them were formally described: Pseudechiniscus juanitae, Minibiotus cf. acontistus, Echiniscus drevfusi and Itaquascon umbellinae (last two species reported for the first time outside the type locality). This study reinforces that meiofaunal diversity and distribution have been underestimated, even in one of the five largest hotspots in the world.

Keywords: Checklist, meiofauna, floating vegetation, moss.

Gastrótricos e tardígrados em um remanescente de Mata Atlântica (Serra do Japi, SP, Brasil)

Resumo: A Serra do Japi, localizada no sudeste do Estado de São Paulo, é considerada uma área prioritária para conservação, por abrigar vestígios de cobertura original de Mata Atlântica. Apesar do número significativo de estudos sobre vertebrados e invertebrados realizados nesta região, a biodiversidade de grupos meiofaunais é completamente desconhecida. Assim, o presente estudo teve como objetivo investigar pela primeira vez Gastrotricha de água doce e Tardigrada limnoterrestre na Reserva Biológica da Serra do Japi. As amostras de sedimentos, perifíton e vegetação flutuante em reservatórios e lagoas naturais, e musgos presentes em troncos de árvores nativas e não nativas foram coletadas em maio de 2019. Foram encontrados cinco morfotipos de gastrótricos, sendo que apenas três deles já foram formalmente descritos: Chaetonotus acanthocephalus, C. dadayi (primeiro registro no Brasil) e Heterolepidoderma mariae (primeiro registro fora da localidade-tipo). Em relação aos tardígrados, foram identificados doze morfotipos, sendo que quatro deles já formalmente descritos: Pseudechiniscus juanitae, Minibiotus cf. acontistus, Echiniscus dreyfusi e Itaquascon umbellinae (as duas últimas espécies relatadas pela primeira vez fora da localidade-tipo). Este trabalho reforça que a diversidade e distribuição da meiofauna têm sido subestimadas, mesmo em um dos cinco maiores hotspots do mundo.

Palavras-chave: Lista de espécies, meiofauna, vegetação flutuante, musgo.

Introduction

Meiofauna can be defined as an assembly of freshwater and marine organisms that pass through a coarse sieve of 500 μ m and are retained by a finer sieve of 44 μ m and do not have close phylogenetic relationships among them (Giere 2009). These animals can be classified as permanent, having an entire life as small organisms, or temporary, having one or more stages of the life cycle with 'meiofaunal' body size (Higgins & Thiel 1988). Beside in the water bodies of rivers and lakes, freshwater meiofauna can be found in distinct environments, such as rooted vegetation, macroalgae fronds, mosses, and some animal structures like worm tubes (Higgins & Thiel 1988, Boeckner et al. 2009, Giere 2009).

Among the 34 phyla of Metazoa, there are at least 23 that have meiofaunal organisms, such as Gastrotricha and Tardigrada (Giere 2009, Cerca et al. 2018). Gastrotricha are microinvertebrates living in marine (e.g. among sand grains and algae) and freshwater (e.g. aquatic plants and psammon) habitats around the world (Balsamo et al. 2014, 2020, Kieneke & Schmidt-Rhaesa 2015). The taxon is divided into two orders: Macrodasyida, with a worm-like body consisting of 380 species, all marine except for four species (Garraffoni et al. 2019a, Balsamo et al. 2020); Chaetonotidae, with a tenpin-shaped body consisting of 480 species, two-thirds of which reported in freshwater (Balsamo et al. 2008, 2014, 2020). Tardigrada, commonly known as water bears, living in marine (e.g. sand grains, rock shore, barnacles), freshwater (e.g. aquatic plants and psammon), and limnoterrestrial (e.g. bryophytes, cushion-forming plants, lichens, algae, soils) habitats (Nelson et al. 2015). The taxon comprises more than 1300 species divided into two valid classes: Heterotardigrada (majority marine); and Eutardigrada (freshwater and limnoterrestrial). The existence of a third class, Apotardigrada (freshwater and limnoterrestrial) has been previously discussed (Guil et al. 2019, Morek et al. 2020).

Due to the meiofaunal body size and challenging identification process, the knowledge about gastrotrichs and tardigrades' diversity in the world is underestimated (Vicente 2010, Appeltans et al. 2012; Balsamo et al. 2014, 2015, 2020, Fonseca et al. 2018, Garraffoni et al. 2019b). Notwithstanding, a few studies have aimed to reveal the richness and distributional patterns of these two freshwater and limnoterrestrial meiofauna taxa in Brazil (de Barros R 1939a, b, 1942a, b, 1943, Kisielewski 1991, Assunção 1999, Pilato et al. 2002b, Garraffoni et al. 2010, 2017a, b, 2019a, Garraffoni & Melchior 2015, Araújo et al. 2013, Rocha et al. 2016, Garraffoni 2017, de Barros RC 2020). This study aims to contribute to this knowledge by presenting the first records of gastrotrichs and tardigrades at the Biological Reserve of Fundação Serra do Japi, an extensive area of Atlantic Forest located in São Paulo State. In addition, this manuscript provides taxonomic notes of the morphotypes and formally described species found at the Reserve.

Material and Methods

1. Study site

Serra do Japi is located in the southeast of São Paulo, bordering four cities (Jundiaí, Pirapora do Bom Jesus, Cajamar and Cabreúva), between the geographical coordinates 23°14′0″S and 46°58′0″W. The elevation has an area of approximately 35,000 hectares and a native vegetation cover of semi-deciduous mesophyllous forests, semi-deciduous forests of altitude, and sparse rocky enclaves (Morellato 1992) and fragments of reforestation with pine and *Eucalyptus* sp., pastures, and small portions of agricultural crops. The altitudinal ranges vary between 700 and 1,000 meters (Pinto et al. 1972). In the coldest month, July, the average temperatures range between 11.8°C and 15.3°C, and in the warmest month, January, between 18.4°C and 22.2°C depending on the altitude (Morellato 1992). The rainfall cycle is variable, increasing in the months of December-January, with values greater than 250 mm per month, and decreasing in the winter, with values lower than 41 mm and 71 mm per month, depending on the location (Morellato 1992).

2. Collecting procedures and Data analysis

Samples were collected from May 18th to 19th, 2019. Gastrotricha specimens were sampled by collecting specimens of the floating vegetation belonging to *Salvinia* sp. from a small artificial reservoir (23°14'42.8''S, 46°56'12.4''W) and natural lagoon (23°14'47.3''S, 46°56'12.4''W) and stored in plastic buckets. Tardigrada specimens were sampled by collecting mosses belonging to family Pottiaceae on native (23°14'21.9''S, 46°56'07.6''W) and from *Sematophyllum galipense* (Müll.Hal.) Mitt. on exotic *Pinus* sp. (23°13'59.8''S, 46°56'01.5''W) trees (Figure 1) and stored in paper bags. Freshwater and limnoterrestrial samples were brought back to the University of Campinas for further analysis.



Figure 1. Map of collection stations. A: Serra do Japi Biological Reserve, B: floating vegetation from an artificial small reservoir, C: natural lagoon, D: exotic *Pinus* sp. tree trunks, E: mosses from the Atlantic Forest.

Water with sediment was sieved (42 μ m mesh) and specimens were encountered by sorting small amounts of sediment poured into Petri dishes under a Zeiss Stemi 2000 stereomicroscope. Mosses were placed in a beaker filled with ddH₂O, and after 20 minutes the water was transferred to Petri dishes and the specimens were scanned under a Zeiss Stemi 2000 stereomicroscope.

Alive specimens were isolated with micropipettes into a glass embryo dish. The gastrotrichs and tardigrades were anesthetized with 2% MgCl, and warm water (70°C), respectively. Subsequently, gastrotrichs were isolated and mounted on glass slides and tardigrades were stored in 70% ethyl alcohol and permanent slides were made using Fluoromount Aqueous Mounting medium. All specimens were documented under a Zeiss Axio Imager M2 light microscope equipped with Differential Interference Contrast optics (DIC) connected to a camera. Photomicrographs were taken using the software ZEN - blue edition. The most representative structures of tardigrades identified up to the specific level were measured. Methods and terminology used to measure the specimens of Echiniscus dreyfusi followed Bartylak et al. (2019); Pseudechiniscus juanitae followed Roszkowska et al. (2020); Itaquascon umbellinae followed Pilato et al. (2002a), Minibiotus cf. acontistus followed Kaczmarek & Michalczyk 2017. Morphometric data was handled using "Echiniscoidea" and "Parachela" templates available from the Tardigrada Register (Michalczyk & Kaczmarek 2013).

Photos of Gastrotricha (due to the fragility of their bodies, they were destroyed during the microscopic examination and are no longer available - Balsamo et al. 2014, 2020, Garraffoni et al. 2019b) and photos and permanent slides of Tardigrada specimens are available at the Zoological Museum "Adão José Cardoso" (ZUEC), at the University of Campinas, Brazil.

Results

At least five morphospecies (38 specimens) of Gastrotricha belonging to two genera of Chaetonotidae were present. Among these five morphospecies, three were attributed to described species (*Chaetonotus dadayi* Schwank, 1990, *Chaetonotus acanthocephalus* Valkanov, 1937 and *Heterolepidoderma mariae* Garraffoni & Melchior, 2015). A total of 12 morphospecies (32 specimens) belonging to two classes of Tardigrada were present. Among these 12 morphospecies, eight were identified at the genus level and four were attributed to described species (*Itaquascon umbellinae* de Barros R, 1939a, *Pseudechiniscus juanitae* de Barros R, 1939b, *Minibiotus* cf. *acontistus* de Barros R, 1942b and *Echiniscus dreyfusi* de Barros R, 1942a). The remaining morphospecies could not be described to species level due to problems in the fixation process (see Discussion).

Phylum Gastrotricha

Order Chaetonotida Remane, 1925 [Rao & Clausen, 1970] Suborder Paucitubulatina d'Hondt, 1971

Family Chaetonotidae Gosse, 1864 [Garraffoni, Araújo, Lourenço, Guidi & Balsamo, 2017b]

Subfamily Chaetonotinae Kisielewski, 1991

Genus Chaetonotus Ehrenberg, 1830

Chaetonotus (Primochaetus) acanthocephalus Valkanov, 1937 Figure 2 (A-C) **Examined material:** One specimen (ZUEC PIC 390). **Remarks:** *C. acanthocephalus* is easily recognized by the presence of five dorsal cephalic scales with long spines, two plates at the side of the hypostomium, and the arrangement of dorsal and lateral spines (e.g. two pairs at the neck, transversal row at the mid-dorsal surface). This species was previously reported in Poland, Germany, Romania, Bulgari (Valkanov 1937, Kisielewski 1981, Schwank 1990) and Brazil (Kisielewski 1991, Garraffoni et al. 2010, Araújo et al. 2013).

Chaetonotus (Chaetonotus) dadayi Schwank, 1990 Figure 2 (D-F)

Examined material: Fourteen specimens (ZUEC PIC 377-389).

Remarks: Von Daday (1905) identified specimens found on the border between Paraguay and Brazil, in Estia Postillon, as *Chaetonotus similis* Zelinka, 1889. Later, Schwank (1990) recognized that the specimens analyzed by Von Daday (1905) had a pair of spines that protruded beyond the adhesive tubes. This feature is not observed in *Chaetonotus similis* and Schwank (1990) described a new species, *C. dadayi*, to accommodate the Paraguayans organisms. Several years later, d'Hondt (2006) found *C. dadayi* in French Guiana and presented the first photographic record of a specimen of this species. The Brazilian specimens are morphologically close to the previous descriptions due to the presence of spined scales of the posterior dorsal region smaller than the trunk ones, lateral spined scales of the furca base longer than adhesive tubes; two pairs of long, thick, and simple spines on the dorsal



Figure 2. DIC photomicrograph of Gastrotricha. A-C: Lateral view of *Chaetonotus (Primochaetus) acanthocephalus*, D, F: Ventral view of *Chaetonotus (Chaetonotus) dadayi*, E: Dorsal view of *Chaetonotus (Chaetonotus) dadayi*. Scale bars: 50 µm.

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region and two pairs of scales with long, thin and simple spines in the ventral region. This is the first report from Brazil.

Chaetonotus sp. 1

Figure 3 (A-C)

Examined material: Four specimens.

Remarks: Mouth terminal; pentalobate head. Trilobate spined scales on dorsal and ventrolateral regions; spines increasing in length along the body; set of nine long and thick spines arranged as 3-3-3 on the middle dorsal region. Ventral interciliary portion covered by small spined scales.

Chaetonotus sp. 2

Examined material: Six specimens.

Remarks: Mouth subterminal; small cephalic hypostomium. Rounded spined double-edge scales on dorsal and ventrolateral regions; dorsal spines increasing in length from head to middle trunk and decreasing until the posterior end; pair of long spines observed at the furcal base. Ventral interciliary portion covered by small rounded spined scales.

Genus Heterolepidoderma Remane, 1927

Heterolepidoderma mariae Garraffoni & Melchior, 2015 Figure 3 (D)



Figure 3. DIC photomicrograph of Gastrotricha. A: Ventral view of *Chaetonotus* sp. 1, B-C: Dorsal view of *Chaetonotus* sp. 1, D: Ventral view of *Heterolepidoderma mariae*. Scale bars: 50 µm.

Examined material: Six specimens (ZUEC PIC 391-396).

Remarks: *Heterolepidoderma mariae* can be easily recognized by specific cuticular ornamentation of the dorsal body surface, keeled scales with three different shapes. It is the first report outside the type locality, Paulínia - São Paulo State, Brazil (Garraffoni & Melchior 2015).

Phylum Tardigrada

Class Heterotardigrada Marcus, 1927 Order Echiniscoidea Richters, 1926 Family Echiniscidae Thulin, 1928

Genus Echiniscus Schultze, 1840

Echiniscus dreyfusi de Barros R, 1942

Figure 4 (A), Table 1

Examined material: One specimen, unidentified sex, (ZUEC PIC 402).

Remarks: The specimen found in Serra do Japi has four features not mentioned in the original description of *Echiniscus dreyfusi*: thick granulation on the dorsal side of the legs; spine present on the first pair of legs; spurs on claws of the third and fourth pairs of legs; the dorsal pair of spines has a striated texture. This species was previously reported in New Guinea (Iharos 1963), Argentina (Claps & Rossi 1988), Japan (Utsugi 1988), and Brazil (de Barros R 1942a). In Brazil, it is the first report outside the type locality, São Vicente City - São Paulo State.

Echiniscus sp.

Figure 4 (B)

Examined material: One specimen, unidentified sex.

Remarks: Body orange and plump. Dorsal plates with large pores irregularly distributed; *spinulosus* type. Trunk appendages in the form of spines; spines D^d and E more robust and rough. There is a small denticle on each D^d spine. Small spine on the first leg pair. Papilla and dentate collar present on the fourth leg pair.

Genus Pseudechiniscus Thulin, 1911

Pseudechiniscus juanitae de Barros R, 1939

Figure 4 (C), Table 2

Examined material: Three specimens, unidentified sex, (ZUEC PIC 399-401).

Remarks: Body orange. Round black eyes present in two specimens. *Cirrus internus, externus* and A present. Clava visible in one specimen. Pseudosegmental plate present. Very visible cuticular granulation on the dorsal plates and legs. Papilla on the fourth pair of legs visible in one specimen. Dentate collar absent. Spurs on claws IV present. Spurs on claws I visible in one specimen. This species was previously reported in Austria (Mihelcic 1962, Maucci 1974), Italy (Ramazzotti & Maucci 1983), Galápagos Islands (Schuster & Grigarick 1966), China (Wang 2009), and Brazil (de Barros R 1939b, 1942a, du Bois-Reymond Marcus 1944).

Class Eutardigrada Richters, 1926 Order Parachaela Schuster, Nelson, Grigarick & Christenberry, 1980 Family Macrobiotidae Thulin, 1928

Genus Paramacrobiotus Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009

Paramacrobiotus sp. 1

Figure 4 (D)

Examined material: Three specimens, unidentified sex.

Remarks: Eyes present in only one specimen. Cuticle smooth. Oral cavity armature (OCA) composed of three bands of teeth. Pharyngeal apophysis present and very visible. Three macroplacoids present and separated by the same distance from each other; the second macroplacoid is the smaller; the third macroplacoid has a small constriction; very small triangular microplacoids present. Claws of the *hufelandi* type.

Paramacrobiotus sp. 2

Figure 4 (E)

Examined material: Four specimens, unidentified sex.

Remarks: Eyes present in two specimens. Cuticle smooth except for very visible granulation present on all legs. Oral cavity armature composed of three bands of teeth. Pharyngeal apophysis present; three macroplacoids present; the second is the smaller and the third is positioned more distant from the others; the third macroplacoid has a small constriction; microplacoids absent. Claws of the *hufelandi* type. Dentate lunules under claws IV.

Paramacrobiotus sp. 3

Examined material: Two specimens, unidentified sex.

Remarks: Eyes absent. Cuticle smooth except for very visible granulation present on all legs. Oral cavity armature (OCA) composed of three bands of teeth. Pharyngeal apophysis present. Three macroplacoids present; the second is the smaller and the third is positioned more distant from the others; the third macroplacoid has a small constriction; very **Table 1.** Measurements $[in \mu m]$ and *sc* values of selected morphological structures of *Echiniscus dreyfusi* de Barros R, 1942. The *sc* is the percentage of scapular plate length (Fontoura et al. 2008).

CHARACTER	μm	sc
Body length	279	575
Scapular plate length	48.5	_
Head appendages lengths		
Cirrus internus	20.5	41.2
Cephalic papilla	8.3	17.1
Cirrus externus	20.0	41.2
Clava	8.5	17.5
Cirrus A	40.8	84.1
Cirrus A/Body length ratio	15%	_
Cirrus int/ext length ratio	100%	_
Body appendages lengths		
Cirrus B	20.5	42.3
Cirrus C	34.5	71.1
Cirrus D	32.0	66.0
Cirrus Dd	30.0	61.9
Cirrus E	37.2	76.6
Spine on leg I length	5.4	11.1
Claw 1 heights		
Branch	13.2	27.2
Claw 2 heights		
Branch	12.5	25.8
Claw 3 heights		
Branch	11.7	24.1
Spur	4.1	8.5
Spur/branch height ratio	35%	_
Claw 4 heights		
Branch	16.3	33.6
Spur	4.3	8.9
Spur/branch height ratio	26%	_



Figure 4. DIC photomicrograph of Tardigrada. A: Dorsal view of *Echiniscus dreyfusi*, B: Ventral view of *Echiniscus* sp. C: Dorsal view of *Pseudechiniscus juanitae*, D: Buccal apparatus of *Paramacrobiotus* sp. 1, E: Buccal apparatus of *Paramacrobiotus* sp. 2. Scale bars: 50 µm.

CHARACTER	μm	sc	μm	sc	μm	sc
Body length	263	793	181	755	301	564
Scapular plate length	33.2	_	24.1	_	53.4	_
Head appendages lengths						
Cirrus internus	19.3	58.0			13.4	25.1
Cephalic papilla						
Cirrus externus	37.8	113.8			28.8	53.9
Clava	7.4	22.3				
Cirrus A			32.3	134.2	40.1	75.0
Cirrus A/Body length ratio		_	18%	_	13%	-
Cirrus int/ext length ratio	51%	-		—	46%	-
Papilla on leg IV length			5.4	22.6		
Claw 1 heights						
Branch			10.0	41.5	11.2	21.0
Spur					3.1	5.7
Spur/branch height ratio		-		—	27%	-
Claw 2 heights						
Branch			8.4	34.7		
Spur						
Spur/branch height ratio		-		_		—
Claw 4 heights						
Branch	11.2	33.7	9.2	38.2		
Spur	3.0	9.0	3.7	15.5		
Spur/branch height ratio	27%	_	41%	_		-

Table 2. Measurements [in µm] and *sc* values of selected morphological structures of *Pseudechiniscus juanitae* de Barros R, 1939b. The *sc* is the percentage of scapular plate length (Fontoura et al. 2008).

small triangular microplacoids present. Claws of the *hufelandi* type. Dentate lunules under claws IV.

Genus Macrobiotus Schultze, 1834

Macrobiotus sp. 1

Figure 5 (A)

Examined material: Four specimens, unidentified sex.

Remarks: Eyes absent. Dorsal cuticle with very visible ornamentation forming polygons like scales of reptiles; thin granulation present on all legs. Bucco-pharyngeal apparatus of the *Macrobiotus* type. Pharyngeal apophysis present; three short and wide macroplacoids present; large microplacoids present. Claws of the *hufelandi* type. Cuticular bars on legs I-III.

Macrobiotus sp. 2

Examined material: Two specimens, unidentified sex.

Remarks: Eyes present. Cuticle smooth. Pharyngeal apophysis presents; three slender macroplacoids present, the third being positioned more distant from the others. Claws of the *hufelandi* type.

Genus Minibiotus Schuster, 1980

Minibiotus cf. acontistus de Barros R, 1942b

Table 3

Examined material: One specimen, unidentified sex (ZUEC PIC 398). **Remarks:** Eyes absent. Cuticle smooth. Buccal tube long and narrow. Three macroplacoids present; microplacoids absent. Claws of the *hufelandi* type (claws Y-shaped; symmetrically arranged with respect to the median plane of the leg; without basal spurs).

Minibiotus sp.

Figure 5 (B)

Examined material: Eight specimens, unidentified sex.

Remarks: Eyes present only in two specimens. Cuticle smooth. Buccal tube long and narrow. Large pharyngeal apophyses present. Three macroplacoids present; microplacoids present and very small. Claws of the *hufelandi* type.

Family Hypsibiidae Pilato, 1969 Subfamily Itaquasconinae Rudescu, 1964

Genus Itaquascon de Barros R, 1939a

Itaquascon umbellinae de Barros R, 1939a

Figure 5 (C), Table 4 Examined material: One specimen, unidentified sex (ZUEC PIC 397). **Table 3.** Measurements [in μ m] and *pt* values of selected morphological structures of *Minibiotus* cf. *acontistus* de Barros R, 1942b. The *pt* index is the ratio of the length of a given structure to the length of the buccal tube expressed as a percentage (Pilato 1981).

CHARACTER	μm	pt
Body length	180	662
Buccal tube length	27.2	—
Pharyngeal tube length	6.8	25.0
Buccopharyngeal tube length	34.0	125.0
Buccal/pharyngeal tube length ratio	400%	_
Stylet support insertion point	25.0	91.9
Buccal tube external width	2.3	8.5
Buccal tube internal width	1.3	4.6
Placoid lengths		
Macroplacoid 1	2.0	7.2
Macroplacoid 2	2.8	10.1
Macroplacoid 3	2.3	8.4
Macroplacoid row	12.2	44.9
Claw 1 heights		
External primary branch	4.9	18.0
External secondary branch	4.7	17.3
Internal primary branch	5.4	19.9
Internal secondary branch	4.8	17.6
Claw 2 heights		
External primary branch	5.8	21.3
External secondary branch	5.1	18.8
Internal primary branch	6.2	22.8
Internal secondary branch	6.0	22.1
Claw 3 heights		
External primary branch	6.7	24.6
External secondary branch	5.0	18.4
Internal primary branch	5.3	19.5
Internal secondary branch	4.3	15.8
Claw 4 heights		
Anterior primary branch	6.8	24.8
Anterior secondary branch	5.6	20.4
Posterior primary branch	8.0	29.4
Posterior secondary branch	7.2	26.5

Remarks: Eyes absent. Cuticle smooth. Buccal tube long and narrow; pharyngeal bulb oval without placoids. Claws of the *Hypsibius* type (the secondary branch and the basal section form a continuous curve; claws of the same leg are different in size and shape). This species was described from Itaquaquecetuba, São Paulo State, Brazil (de Barros R 1939a), and reported in Argentina (Maucci 1988). In Brazil, this is the first report outside the type locality.

Genus Adropion Pilato, 1987

Adropion sp.

Figure 5 (D) **Examined material:** One specimen, unidentified sex.

Remarks: Eyes absent. Cuticle smooth. Cuticular thickening between the buccal tube (rigid) and the pharyngeal tube (flexible) absent. Pharyngeal apophyses are small. Three macroplacoids present; microplacoids present. Claws of the *Hypsibius* type.

Discussion

Meiofauna organisms are represented in ½ of all known metazoan taxa (Giere 2009, Cerca et al. 2018) and it is impossible to apply a unique methodological protocol to perform fixation and preparation for all meiofaunal taxa (Fonseca et al. 2018). As a consequence, a large amount of these tiny animals, that have small, fragile bodies, remains poorly studied by researchers since the process of sorting and identification is intensive and time sensitive (Appeltans et al. 2012, Fonseca et al. 2018, Garraffoni et al. 2019b, Balsamo et al. 2020).

The examination of living microscopic organisms requires patience from the taxonomists, especially those that are beginners in meiofaunal identification. The small size and transparency of the body of meiofaunal individuals make them difficult to be discovered among sediments and for subsequent steps such as collecting them by pipette and isolating them for further studying (Giere 2009, Balsamo et al. 2014, 2020, Nelson et al. 2015). Furthermore, gastrotrichs must be studied alive and are frequently destroyed or even lost during the process of preparation for observations under light microscopy (Balsamo et al. 2014, 2020, Garraffoni et al. 2019b).

If the specimens are not properly fixed, they can easily vanish or not stay in a suitable position for future observations of the morphological features (Nelson et al. 2015, Fonseca et al. 2018, Garraffoni et al. 2019b, Balsamo et al. 2014, 2020). In these cases, due to losses of important morphological information, the accuracy of the species identification decreases, and misidentifications are inevitable (Schill 2018). Even in tardigrades that have an exocuticle that remains preserved after the fixation process, morphological information of taxonomic importance can be lost (Morek et al. 2016).

This study is a direct result of undergraduate lectures that aimed to teach not only meiofaunal diversity but also how to collect, sort, and identify these tiny animals, using gastrotrichs and tardigrades as study models. Thus, all the problems described above were considered and largely overcome by the students during the lectures. As a result, we found at least five morphospecies of Gastrotricha and 12 of Tardigrada, but within these 17 morphospecies, only seven of them (41,2%) were identified at a specific level. It is important to highlight that we could not find more morphospecies of Gastrotricha because many specimens were poorly fixed or deteriorated, preventing proper identification beyond the genus level.

Regarding the gastrotrichs, two of the three identified species and all unidentified morphotypes belong to the most specious genus in the taxon, *Chaetonotus* Ehrenberg, 1830 with more than 250 species formally described (Balsamo et al. 2020). The species *Chaetonotus dadayi* was found in Brazil for the first time and these samples will be important to provide material for a near future redescription of *this species* providing detailed morphological and ecological data.

The morphotype *Chaetonotus* sp. 1 has a remarkable feature of nine long spines arranged in three subsequent groups of three spines in the middle of the trunk on the dorsal surface. These conspicuous spines in the middle of the trunk are found in other representatives of this taxon and can be arranged in a "belt", in a subsequent transverse row, or inserted in large nearby scales forming a grouping (Araújo et al.



Figure 5. DIC photomicrograph of Tardigrada. A: Dorsal view of *Macrobiotus* sp 1. B: Buccal apparatus of *Minibiotus* sp. C: Ventral view of *Itaquascon umbellinae*. D: Buccal apparatus of *Adropion* sp. Scale bars: 50 µm.

Table 4. Measuremen	s [in μm] o	of selected	morphological	structures
of Itaquascon umbelli	ae de Barr	os R, 1939	a.	

CHARACTER	μm
Body length	243
Buccal tube external width	3.9
Buccal tube internal width	3.3
Claw 1 heights	
External	9.0
External primary branch	6.7
External secondary branch	5.0
Internal	6.1
Claw 2 heights	
External	10.6
External primary branch	7.5
External secondary branch	5.4
Internal	5.9
Claw 3 heights	
External	9.9
External primary branch	7.2
External secondary branch	4.5
Internal	5.8
Claw 4 heights	
External	11.4
External primary branch	8.8
External secondary branch	6.3
Internal	6.4

2013). Kisielewski (1991) reported two species of *Chaetonotus* in Brazil with long spines on the dorsal region, *C. bisacer* and *C. succinctus*. Representatives of these species have spines arranged in a "belt" shape, differing from *C.* sp.1, which presents three spines closely inserted in three subsequent groups of spines. Araújo et al. (2013) also reported an unidentified species of *Chaetonotus* with long spines on the dorsal region, but with five and not nine as described for the present one.

Due to the shape and distribution of the dorsal and ventral scales and a pair of spines over the furcal base, the morphotype *Chaetonotus* sp. 2 is similar to two species reported for Brazil by Kisielewski (1991), *C. intermedius* and *C. breviacanthus*. However, *C.* sp. 2 differs from both species by the presence of a double-edge scale and by the pair of long spines at the furcal base. In comparison to *C. intermedius*, it was not possible to visualize the two pairs of ventral terminal scales and the cuticular pharyngeal rod. Additionally, in comparison to *C. breviacanthus*, conspicuous protuberance at the anterior edges of the hypostomium and the posterior "U" edge of the scales were not observed in any specimens of *C.* sp.2.

Despite the fact that tardigrades have a rigid and armored integument and morphological investigations of living individuals are not required, from the 12 morphotypes of Tardigrada found in the present study, eight were not identified to species level. This occurred because in many species the study of adult morphology alone is not enough for species identification (Nelson et al. 2015, Schill 2018). Very often it is necessary to analyze the eggs because they have essential taxonomic significance and show species-specific ornamentation. In particular, within the taxon Macrobiotidae, limnic and terrestrial tardigrades belonging to the genera *Minibiotus* Schuster, 1980, *Macrobiotus* Schultze, 1834 and *Paramacrobiotus* Guidetti, Schill, Bertolani, Dandekar & Wolf, 2009 (see Dastych 1998, Meyer & Hinton 2009, Kaczmarek et al. 2017), can only be properly identified if the eggs are available.

The genus *Minibiotus* has 50 described species morphologically diverse (Degma et al. 2020). According to recent studies, the results obtained with the use of integrative approach using genetic and detailed morphological data, e.g. in the description of the species *Minibiotus ioculator* Stec, Kristensen and Michalczyk 2020a, can clarify the species relationships and contribute with information for status polyphyletic of the group (Stec et al. 2020a).

The only four species of *Minibiotus* genus reported in Brazil were: *Minibiotus acontistus* (de Barros R 1942), *Minibiotus intermedius* (Plate 1888), *Minibiotus julietae* (de Barros R 1942) and *Minibiotus marcusi* (de Barros R 1942) (de Barros R 1942b, du Bois-Reymond Marcus 1944, Kaczmarek et al. 2015). Two *Minibiotus* morphospecies were sampled in the present study and one of them was very similar to *M. acontistus* described by de Barros R (1942b). Among the genus *Macrobiotus*, one of the most specious groups within Tardigrada, we were only able to highlight that *Macrobiotus* sp. 1 shows a very distinct dorsal cuticle ornamentation.

Three morphospecies of the genus *Paramacrobiotus* were found: *Paramacrobiotus* sp. 1, *Paramacrobiotus* sp. 2 e *Paramacrobiotus* sp. 3. Despite that species belonging to this genus have a very restricted biogeographic distribution range (Kaczmarek et al. 2017), for an accurate description of any *Paramacrobiotus* species it is important to use an integrative taxonomy approach (Stec et al. 2020b). Furthermore, recentely the subgeneric classification was revisited and nomenclature acts and diagnoses were changed (Marley et al. 2018).

The genus *Adropion* Pilato, 1987 is currently composed of 15 species (Degma et al. 2020), and considered a polyphyletic group, represented by three independent evolutionary lineages (Gasiorek & Michalczyk 2020). The unique species reported in Brazil was *Adropion scoticum* Murray, 1905 (de Barros R 1943, du Bois-Reymond Marcus 1944, Kaczmarek et al. 2015). However, we only found a single specimen and a detailed observation of the morphological traits was not possible and *A. scoticum* is considered a complex of species and a reanalysis of the taxon is necessary with an integrative taxonomy approach (Kaczmarek et al. 2015, Duenãs-Cedillo et al. 2020). Thus, we decided to keep as *Adropion sp*.

The last morphotype, *Echiniscus* sp. is very similar to *Echiniscus* succineus Gąsiorek & Vončina 2019, only reported for the type locality in Madagascar. However, the specimen found in Serra do Japi differs from *E. succineus* by the presence of a small denticle on spines D^d (absent in *E. succineus*) and lacks epicuticular thickenings at the edges of the dorsal plates (present in *E. succineus*). Given these morphological differences and considerable geographic distance, they are probably different species.

It is known that Brazilian freshwater and limnoterrestrial meiofauna organisms are poorly studied and their biodiversity is still underestimated (Garraffoni et al. 2017a, Kaczmarek et al. 2015, de Barros RC 2020). This perspective will only change if the number of surveys increases in the coming years. As the number of taxonomists specialized in these groups is very low in Brazil, the opportunity to show these lesser-known taxa to undergraduate students can potentially inspire the next generation of meiofaunal researchers. Despite some problems in sorting and identification routines due to students' inexperience, this study achieved important goals of teaching and provided an immersive experience for the students interested in these tiny animals.

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Author Contributions

Emiliana Brotto Guidetti: Contribution to data collection, data analysis and interpretation, manuscript preparation, and critical revision.

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André Rinaldo Senna Garraffoni: Contribution in the concept and design of the study, data collection, data analysis and interpretation, manuscript preparation, critical revision, and funding acquisition.

Conflicts of Interest

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

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Lizards from the Alto Sertão region of Sergipe state, northeastern Brazil

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Abstract: We present the first detailed inventory of the lizard fauna of Alto Sertão region, west of Sergipe state, based on three field expeditions carried out by the authors and specimens deposited at the Herpetological Collection of Universidade Federal de Sergipe. The three inventories were carried out between 2010 and 2016, in the municipalities of Canindé de São Francisco, Poço Redondo, Monte Alegre de Sergipe, Nossa Senhora da Glória, and Porto da Folha. In total, we sampled 9,485 lizards of 19 species (10 families) of which 10 are new records for the study area. The most abundant species were *Ameivula ocellifera, Tropidurus hispidus* and *T. semitaeniatus*. Thirteen species were considered as widely distributed in Caatinga, five presented a relictual distribution and one was exotic. We suggest that future research focus mainly on non-sampled areas and should prioritize the municipalities of Gararu, Nossa Senhora da Glória and Nossa Senhora de Lourdes since they are localities with little or no sampling records. *Keywords: Squamata; Caatinga; Seasonally Dry Tropical Forest; Species list.*

Lagartos da região do Alto Sertão de Sergipe, nordeste do Brasil

Resumo: Apresentamos o primeiro inventário detalhado da fauna de lagartos da região do Alto Sertão sergipano, nordeste do Brasil, a partir de três expedições de campo realizadas pelos autores e exemplares depositados na Coleção Herpetológica da Universidade Federal de Sergipe. Os três inventários foram realizados entre 2010 e 2016, nos municípios de Canindé de São Francisco, Poço Redondo, Monte Alegre de Sergipe, Nossa Senhora da Glória e Porto da Folha. No total, foram amostrados 9.487 lagartos de 19 espécies (10 famílias), das quais 10 são novos registros para a área de estudo. As espécies mais abundantes foram *Ameivula ocellifera, Tropidurus hispidus e T. semitaeniatus*. Treze espécies foram consideradas com ampla distribuição na Caatinga, cinco apresentaram distribuição relictual e uma exótica. Sugerimos que futuras pesquisas se concentrem principalmente em áreas não amostradas e priorizem os municípios de Gararu, Nossa Senhora da Glória e Nossa Senhora de Lourdes por se tratarem de localidades com pouca ou nenhuma amostragem.

Palavras-chave: Squamata; Caatinga; Floresta Tropical Sazonalmente Seca (FTSS); Lista de espécies.

Introduction

The South American dry open diagonal extends from southwest to northeast of the continent, across Chaco, Cerrado and Caatinga vegetations (Ab'Sáber 1974, 1977, Werneck 2011). The Caatinga is an environment exclusively found in northeastern Brazil, covering 912,529 km² (Prado 2003, Silva et al. 2017). Its topography varies from a flattened to slightly wavy surface, with residual scattered hills and shallow soils, which are often stony with some areas of exposed solid rock (Ross 2006, Rodal et al. 2013). The Caatinga is characterized by a semi-arid climate, with high temperatures and low annual precipitation (Trovão et al. 2007, Rodal et al. 2013); precipitation is highly seasonal and unpredictable with 50 to 70% of the annual rainfall occurring in three consecutive months (Santos & Andrade 1992, Rodal et al. 2013). The combination of unique geomorphological, pedological, and climatic conditions (Fernandes 2006) results in a xeric formation dominated by small shrubs, cacti, and bromeliads (Cardoso & Queiroz 2007). However, there are forests with a rich flora and medium to large trees that are able to form a canopy, currently classified as a Seasonally Dry Tropical Forest (Pennington et al. 2009, Werneck 2011, Silva et al. 2017).

The Caatinga contains unique biodiversity with high endemism; yet, it is seriously threatened by unrestrained habitat degradation (Rodrigues 2003, Mesquita et al. 2017). Of all the semi-arid regions of the world, it is the most populated, with about 27 million people that are mostly low-income, which makes them dependent on the exploitation of natural resources (Silva et al. 2017). Thereby, 46% of Caatinga's original composition is already deforested (MMA 2020). This scenario is aggravated by its poor network of protected areas; currently, protected areas cover only 7.5% of the Caatinga, with only 1% composed of restricted use areas (Tabarelli & Vicente 2004, MMA 2020).

For a long time, lizard fauna of Caatinga was considered to be undersampled, comprising a large proportion of widely distributed species and studies restricted to a few sampling sites (Rodrigues 2003). However, several studies have been published in the past 15 years and have contributed substantially to increasing our knowledge of the species richness and distribution in this region (Mesquita et al. 2017). Mesquita et al. (2017) reported 79 lizards species, similar to that recorded for the adjacent Cerrado (76 species; Brites et al. 2009; Nogueira et al. 2011). Recent studies also have shown moderate to high levels of endemism (Rodrigues 2000, Magalhães et al. 2014, Mesquita et al. 2017). In Sergipe state, little is known about the diversity of lizard fauna. The published studies are limited to some records and deal mainly with the expansion of the geographical distribution of specific species and some small reports of natural history (Delfim et al. 2006, Gouveia et al. 2010, De-Carvalho et al. 2012, Amora et al. 2014, Ferreira et al. 2014, Moura et al. 2015, Caldas et al. 2016a, Mikalauskas et al. 2017). More detailed ecological studies were conducted only in semiarid caatinga of the Monumento Natural Grota do Angico, municipality of Poço Redondo. The first two deal with the population dynamics and behavior of two Tropidurus species (Santana et al. 2011, Gomes et al. 2015) and the last relies on diet composition of six lizard species (Ferreira et al. 2017). To date, no species list has been published in Caatinga areas from Sergipe state. Studies that characterize the diversity of the local herpetofauna, as well as long-term studies of community dynamics, are essential to subsidize conservation planning and management for the sustainable use of natural resources (Silveira et al. 2010). Herein, we present a detailed inventory of lizard species that occur in the Caatinga from the Alto Sertão region of Sergipe state, based on field expeditions carried out by the authors and specimens deposited at scientific collections.

Material and Methods

1. Study area

The Alto Sertão region of Sergipe is part of the Caatinga ecoregion called "Depressão Sertaneja Meridional" (Ab'Sáber 1974, Velloso et al. 2002, Silva et al. 2017), and encompasses seven municipalities: Canindé de São Francisco, Gararu, Monte Alegre de Sergipe, Nossa Senhora da Glória, Nossa Senhora de Lourdes, Poço Redondo, and Porto da Folha (Figure 1). The topography is predominantly smooth and wavy, cut by narrow valleys with dissected slopes (Sá et al. 2004). The vegetation is mainly composed of trees and shrubs, where *Poincianella, Aspidosperma,* and *Jatropha* predominate (Andrade-Lima 1981; Figure 2). According to the Köppen classification, the local climate is BSh, a mixture of arid and semi-arid regions (Alvares et al. 2013). Temperatures are generally high, ranging from 26 to 28 °C and the average precipitation is approximately 500 mm per year (Nimer 1972). Rainfall is seasonal, occurring from April to August (autumn-winter), and the dry season occurs during spring-summer (Nimer 1972, Santos & Andrade 1992).

2. Data collection

We conducted three inventories between 2010 and 2016. The first one had two stages, which lasted 20 days each. They were performed between 18 to 26 September 2010 and 5 to 15 April 2011, in dry and rainy seasons, respectively. The sampling sites were at the municipalities of Canindé do São Francisco (09°38'31"S, 37°47'16"W), Poço Redondo (09°48'21"S, 37°41'06"W), Monte Alegre de Sergipe (10°01'37"S, 37°33'43"W) and Nossa Senhora da Glória (10°13'06"S, 37°25'13"W; Figure 1). We used pitfall traps with drift fences and active search to capture lizards. In each locality, 25 traps were installed linearly, where each trap had four 20 L buckets buried at ground level. They were arranged in a Y-shape and interconnected by five meters long plastic fences, fixed on wooden stakes. The traps were approximately 20 m apart from each other and were checked daily. We also performed active searches during the day and night on random tracks.

In the second inventory, monthly expeditions of five consecutive days each were conducted between January 2010 and December 2011 at Monumento Natural Grota do Angico (09°39'S, 37°40'W; Figure 1), municipality of Poço Redondo. The sampling methods were the same as above, but 32 pitfall traps were installed and divided into three transects with a minimum distance of 1 km between them. Active searches were performed whenever possible.

The third inventory was carried out between 2015 and 2016. Two expeditions were performed in each locality, lizards were caught during the rainy season, between September 2015 and February 2016, and during the dry season, between April and August 2016. The sampled sites were: Fazenda Jerimum in Canindé de São Francisco (9°38'58.44'S, 37°44'5.50"W); Fazenda do Senhor João (9°41'10.56"S, 37°40'59.82"W), Fazenda Angico (9°40'15.72"S, 37°42'23.04"W) and Monumento Natural Grota do Angico (9°39'54.42"S, 37°40'44.10"W), in Poço Redondo; and Comunidade Mocambo in Porto da Folha. The latter was separated in two different locations (Figure 1), one composed by arboreal Caatinga
Lizards from the Alto Sertão of Sergipe, Brazil



Figure 1. Map of South America showing the location of the study site. The outlined map on the right shows the municipalities of Sergipe state, with the Alto Sertão region shaded in blue and the eleven sampling sites in pink, orange and yellow. Sites 1-4 (pink squares) refer to the first inventory: 1) Canindé de São Francisco; 2) Poço Redondo; 3) Monte Alegre de Sergipe; 4) Nossa Senhora da Glória. Point 5 (orange triangle) refers to the second inventory carried out at the Monumento Natural Grota do Angico and points 6-11 (yellow circles) refer to the third inventory: 6) Fazenda Jerimum; 7) Fazenda do Senhor João; 8) Fazenda Angico; 9) Monumento Natural Grota do Angico; 10) Arboreal Caatinga in Comunidade Mocambo; 11) Recently abandoned pastures in Comunidade Mocambo.

(9°46'59.46"S, 37°26'0.36"W) and the other comprised of recently abandoned pastures (9°48'36.70"S, 37°25'59.50"W). Lizards were sampled with pitfall traps with drift fences, arranged as in the second inventory, and active searches were made throughout the day and night. All animals were captured or recorded.

Studies were performed with permits from IBAMA (189/2010-CGFAP), Secretaria do Meio Ambiente e Recursos Hídricos (SEMARH) of the state of Sergipe (2010.04.1008/00104-002 and 032.000.01920/2011-1) and SISBIO-ICMBio (48122-2). Lizards collected in all inventories were deposited in the Coleção Herpetológica da Universidade Federal de Sergipe (CHUFS) or Coleção Herpetológica da Universidade Federal da Paraíba (CHUFPB), and a list of the vouchers is presented in Appendix. The number of specimens collected in each inventory followed the limit imposed by our respective license. However, the other animals captured and released were also computed, since they were identified and marked. Recapture data is not considered in our analysis. Finally, records from CHUFS were also used as complementary data.

3. Statistical analyses

To evaluate the sampling effort, we built an individual-based rarefaction curve (Gotelli & Colwell 2001) coupled with the nonparametric richness estimator Chao2P, using EstimateS v. 9.10 and Microsoft Excel (Colwell 2006, Lopez et al. 2012). The curve was built using 100 randomizations of the original data without replacement. The Chao2P is a factor correction based in Chao2 estimator able to reduce the bias under sampling conditions.

Results

We performed a detailed inventory of Caatinga lizards from Caatinga from the Alto Sertão region of Sergipe and obtained data from 9,485 individuals, representing 19 species and 10 families (Table 1; Figures 3-5). Teiidae and Gekkonidae were the most represented families, with three species each (Table 1). Moreover, *Ameivula ocellifera* (47.95%),



Figure 2. Habitats sampled during the lizards survey at the Alto Sertão region of Sergipe state, Brazil. A, B) Typical arboreal and arbustive Caatinga vegetation in rainy and dry seasons, respectively. C, D) Pitfall trap in open habitat during the rain and dry seasons, respectively. E) The bedrocks of dry streams. F) Rocky outcrops.

Tropidurus hispidus, (19.00%) and *T. semitaeniatus* (13.85%) were the most abundant species (Figure 6). The shape of the rarefaction curve and the estimated value of Chao2P indicated our sampling was adequate and had a low probability of new species being collected with additional samples (Figure 7). Finally, considering the Caatinga distribution patterns proposed by Rodrigues (2003), we observed that 73.68% of the sampled species were widely distributed (14 species, one being invasive) and 26.32% had a relictual distribution (five species; Table 1).

Discussion

We here present ten new records of lizards for the Alto Sertão region of Sergipe. The species are *Hemidactylus brasilianus*, *H. mabouia* (Gekkonidae), *Acratosaura mentalis, Vanzosaura multiscutata* (Gymnophthalmidae), *Iguana iguana* (Iguanidae), *Psychosaura agmosticha*, *P. macrorhyncha* (Mabuyidae), *Polychrus acutirostris* (Polychrotidae), *Coleodactylus meridionalis* (Sphaerodactylidae), *Ameiva ameiva* and *Salvator merianae* (Teiidae). These species already have their occurrence registered for the Caatinga region, but has no previous records for Caatinga from Alto Sertão region of Sergipe state.

The species richness observed in the Alto Sertão region (n = 19) was higher or equal than that from eight studies conducted in the Caatinga (Rodrigues 1986, Vitt 1995, Borges-Nojosa & Cascon 2005, Freire et al. 2009, Garda et al. 2013, Cavalcanti et al. 2014, Magalhães et al. 2015, Caldas et al. 2016b), which averaged 17 ± 1.30 lizard species. We highlight PARNA Chapada Diamantina (n = 15; Magalhães et al. 2015) and Estação Ecológica Raso da Catarina (n = 19; Garda et al. 2013) as they are the closest to the Alto Sertão region of Sergipe State. However, the richness found in the present study is lower than that recorded for the sand dunes of the São Francisco river (n = 30 species; Rodrigues 1996), PARNA Catimbau (n = 25; Pedrosa et al. 2014), and Araripe bioregion (n = 27; Ribeiro et al. 2012). This variation in species richness may be result of different sampling efforts, differences in sample sizes or simply intrinsic differences of the locations.

Although our sampling effort seemed adequate, other species could be registered with the addition of new sampling points (Cornell & Lawton 1992). For example, *Psilops paeminosus* was previously recorded in Canindé de São Francisco (Delfim et al. 2006), but we did not record this species. Other species have also been observed in nearby locations and were not found in this study, such as *Tropidurus cocorobensis*, which occurs in sandy areas between Bahia and Pernambuco (Rodrigues 2003), and *Micrablepharus maximiliani*, whose type locality is the state of Sergipe and has a wide distribution in Caatinga (Rodrigues 1996, Moura et al. 2010). Thus, we suggest that new samplings be carried out in unsampled areas of the municipalities in the Alto Sertão region of Sergipe state. New efforts should prioritize the municipalities of Gararu, Nossa Senhora da Glória and Nossa Senhora de Lourdes since they are localities with little or no sampling records.

The lizards Ameivulla ocellifera, Tropidurus hispidus, and T. semitaeniatus were predominant in the study area. These species are widely distributed throughout Caatinga, and A. ocellifera may also occur in coastal Restingas, being more common in open areas, occupying sandy soils and high insolation (Vitt 1995, Werneck et al. 2009, Oliveira et al. 2015). Tropidurus hispidus is widely distributed throughout Caatinga, Cerrado, and Restingas (Rodrigues 1987). It is considered a habitat-generalist and can be found on rock surfaces, forest edges, tree trunks, fences, and walls, among other substrates (Rodrigues 1987, Vitt et al. 1997, Van-Sluys et al. 2004, Carvalho et al. 2005). Although, T. semitaeniatus is widespread in Caatinga, it has high fidelity to rocky microhabitats (Freitas & Silva 2007, Gomes et al. 2015). Niche differences in microhabitat use, added to trophic and temporal differences, can be the main reason for the high abundance of these species, since smaller overlays can decrease the effect of competition between them (Pianka 1973, Giacomini 2007).

Ten other species recorded in this study are also considered widely distributed in Caatinga. Ameiva ameiva, Iguana iguana, Polychrus acutirostris, Salvator merianae and Phyllopezus pollicaris are distributed throughout the dry diagonal in South America, including the Caatinga, Cerrado, and Chaco (Vanzolini et al. 1980, Werneck & Colli 2006, Delfim & Freire 2007, Garda et al. 2012). The teiids A. ameiva and S. merianae occur in different vegetation types, but these lizards seem prefer open vegetation (forest ecotones or disturbed areas) and forest borders, which receive more direct sunlight (natural or not; Vitt & Colli, 1994, Werneck et al. 2009, Sales et al. 2011). Iguana iguana often use vegetation microhabitats close to bodies of water (Lara-López & González-Romero, 2002). Polychrus acutirostris prefers a heterogeneous arboreal environment, but predominantly lower tree branches and twigs and are often found near human habitations (Vanzolini 1974; Renous et al. 2010). Phyllopezus pollicaris has been frequently associated with rocky outcrops, but can be found within a wide range of microhabitats, like trees trunks, fallen logs and human

Table 1. Lizard species from Alto Sertão region of Sergipe state, Brazil. Municipalities = CSF - Canindé de São Francisco; MA - Monte Alegre de Sergipe; NSG - Nossa Senhora da Glória; PF - Porto da Folha; PR - Poço Redondo. Distribution Pattern (DP) = WD - widely distributed; WD(I) - widely distributed (invasive species); RD - relictual distribution; SFR - related with paleoquaternary sandy dunes from São Francisco River. = CHUFS - Herpetological Collection of the Universidade Federal de Sergipe; PS - Present study; 1 - De-Carvalho et al. 2012; 2 - Santana et al. 2011; 3 - Amora et al. 2014; 4 - Ferreira et al. 2014; 5 - Gomes et al. 2015; 6 - Moura et al. 2015; 7 - Caldas et al. 2016a; 8 - Ferreira et al. 2017; 9 - Mikalauskas et al. 2017. * = visual record; ** = not collected.

Taxon	Municipalities	DP	Source
Anguidae			
Diploglossus lessonae (Peracca 1890)	MA	RD	7, PS
Gekkonidae			
Hemidactylus brasilianus (Amaral, 1935)	CSF, PR	WD	PS
Hemidactylus mabouia (Moreau de Jonnès, 1818)	CSF, MA, PR	WD(I)	PS
Lygodactylus klugei (Smith et al., 1977)	CSF, MA, NSG, PF, PR	WD	8, CHUFS, PS
Gymnophthalmidae			
Acratosaura mentalis (Amaral, 1933)	CSF, MA, PF, PR	RD	PS
Vanzosaura multiscutata (Amaral, 1933)	CSF, MA, NSG, PF, PR	WD	PS
Iguanidae			
Iguana iguana (Linnaeus, 1758)	CSF*, MA, PF, PR	WD	CHUFS, PS
Mabuyidae			
Brasiliscincus heathi (Schmidt and Inger, 1951)	CSF, MA, PF, PR	WD	8, CHUFS, PS
Psychosaura agmosticha (Rodrigues, 2000)	PR	RD	PS**
Psychosaura macrorhyncha (Hoge, 1947)	MA	RD	PS
Phyllodactylidae			
Gymnodactylus geckoides (Spix, 1825)	CSF, MA, PF, PR	WD	1, 8, CHUFS, PS
Phyllopezus pollicaris (Spix, 1825)	CSF, PF, PR	WD	3, CHUFS, PS
Polychrotidae			
Polychrus acutirostris (Spix, 1825)	PF*, PR	WD	CHUFS, PS
Sphaerodactylidae			
Coleodactylus meridionalis (Boulenger, 1888)	MA, PF	RD	PS
Teiidae			
Ameiva ameiva (Linnaeus, 1758)	MA, PF, PR	WD	PS
Ameivula ocellifera (Spix, 1825)	CSF, MA, NSG, PF, PR	WD	6, 8, CHUFS, PS
Salvator merianae (Duméril and Bibron, 1839)	CSF*, PF*, PR	WD	PS
Tropiduridae			
Tropidurus hispidus (Spix, 1825)	CSF, MA, NSG, PF, PR	WD	2, 4, 5, 8, CHUFS, PS
Tropidurus semitaeniatus (Spix, 1825)	CSF, MA, PF, PR	WD	2, 5, 8, 9, CHUFS, PS

edifications (Vanzolini et al. 1980, Vitt 1995, Recoder et al. 2012). This species has also been recorded in the Atlantic Forest, a humid forest located in Brazilian coast (Feio & Caramaschi, 2002).

Brasiliscincus heathi, Hemidactylus brasilianus and Gymnodactylus geckoides, in addition to the Caatinga, also permeate neighbor environments, such as the Cerrado and Atlantic Forest (Mesquita et al. 2006, Freitas & Silva 2007, Freitas 2014, Travassos et al. 2015), while *B. heathi* preferentially inhabits areas with bromeliads (Rodrigues 2003). *Hemidactylus brasilianus* can be found both in trees and in the soil and *G. geckoides* are most commonly seen under logs fallen or rocks and moving in litter (Vanzolini et al. 1980, Vitt 1995, Souza et al. 2013, Mesquita et al. 2017). Finally, despite occurring almost exclusively in Caatinga, *Lydogactylus klugei* also inhabit trees in SDTF enclaves of

the Cerrado and *V. multiscutata* has a single isolated population in a dry forest habitat in the eastern Cerrado (Recoder et al. 2014). In Caatinga, *V. multiscutata* occur in litter and in other areas with little vegetation cover, but in areas with high cacti abundance, since they seem to prefer clumps in this type of plants (Delfim & Freire 2007).

Five species present relictual distribution in Caatinga. *Psychosaura agmosticha* and *P. macrorhyncha* are more associated with bromeliads, especially *Bromelia laciniosa* (Rodrigues 2003). These species are also distributed in Restinga, an ecosystem associated with the Atlantic Forest, occurring all along the Brazilian coast (Rodrigues 2003, Dias & Rocha 2014). Ultimately, *Acratosaura mentalis, Coleodactylus meridionalis* and *Diploglossus lessonae* are closely associated with forest environments, be can found in leaf litter (Rodrigues et al. 2005, Mesquita et al. 2017).



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Figure 3. Lizards from Alto Sertão region of Sergipe state, Brazil: A) *Diploglossus* lessonae; B) *Hemidactylus brasilianus*; C) *Hemidactylus mabouia*; D) *Lygodactylus* klugei; E) *Acratosaura mentalis*; F) *Vanzosaura multiscutata*; G) *Iguana iguana*; H) *Brasiliscincus heathi.* A, C, F, G–specimens were recorded in municipality of Monte Alegre; B, D, E, H – specimens were recorded in municipality of Poço Redondo. Photos by Daniel Mesquita, Daniel Santana and Renato Faria.

This type of distribution may be related to the theory of Pleistocene refuges, which postulates that the decline of forests and expansion of xeric environments during the Quaternary promoted the isolation of these species locked into the relictual vegetation where they occurred previously (Haffer 1969, Vanzolini & Williams 1970, Vanzolini & Williams 1981). *Acratosaura mentalis* occurs in mesic habitats and relictual forests in the Caatinga, in semidecidual forests in the Cerrado and in the agreste, a transition zone between the Atlantic Forest and the semi-arid Caatinga, in which species from both biomes coexist (Queiroz & Lema 1996, Rodrigues et al. 2009, De-Carvalho et al. 2010); *C. meridionalis* is a typical Atlantic Forest species (Vanzolini et al. 1980); and *D. lessonae* occurs in all of northeastern Brazil, both in the Atlantic Forest, in the agreste and in the caatingas (Vanzolini et al. 1980, Caldas et al. 2016a).

The only introduced species recorded in this work is *Hemidactylus mabouia*. It is dispersed from Africa and is widely distributed in the Caribbean, South America, and Florida (Vanzolini 1978, Gamble et al. 2011). It is generally found in urban environments but can also be found in several Brazilian environments (Rödder et al. 2008).

Finally, lizard fauna of the Caatinga in Sergipe is still poorly known when compared to other states that are also located in this environment (Rodrigues 2003). Although recent studies have been made about



Figure 4. Lizards from Alto Sertão region of Sergipe state, Brazil: A) *Psychosaura agmosticha*; B) *Psychosaura macrorhyncha*; C) *Gymnodactylus geckoides*; D) *Phyllopezus pollicaris*; E) *Polychrus acutirostris*; F) *Coleodactylus meridionalis*; G) *Ameiva ameiva*; H) *Ameivula ocellifera*. A, D, E, G, H – specimens recorded in municipality of Poço Redondo; B, C, F – specimens recorded in municipality of Monte Alegre. Photos by Daniel Mesquita, Daniel Santana and Renato Faria.



Figure 5. Lizards from Alto Sertão region of Sergipe state, Brazil: A) Salvator merianae, adult; B) Salvator merianae, juvenile; C) Tropidurus semitaeniatus; D) Tropidurus hispidus. Specimens recorded in municipality of Poço Redondo. Photos by Renato Faria and Stéphanie Rocha.



Figure 6. Relative and absolute abundances of the lizard species of the Alto Sertão region of Sergipe state, Brazil (N = 9,485). The numbers next to the black bars refer to the absolute abundances of each species.



Figure 7. Individual-based rarefaction curve (line) and Chao2P species estimator (circles) for lizard species recorded in Alto Sertão region of Sergipe state, Brazil.

ecology and geographical distribution of lizards, much work should be carried out for achieving more knowledge on Caatinga's saurofauna. Therefore, we suggest that future research focus mainly on non-sampled areas, due to this prior knowledge of species distribution, so that more complex studies can be developed.

Supplementary Material

The following online material is available for this article: Appendix

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Conflicts of Interest

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

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Brown booby (Sula leucosgaster) envenomation and death caused by toadfish (Porichthys porosissimus) ingestion in Southeastern Brazil

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Abstract: A case report of six brown booby *(Sula leucogaster)* envenomation following the ingestion of toadfish *(Porichthys porosissimus)* specimens in Rio de Janeiro, Southeastern Brazil is discussed herein. Several macroand microscopic pathological alterations were observed in the examined brown booby specimens, and rapid envenomation (<24 h) is suggested due to the digestion stage of the fish found in their gastrointestinal tracts. This is the first report to mention the death of adult and juvenile brown boobies due to envenomation by the toadfish *P. porosissimus.* Further studies assessing the role of discarded fish as a driving force of negative effects on seabirds, such as envenomations, for example, along the southeastern coast of Brazil are recommended. *Keywords: Fatal ingestion; poisonous fish; seabirds; Western Atlantic*

Envenenamento e morte de atobás-marrons (Sula leucogaster) causados pela ingestão de magangá-liso (Porichthys porosissimus) no sudeste do Brasil

Resumo: Um relato de caso sobre o envenenamento de seis indivíduos de atobás-marrons (Sula leucogaster) após a ingestão de espécimes de mangagá-liso (Porichthys porosissimus) no Rio de Janeiro, sudeste do Brasil, é discutido neste estudo. Diversas alterações patológicas macro e microscópicas foram observadas nos espécimes de atobámarrom examinados, e um envenenamento rápido (<24 h) é sugerido devido ao estágio de digestão dos peixes encontrados em seus tubos digestórios. Este é o primeiro registro que menciona a morte de indivíduos juvenis e adultos de atobás marrons por envenenamento devido a ingestão do magangá-liso P. porosissimus. Novos estudos avaliando o papel dos peixes descartados como uma força motriz dos efeitos negativos sobre as aves marinhas como, por exemplo, envenenamentos, ao longo da costa sudeste do Brasil são recomendados. **Palavras-chave:** Ingestão fatal; peixes venenosos; aves marinhas; Oeste Atlântico.

Introduction

The toadfish, *Porichthys porosissimus* (Cuvier, 1829) (Teleostei, Batrachoididae) is found in in marine and brackish environment in the Southwest Atlantic, ranging from Espírito Santo, Brazil to eastern Argentina (Floeter et al. 2003, Figueiredo et al. 2002). This species is routinely caught as by-catch, especially through shrimptrawling activities (Vianna et al. 2000)discarded due to their having no commercial va. Typical toadfish are nocturnal and bury themselves in sand or mud in the intertidal zone during the day and inhabit the water column by (Vianna et al. 2000)discarded due to their having no commercial va. Most present corporal bioluminescent photophores on the lateral of the body and head, probably reproductive in nature, and some species, have venomous dorsal spines and are capable of inflicting serious injuries (Cormier et al. 1967, Greenfield et al. 2008).

Among toadfish, *Porichthys porosissimus* has been recently proven venomous after much discussion in the literature, although only one study on envenoming by this species is available in the literature, regarding two human cases (Lopes-Ferreira et al. 2014). No reports concerning wildlife envenomation have been reported to date.

In this context, this study consists in a case report of the death of six brown booby (*Sula leucogaster*) individuals following the ingestion of this venomous toadfish in Rio de Janeiro, Southeastern Brazil. Evidence of Brown booby tissue alterations are discussed.

Material and methods

The individuals in the present study were found dead on beaches in the Região dos Lagos area, on the coast of the state of Rio de Janeiro, through the Campos Basin Beaches Monitoring Project. The cases were detected in July and August 2019, in the municipalities of Arraial do Cabo, Cabo Frio and Armação dos Búzios (Figure 1). The carcasses were classified as "fresh" or "moderately fresh" (Van Franeker 2004) and were processed and necropsied at the Araruama Rehabilitation and Depetrolization Center.

Carcass weights were determined using a digital scale. Total length measurements were performed using a tape measure. Sexing was determined by means of sexual dimorphism, represented by the physical characteristics and plumage present in adults, and by the gonads in the juvenile individual (Schreiber & Norton 2002).



Figure 1. Map indicating the locations of the Brown Booby samplings, Rio de Janeiro, Southeastern Brazil.

The necroscopic exams were performed according to Work (2000). The organs were individually analyzed, and macroscopic changes were described according to the findings, categorized as musculoskeletal, respiratory, cardiovascular, digestive, urinary, reproductive, hemolymphatic, endocrine and nervous system alterations. All information was recorded by photographs, and autopsy reports were completed. Organ samples classified as "fresh" were collected for histopathological evaluations. The samples were first fixed in 10% neutral buffered formaldehyde for at least 48 hours and subsequently sent to the Animal Morphology and Pathology Laboratory at the Darcy Ribeiro Norte Fluminense State University (UENF), cleaved and taken to a tissue processor (Leica ASP300S) for dehydration, clarification, paraffin impregnation and subsequent block inclusion. After the inclusion process, serial 5 µm slices were obtained and stained with hematoxylin and eosin (HE). An optical Leica DM4B microscope (Leica microsystem CMS GmbH, Wetzlar) was used for the microscopic analysis and photomicrographs.

Toadfish specimens found in the digestive tract of the brown boobies were preserved in alcohol following necropsies. Identification was confirmed as *P. porosissimus* after comparison with specimens deposited at the Ichthyological Section of the Museu Nacional/UFRJ. The specimens corroborate previous descriptions for the species (Fishbase 2019, Nakamura et al. 1986, Figueiredo & Menezes 1980) namely scaleless, exhibiting four lateral lines and displaying two dorsal spines, between 34-36 dorsal soft rays, no anal spines, and 33 anal soft rays. The upper side of the specimens' body was greyish brown, silvery on the side and yellowish on the belly, containing seven dark broad bands on the upper portion of body. The tips of each fin were blackish, dark blotches were noted on the fin membrane of the dorsal and pectoral fins and the pelvic fin was yellowish. Two specimens are now stored at the Biodiveristy Collection (*Coleção de Biodiversidade*), IOC/Fiocruz under numbers "IOC/Peixes 069" and "IOC/Peixes 070".

Results and discussion

The Brown booby (*Sula leucogaster*) inhabits tropical and subtropical regions and breeds along the Brazilian coast (Cunha et al. 2012). When juvenile, this species travels up to 2,000 km from their hatching site, and after approximately three years return to their original colonies to breed (Nelson 1978, Baumgarten 2003). This species is considered a top predator, foraging primarily on fish and squid residing near their breeding colonies (Alves et al. 2004, Coelho et al. 2004).

From February to November 2019, a total of 91 brown booby specimens were necropsied at the Araruama Rehabilitation Center. A total of 43 individuals died during treatment and 48 were already found dead. Of this total, six presented envenomation by *P. porosissimus* (6.6%). Of the six envenomated examined specimens, five were adults (three females and two males) and one juvenile (male). Females weighed 1.0-1.2 kg (1.1 kg \pm 0.1), averaging 75.97 cm in total length, and males weighed 0.8-1.1 kg (0.93 kg \pm 0, 15), with an average total length of 76.9 cm.

Figure 2 exhibits a *P. porosissimus* specimen observed in the gastroesophageal lumen of a brown booby specimen, and different histopathological brown booby alterations.

Macroscopic findings were observed in the cutaneous, respiratory, and digestive systems. Of the six individuals, three presented severe subcutaneous hemorrhage in the cervical region, severe esophageal congestion, and hemorrhagic cavitary effusion in the celomatic cavity. The other specimen presented hemorrhagic fluid, over-hyperemic mucosa, and areas of mild edema in the esophageal and gastric mucosae. All six necropsied brown boobies with such lesions had one or more *P. porosissimus* specimens in the gastrointestinal tract, and no piercing-cutting lesions were detected. In two specimens, toadfish were found



Figure 2. (A) *P. porosissimus* in the gastroesophageal lumen. Note the esophagitis associated with hemorrhagic exudate (arrow); (B) Slight heterophilic (arrow) inflammatory infiltrate, randomly distributed in the esophageal lamina-propria; (C) Severe parabronchial hemorrhage (asterisk) and diffuse congestion. Mild macrophages that contain a fine black pigment (carbon).

only in the stomach and, in the others, in the esophagus and stomach. All individuals presented hemorrhage and severe pulmonary congestion.

Microscopically, a fibrinemorrhagic exudate over the esophageal mucosa was observed, as well as a slight heterophilic inflammatory infiltrate randomly distributed in its own lamina, associated with diffusely ectatic blood vessels, markedly and diffusely congested in the esophageal submucosa. Pulmonary blood capillaries filled with blood, markedly ectatic associated with marked multifocal parabronchial hematic overflow, a diffuse marked interstitial edematous accumulation and moderate to diffuse edematous edema were also observed. Aerial capillaries were multifocal and moderately collapsed.

Due to the decomposition stage and digestion process of the fish, accurate measurements could not be performed. However, all specimens were approximately 10 cm in total length, and possibly young specimens, given that the largest *P. porosissimus* recorded in the literature measured 32 cm (Figueiredo & Menezes 1978).

Marine fish present an enormous diversity and complexity of venoms and poisons, such as in the Scorpaenidae, Tetraodontidae, Dasyatidae and Batrachoididae families (Smith & Wheeler 2006). The absence of a venom gland attached to *P. porosissimus* dorsal and opercular spines, and the fact that the spines are solid and not hollow has led researchers to classify this species as non-venomous (Lopes-Ferreira et al. 2014). However, the presence of a pectoral axillary gland (Collette & Russo 1981), glandular tissue on the pectoral fin (Walker & Rosenblatt 1988) and two curved opercular spines containing a yellow mass, probably the venom glandular tissue (Lane 1967), have indicated probable venomous status. This was confirmed in a study carried out in 2014, regarding two human cases and laboratory mice assessments (Lopes-Ferreira et al. 2014).

Mice exposed to P. porosissimus spine extract displayed edematogenic and nociceptive responses, the former indicating severe inflammatory activity, through classical cellular recruitment initiated by neutrophils followed by macrophages and increased leukocyte rolling, with a slightly increase in adherent cells to the endothelium, while nociception induced during the inflammatory period (15-40 min) was associated with the augmented rolling and adhesion of leukocytes to the endothelium (Lopes-Ferreira et al. 2014). Exposure to P. porosissimus spine extracts also decreased the blood flow and caliber of vessels participating in microcirculation (Lopes-Ferreira et al. 2014). These edematogenic and nociceptive responses and organ alterations are similar to inflammatory responses induced by other venomous fish, such as other Batrachoididae species like T. nattereri and T. maculosa, Potamotrygonidae freshwater stingrays and Ariidae marine catfish (Magalhães et al. 2006, Monteiro dos Santos et al. 2011, Junqueira et al. 2007, Sosa-Rosales et al. 2005)local, and intense pain, soft tissue edema, and a variable extent of bleeding. The present study was carried out in order to describe the principal biological and some biochemical properties of the Brazilian Potamotrygon fish venoms (Potamotrygon cf. scobina and P. gr. orbignyi.

The only account in the literature regarding *P. porosissimus* spine puncture effects indicates intense and continuous pain, lasting about 2 hours (Lopes-Ferreira, et al., 2014), and a previous citation about this venom indicates arteriolar constriction, although no further details are given (Lane 1967). One study attributed the death of five procellariiform species – Atlantic Yellow-nosed Albatross (*Thalassarche chlororhynchos*), Cory's Shearwater (*Calonectris diomedea*), Sooty

Shearwater (*Ardenna grisea*), Great Shearwater (*Ardenna gravis*) and Manx Shearwater (*Puffinus puffinus*), in southern Brazil to the ingestion of *P. porosissimus* and subsequent suffocation (Benemann et al. 2016)we recorded five procellariiform species - Atlantic Yellow-nosed Albatross (Thalassarche chlororhynchos. The authors only briefly mention that the potential effect of *P. porosissimus* toxins might have contributed to the evaluated seabird deaths, and it is important to note that they observed only 22 cases during 6 years of surveys (63 surveys).

In the present study, envenomation by *P. porosissimus* through spine punctures was observed in six brown booby carcasses found on the eastern coast of Rio de Janeiro state, Brazil. The pathological changes detected in the examined specimens are in accordance with the findings by Lopes-Ferreira (2014), suggesting that the effect of the poison in contact with the bird's gastric mucosa resulted in negative flight and fishing activity effects, possibly causing trauma, consequent drowning and death, mainly due to severe pain. It is also suggested that the death of these seabirds occurs acutely (<24 hours), due to the digestion stage of the fish found in their gastrointestinal tracts (Hilton et al. 2000). The present report is the first to attribute the venomous toadfish *P. porosissimus* as the cause of death of adult and juvenile brown boobies (*S. leucogaster*). Further studies should evaluate this negative relationship between fish and seabirds along the Brazilian coast.

Author contributions

Rachel Ann Hauser-Davis: 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.

Amanda Dias Tadeu: 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.

Ana Carolina da Silva Scarelli: 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.

Hassan Jerdy: 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.

Eulogio Carvalho: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Sergio Carvalho Moreira: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Salvatore Siciliano: 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.

Conflict of interest

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

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From the headwaters to the Iguassu Falls: Inventory of the ichthyofauna in the Iguassu River basin shows increasing percentages of nonnative species

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Abstract: Knowledge of the ichthyofauna of a hydrographic basin is the minimum necessary condition for the implementation of any measures for the exploration, management or preservation of water and fishing resources. Despite its relevance, the number of fish species across the Iguassu River basin is still uncertain. Thus, the objective of this study was to compile the fish species that occur in the extensive stretch of the basin above the Iguassu Falls. In addition, we recorded the level of threat of extinction for native species, the origin of nonnative species, and their main vectors of introduction. To achieve this goal, a survey was carried out through consultations with ichthyological collections as well as online databases. Also, a literature review was conducted using the search platforms Thomson Reuters, SciELO and Elsevier's ScienceDirect to locate all articles published by March 2020 that addressed the topic "ichthyofauna in the Iguassu River basin". The survey compiled a total of 133 fish species distributed in nine orders, 29 families and 72 genera. Seventy-nine fish species were recorded that occur throughout the entire length of the basin, 119 species that occur in the hydrographic units of the middle and lower Iguassu River (40 exclusive) and 93 species that occur in the hydrographic unit of the upper Iguassu River (14 exclusive). The endemism rate shown here for the Iguassu River basin (approximately 69%) contrasts with the 40 nonnative fish species recorded (approximately 30% of the total species in the basin). Successive impoundments, reductions in habitat quality and the increase in the number of nonnative species are the main threats to native species, especially to the endemic species; approximately 20% of these species were listed in some category of threat of extinction. We emphasize that constant monitoring of ichthyofauna is necessary to discover putatively undescribed species, as well as for the application of management strategies to mitigate the negative effects and promote the control of the spread of nonnative species.

Keywords: Extinction, risk; Fish; Introduced, species; List of species; Paraná, State.

Das cabeceiras às Cataratas do Iguaçu: inventário da ictiofauna da bacia do rio Iguaçu mostra aumento na porcentagem de espécies não nativas

Resumo: Conhecer a ictiofauna de uma bacia hidrográfica compreende condição mínima necessária para que se possam implantar quaisquer medidas de exploração, manejo ou preservação dos recursos hídricos e pesqueiros. Apesar de sua relevância, o número de espécies de peixes de toda a bacia hidrográfica do rio Iguaçu ainda é incerto. Assim, o objetivo deste estudo foi compilar as espécies de peixes que ocorrem no extenso trecho da bacia acima das Cataratas do Iguaçu. Além disso, registramos o nível de ameaça de extinção às espécies nativas, a origem das espécies não nativas e suas principais vias de introdução. Para isso foi realizado um levantamento por meio de consultas a coleções ictiológicas, bem como aos bancos de dados online, além de revisão de literatura por meio do uso das plataformas de buscas Thomson Reuters, Scielo e Elsevier – ScienceDirect, que abordavam o tópico "ictiofauna da bacia do rio Iguaçu" e o período de tempo incluiu todos os trabalhos publicados até março de 2020.

O levantamento compilou ao todo 133 espécies, distribuídas em nove ordens, 29 famílias e 72 gêneros. Em toda a extensão da bacia foram registradas 79 espécies de peixes. Nas unidades hidrográficas do médio e baixo rio Iguaçu foram registradas 119 espécies (40 exclusivas) e na unidade hidrográfica do alto rio Iguaçu foram registradas 93 espécies (14 exclusivas). A taxa de endemismo para a bacia do rio Iguaçu (aproximadamente 69%) contrasta com as 40 espécies de peixes não nativos registradas (aproximadamente 30% do total de espécies na bacia). Os sucessivos barramentos, a perda de qualidade de habitats e o aumento no número de espécies não nativas são ameaças às espécies autóctones, especialmente às endêmicas, as quais apresentam aproximadamente 20% listadas em alguma categoria de ameaça ao risco de extinção. Ressaltamos que o monitoramento constante da ictiofauna é necessário para a descoberta de espécies supostamente não descritas, bem como para a aplicação de estratégias de manejo para mitigar os efeitos negativos e promover o controle da disseminação de espécies não nativas. *Palavras-chave: Risco de extinção; Peixes; Espécies, introduzidas; Lista de espécies; Estado do Paraná.*

Introduction

The increase in human population and, consequently, human activities has increasingly changed ecosystems around the world, especially aquatic ones (Azevedo-Santos et al. 2019). Aquatic environments form a mosaic of habitats, from headwaters in mountainous regions to estuaries, shallow coastal habitats, reefs and seas (Arthington et al. 2016). For these environments, more than 30,000 fish species have already been described (Nelson et al. 2016), many of which are in danger of becoming locally or globally extinct (Darwall & Freyhof 2015). In this way, for the human use of natural resources from aquatic ecosystems, knowledge of the local ichthyofauna is the minimum necessary condition for the implementation of any measures of exploration, management or preservation for water and fish resources (Cavalli et al. 2018).

In different freshwater environments from different hydrographic basins, ichthyofaunal sampling has been performed and improved over the years; however, several species are still unknown to science (Langeani et al. 2007, Ota et al. 2015). Thus, these species are in danger of disappearing even before they are described and their real geographic distributions are known (Hortal et al. 2015). For the Iguassu River basin, which is one of the main tributaries of the left margin of the Paraná River, new inventories have revealed possible new species (Frota et al. 2016a, for example), many of which are endemic and could fall into categories with a marked risk of extinction.

The high endemism of the ichthyofauna in the hydrographic basin of the Iguassu River makes it a freshwater 'ecoregion' (Zawadzki et al. 1999, Abell et al. 2008, Baumgartner et al. 2012). This characteristic was possibly due to the isolation promoted by the formation of the Iguassu Falls during the Cretaceous (c. 22 Ma), which separated the ichthyofauna upstream of the Iguassu Falls from those downstream (Parolin et al. 2010). In addition, there are several waterfalls and rapids along the main channel and tributaries that contributed to the isolation of fish populations and, consequently, to the speciation process (Garavello et al. 1997, Baumgartner et al. 2012, Maack 2012, Frota et al. 2016a). As the area has high species richness with a high proportion of endemic fish species, the Iguassu River basin comprises a crucial site for preservation, since local extinctions would certainly result in global species extinctions (Baumgartner et al. 2012). The first studies on fish in the Iguassu River were carried out by Haseman (1911a, b), who described 13 fish species. Despite their significance, the ichthyofauna of the entire hydrographic basin of the Iguassu River are still little known when compared to the ichthyofauna of other large hydrographic systems, for example, the upper Paraná River basin (Delariva et al. 2018). In addition, the construction of dams and the introduction of nonnative fish species, mainly from sport fishing (Ribeiro et al. 2017) and aquaculture (Agostinho et al. 1999, ICMBio 2018), have threatened the native fish species of the Iguassu River (Agostinho et al. 1999, Daga & Gubiani 2012, Daga et al. 2016, Gubiani et al. 2018).

Ichthyofaunal surveys have been carried out in a segmented manner in the Iguassu River basin, especially in the upper and lower stretches of the basin (Baumgartner et al. 2012). In a catalog, Severi & Cordeiro (1994) registered 47 fish species for the Iguassu River basin, while Garavello et al. (1997) recorded 52 fish species only for the Segredo reservoir region. Ingenito et al. (2004), in an ichthyofaunal survey carried out in the upper Iguassu River, recorded the occurrence of 41 fish species that had not previously been mentioned, increasing the total number of species recorded for the entire Iguassu River basin to 84. Baumgartner et al. (2006) registered 41 fish species in the area of influence of the Salto Osório Reservoir, and Baumgartner et al. (2012) increased the number of fish in the region corresponding to the lower Iguassu River to 106 fish species. Therefore, it is noted that there is still no compilation of all studies and sampling efforts carried out that make it possible to report the total number of known fish species for the Iguassu River basin, especially in the extensive stretch of the basin above the Iguassu Falls.

In view of the above, the present study aimed to compile the fish species that occur in the entire Iguassu River basin, from the headwaters to the Iguassu Falls, by reviewing the species lists published in scientific articles and books, as well as the species registration in ichthyological collections. In this study, we recorded the endemic fish species for the middle/lower and upper Iguassu River basin, as well as the nonnative fish species and their origin. We investigated the threat level of the native fish species according to the categories of the International Union for Conservation of Nature (IUCN 2012), and we established the main vectors of introduction of the nonnative fish species. In this way, we hope to provide support for strategies for the conservation of ichthyofauna in the Iguassu River basin.

Material and Methods

1. Study area

The Iguassu River is formed by the junction of the Iraí and Atuba rivers on the border between the municipalities of Curitiba and Pinhais on the first of the Paraná plateaus, from where it flows over 1,320 km until flowing into the Paraná River close to the city of Foz do Iguaçu in the Paraná State (SEMA 2010). This river is considered one of the main tributaries of the left margin of the Paraná River, and its mouth is located downstream of the Itaipu Dam (Baumgartner et al. 2006). In addition, the Iguassu River is considered the largest river in the Paraná State as well as the river with the largest drainage basin, with an area of 72,000 km², of which 79% belongs to the Paraná State, 19% to the Santa Catarina State and 2% to Argentina (Eletrosul 1978).

Although the limits are not well established, the hydrographic basin of the Iguassu River can be subdivided into three hydrographic units (Figure 1): the upper, middle and lower Iguassu, which represent the first, second and third Paraná plateaus, respectively (Baumgartner et al. 2012, Maack 2012). The Iguassu Falls (Figure 1) are located in Iguassu National Park (hydrographic unit of the lower Iguassu River) and are considered the largest falls on the planet in terms of water volume, which flows at approximately 1,551 m³.s⁻¹ (SEMA 2010). In this region, the Iguassu River reaches an approximate width of 1,200 m, running in a deep canyon for the rest of its course until its mouth on the Paraná River (Maack 2012). Thus, due to ichthyofaunal isolation promoted by the formation of the Iguassu Falls, our compilation considered the occurrence of fish species from the headwaters of the upper Iguassu River to the Iguassu Falls.

Land use is quite diverse across the basin. In the upper Iguassu River, there is a large resident population that is mainly occupied by industrial, commercial, and service activities. In its course in the interior of the Paraná State, agriculture is predominant, with some areas of intensive agriculture in the region of the municipality of Guarapuava up to the border with the Santa Catarina State to the south of the basin.



Figure 1. Map of the Iguassu River basin, Paraná State, Brazil. Yellow dots indicate the sampling sites within the basin that were georeferenced and cataloged in the ichthyological collections. Each point may correspond to more than one sampling site. The boundaries between the middle/lower and upper hydrographic units are represented by red diamonds. The red star indicates the location of the Iguassu Falls.

In the middle and lower stretches of the Iguassu River, there is a high concentration of forest cover (SEMA 2010), and the unevenness of this region favors hydroelectric use. There are 12 large reservoirs in the middle/lower and another three in the upper Iguassu River basin (Daga et al. 2016). As it is located in an area of rugged relief with several rivers, rapids, and waterfalls, the hydrographic basin of the Iguassu River has greatly influenced the geographical distribution of several groups of organisms, promoting a high degree of endemism of the fish species that inhabit it (Baumgartner et al. 2012).

2. Database

The survey of the fish species found in the hydrographic basin of the Iguassu River, in the long stretch above the Iguassu Falls, was carried out by consulting the ichthyological collections of the following institutions: Londrina State University Museum in Londrina (MZUEL), the Museum of Zoology of the University of São Paulo in São Paulo (MZUSP), the Capão da Imbuia Natural History Museum in Curitiba (MHNCI), the PUCRS Museum of Science and Technology in Porto Alegre (MCP), the Nupélia Ichthyology Collection of the State University of Maringá in Maringá (NUP), National Museum of Rio de Janeiro in Rio de Janeiro (MNRJ), and the Ichthyology Collection of GERPEL of the Western Paraná State University in Toledo (CIG). The species records (Figure 1) of these collections came from the online databases Species Link (http://www.splink.org.br/), Fishnet2 (http://www.fishnet2.net/search. aspx) and SiBBr (https://ala-hub.sibbr.gov.br/ala-hub/occurrences/ search), which were accessed in May 2020. Personal communications with the professionals responsible for the ichthyological collections were also carried out. In addition, to complement the information, bibliographical research was performed in March 2020 using articles in the Thomson Reuters (ISI Web of Knowledge, apps.isiknowledge. com), Elsevier's ScienceDirect (http://www.sciencedirect.com), and SciELO (http://www.scielo.org) databases that addressed the topic of "ichthyofauna of the Iguassu River basin". The search terms in the "topic" field were "fish* OR ichthyo* OR checklist AND Iguassu River basin", and the searched timespan included all years up to the date of the search. The search was then refined according to the following research areas: environmental sciences, ecology, zoology, freshwater biology, biodiversity, conservation, and fisheries and water resources. In addition, all articles that included lists of fish species of the Iguassu River basin that were published in the journal Check List: Journal of Species Lists and Distributions, which is not indexed in the aforementioned databases, were also included in our review. For this, the search was carried out using the option "search for articles" on the journal website (http://www.checklist.org.br/search), and all categories and volumes were searched. The studies included in this bibliographical research contained a list of fish species caught in the Iguassu River or in its tributaries in the stretch above the Iguassu Falls. Nonrelated articles were excluded based on their title, abstract or, if necessary, after a careful reading of the entire text.

To identify the origin, the fish species were classified as autochthonous (endemic or naturally occurring fish species in the Iguassu River basin) and nonnative. For the classification of nonnative fish species, the recommendation of Ellender & Weyl (2014) was adopted, which separates them into extralimital species (from other hydrographic basins in the Neotropical region) and alien species (from other biogeographic regions).

Nonnative fish species were classified according to the possible vectors of introduction into five groups: aquaculture (species widely used in fish farms in the region, introduced intentionally or accidentally); aquarism (ornamental fish species, introduced intentionally or accidentally); stocking (species from stocking in reservoirs); baiting (species used as bait in fishing activities, introduced intentionally or accidentally) and sport fishing (species introduced for sport fishing). The threat level for each autochthonous fish species was set according to the Portaria do Ministério do Meio Ambiente, nº 445 (December 17, 2014) (BRASIL 2014), which was amended by Decree nº 98 (April 28, 2015) (BRASIL 2015) and by the Red Book of Endangered Brazilian Fauna (ICMBio 2018). These regulations classify the endangered species of fish and aquatic invertebrates from the Brazilian fauna with the following categories: extinct in the wild (EW), critically endangered (CR), endangered (EN), and vulnerable (VU). Finally, considering that the middle stretch of the Iguassu River basin is short, with weakly established limits and presents a similar fish species composition with the lower stretch (Ingenito et al. 2004, Baumgartner et al. 2012), we compartmentalized the species distribution inventoried for the middle/ lower and upper stretches of the basin.

3. Fish identification

Identification follows Ingenito et al. (2004), Baumgartner et al. (2012), Garavello et al. (2012), and by comparison of the specimens with original descriptions. Whenever possible, the determinations of the fish species were checked by specialists of each taxonomic group. Fish species were classified based on Van der Laan et al. (2020), except for Astyanax and Psalidodon that follow Terán et al. (2020). Species names validity was based on Fricke et al. (2020). Some species recorded in the collections or literatures analyzed were reexamined and identifications were corrected: Astyanax fasciatus (Cuvier, 1819) is *Psalidodon bifasciatus* (Garavello & Sampaio, 2010); A. aff. scabripinnis (Eigenmann, 1921) is A. totae Ferreira Haluch & Abilhoa, 2005 (see Haluch & Abilhoa 2005) or A. eremus Ingenito & Duboc, 2014 (see Ingenito & Duboc 2014); Bryconamericus sp. and Diapoma aff. alburnum (Hensel, 1870) are Diapoma sp.; Characidium sp. 2 is C. travassosi Melo, Buckup & Oyakawa 2016; Corydoras aff. paleatus (Jenyns, 1842) is Corydoras sp.; Crenicichla yaha Casciotta, Almirón & Gómez, 2006 is C. tesay Casciotta & Almirón, 2009 (see Piálek et al. 2015); C. tesay from Jordão and Areia river basins (sensu Frota et al. 2016a) is Crenicichla sp. (see Říčan et al. 2017); Geophagus brasiliensis (Quoy & Gaimard, 1824) is Geophagus iporangensis Haseman, 1911 (see Argolo et al. 2020); Glandulocauda melanopleura Eigenmann, 1911 is G. caerulea Menezes & Weitzman, 2009 (see Menezes & Weitzman 2009); Gymnogeophagus setequedas Reis, Malabarba & Pavanelli, 1992 is G. taroba Casciotta, Almirón, Piálek & Říčan, 2017 (see Casciotta et al. 2017); Hisonotus sp. is H. yasi (Almirón, Azpelicueta & Casciotta, 2004); some individuals identified as Hoplias aff. malabaricus (Bloch, 1794) are H. misionera Rosso, Mabragaña, González-Castro, Delpiani, Avigliano, Schenone & Díaz de Astarloa, 2016; Megaleporinus aff. elongatus (Valenciennes, 1850) is M. obtusidens (Valenciennes, 1837); M. obtusidens is M. piavussu (Britski, Birindelli & Garavello, 2012), Pareiorhaphis sp. is P. parmula Pereira, 2005, and *Phalloceros caudimaculatus* (Hensel, 1868) is P. harpagos Lucinda, 2008 or P. spiloura Lucinda, 2008.

Results

The survey on the ichthyofaunal diversity of the Iguassu River hydrographic basin, from its sources to the Iguassu Falls, revealed a total of 133 fish species distributed in nine orders, 29 families and 72 genera (Table 1). Siluriformes (51 species) and Characiformes (48 species) were the most representative orders, comprising approximately 74% of the total species recorded in the basin (Figure 2). The families that showed the highest species richness were Characidae (28 species), Loricariidae (17 species), Cichlidae (13 species), Trichomycteridae (12 species), Anostomidae and Heptapteridae (seven species each), which composed approximately 63% of all species (Figure 2).

The fish species distribution in the middle/lower and upper stretches of the Iguassu River basin revealed that 79 fish species (approximately 59% of the total) were present across the entire length of the basin (Table 1). Of the total species, 119 fish species occurred in the middle/lower Iguassu (approximately 89% of the total fish species), and 40 of these fish species (approximately 30% of the total) were exclusive to this stretch of the basin (Table 1). In the upper Iguassu River, 93 fish species were recorded (approximately 70% of the total fish species), and 14 of these fish species (approximately 11% of the total) were exclusive to this stretch of the basin (Table 1).

The species origin classification revealed that of the 133 recorded fish species, 93 were considered autochthonous (approximately 70% of the total fish species) and 40 were nonnative (approximately 30% of the total fish species). Among the nonnative fish species, 30 species were classified as extralimital (approximately 23% of total fish species and 75% of nonnative fish species), and the other 10 species were classified as aliens (approximately 8% of total fish species and 25% of the nonnative fish species). The main vector of introduction of nonnative fish species was aquaculture.

Table 1. Iguassu River basin ichthyofauna recorded above the Iguassu Falls according to species, voucher specimens, the origin of each species, threat level, introduction vector, and distribution along the middle/lower and upper sections of the basin. Abbreviations are: CAS, California Academy of Sciences; CIG, Coleção Ictiológica do Gerpel; FMNH, Field Museum of Natural History; MACN, Museo Argentino de Ciencias Naturales; MCP, Museu de Ciências e Tecnologia da Pontificia Universidade Católica do Rio Grande do Sul; MHNCI, Museu de História Natural do Capão da Imbuia; MLP, Museo de La Plata; MNRJ, Museu Nacional do Rio de Janeiro; MZUEL, Museu de Zoologia da Universidade Estadual de Londrina; MZUSP, Museu de Zoologia da Universidade de São Paulo; NUP, Coleção Ictiológica do Nupélia; CR: Critically Endangered species; EN: Endangered species; VU: Vulnerable species. Autochthonous*: Endemic species from Iguassu River basin; Nonnative: Extralimital species; Nonnative*: Alien species. The symbol # refers to species added to the list due to personal observation of the authors and that do not have material registered in the consulted ichthyological collections.

	Species	Voucher	Origin/Threat level	Introduction vector	Middle/ Lower Iguassu	Upper Iguassu
	CYPRINIFORMES					
	Cobitidae					
1	Misgurnus anguillicaudatus (Cantor, 1842)	MHNCI 9076	Nonnative [•]	Aquarism		Х
	Cyprinidae					
2	Cyprinus carpio Linnaeus, 1758	NUP 1811	Nonnative [•]	Aquaculture	Х	Х
	Xenocyprididae					
3	Ctenopharyngodon idella (Valenciennes, 1844)	NUP 11141	Nonnative [•]	Aquaculture	Х	Х
4	Hypophthalmichthys molitrix (Valenciennes, 1844)	NUP 2383	Nonnative [®]	Aquaculture	Х	Х
5	Hypophthalmichthys nobilis (Richardson, 1845)	NUP 2056	Nonnative [®]	Aquaculture	Х	Х
	CHARACIFORMES					
	Anostomidae					
6	Leporinus amae Godoy, 1980	CIG 3094	Nonnative	Aquaculture	Х	
7	Leporinus friderici (Bloch, 1794)	NUP 11872	Nonnative	Aquaculture	Х	Х
8	Leporinus octofasciatus Steindachner, 1915	NUP 12787	Nonnative	Aquaculture	Х	Х
9	Megaleporinus macrocephalus (Garavello & Britski, 1988)	NUP 3252	Nonnative	Aquaculture	Х	Х
10	Megaleporinus obtusidens (Valenciennes, 1837)	NUP 12788	Nonnative	Aquaculture	Х	Х
11	Megaleporinus piavussu (Britski, Birindelli & Garavello, 2012)	MZUEL 15983	Nonnative	Aquaculture	Х	Х
12	Schizodon borellii (Boulenger, 1900)	MZUEL 17941	Nonnative	Aquaculture	Х	
	Bryconidae					
13	Brycon hilarii (Valenciennes, 1850)	NUP 3245	Nonnative	Aquaculture	Х	Х
14	Brycon orbignyanus (Valenciennes, 1850)	CIG 3516	Nonnative	Aquaculture	Х	
15	Salminus brasiliensis (Cuvier, 1816)	MZUEL 13302	Nonnative	Sport-fishing	Х	Х
	Characidae					
16	Astyanax dissimilis Garavello & Sampaio, 2010	NUP 17791	Autochthonous*		Х	Х
17	Astyanax eremus Ingenito & Duboc, 2014	NUP 13501	Autochthonous*/CR			Х
18	Astyanax jordanensis Vera Alcaraz, Pavanelli & Bertaco, 2009	NUP 5252	Autochthonous*/VU		Х	

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	Species	Voucher	Origin/Threat level	Introduction vector	Middle/ Lower Iguassu	Upper Iguassu
19	Astyanax lacustris (Lütken, 1875)	NUP 17521	Autochthonous		Х	Х
20	Astyanax minor Garavello & Sampaio, 2010	NUP 16888	Autochthonous*		Х	Х
21	Astyanax serratus Garavello & Sampaio, 2010	NUP 16030	Autochthonous*		Х	Х
22	Astyanax totae Ferreira Haluch & Abilhoa, 2005	MHNCI 10305	Autochthonous*			Х
23	Astyanax varzeae Abilhoa & Duboc, 2007	MCP 40535	Autochthonous*			Х
24	Astyanax sp. 1	NUP 3706	Autochthonous*		Х	
25	Astyanax sp. 2	NUP 3048	Autochthonous*		Х	
26	Bryconamericus ikaa Casciotta, Almirón & Azpelicueta, 2004	NUP 15987	Autochthonous*		Х	Х
27	Bryconamericus pyahu Azpelicueta, Casciotta & Almirón, 2003	NUP 19031	Autochthonous*		Х	Х
28	Charax stenopterus (Cope, 1894)	NUP 16033	Nonnative	Aquaculture		Х
29	Diapoma sp.	NUP 6620	Autochthonous*		Х	Х
30	Glandulocauda caerulea Menezes & Weitzman, 2009	MNRJ 5642	Autochthonous*/EN			Х
31	Hasemania maxillaris Ellis, 1911	FMNH 54303	Autochthonous*		Х	Х
32	Hasemania melanura Ellis, 1911	FMNH 54384	Autochthonous*		Х	Х
33	Hyphessobrycon bifasciatus Ellis, 1911	MHNCI 10621	Autochthonous			Х
34	Hyphessobrycon griemi Hoedeman, 1957	MHNCI 10622	Autochthonous		Х	Х
35	Hyphessobrycon reticulatus Ellis, 1911	NUP 15684	Autochthonous		Х	Х
36	Hyphessobrycon taurocephalus Ellis, 1911	FMNH 54389	Autochthonous*			Х
37	Oligosarcus longirostris Menezes & Géry, 1983	NUP 15881	Autochthonous*		Х	Х
38	Roeboides descalvadensis Fowler, 1932	MZUEL 16357	Nonnative	Aquarism	Х	
39	Mimagoniates microlepis (Steindachner, 1877)	NUP 15549	Autochthonous		Х	Х
40	Psalidodon bifasciatus (Garavello & Sampaio, 2010)	MHNCI 12340	Autochthonous		Х	Х
41	Psalidodon gymnodontus Eigenmann, 1911	NUP 6843	Autochthonous*		Х	Х
42	Psalidodon gymnogenys (Eigenmann, 1911)	FMNH 54707	Autochthonous*/EN		Х	Х
43	Undescribed genus sp.	NUP 12783	Autochthonous*		Х	
	Crenuchidae					
44	Characidium travassosi Melo, Buckup & Oyakawa, 2016	MCP 22605	Autochthonous		Х	
45	Characidium sp.	NUP 15876	Autochthonous*		Х	Х
	Curimatidae					
46	Cyphocharax cf. santacatarinae (Fernández - Yépez, 1948)	NUP 11205	Autochthonous		Х	
47	Steindachnerina brevipinna (Eigenmann & Eigenmann, 1889)	NUP 11487	Nonnative	Baiting	Х	
	Erythrinidae					
48	Hoplias intermedius (Günther, 1864)	#	?		Х	Х
49	Hoplias aff. malabaricus (Bloch, 1794)	NUP 11855	Autochthonous		Х	Х
50	Hoplias misionera Rosso, Mabragaña, González-Castro, Delpiani, Avigliano, Schenone & Díaz de Astarloa, 2016	NUP 2074	Autochthonous		Х	
	Parodontidae					
51	Apareiodon vittatus Garavello, 1977	NUP 12097	Autochthonous*		Х	Х
	Prochilodontidae					
52	Prochilodus lineatus (Valenciennes, 1837)	NUP 3251	Nonnative	Reservoir	Х	Х
	Serrasalmidae					
53	Piaractus mesopotamicus (Holmberg, 1887)	NUP 21149	Nonnative	Aquaculture	Х	Х

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	Species	Voucher	Origin/Threat level	Introduction vector	Middle/ Lower Iguassu	Upper Iguassu
	GYMNOTIFORMES					
	Apteronotidae					
54	Apteronotus ellisi (Alonso de Arámburu, 1957)	NUP 3253	Nonnative	Baiting	Х	Х
	Gymnotidae					
55	Gymnotus inaequilabiatus (Valenciennes, 1839)	NUP 3752	Nonnative	Baiting	Х	Х
56	Gymnotus sylvius Albert & Fernandes-Matioli, 1999	NUP 19035	Nonnative	Baiting	Х	Х
	SILURIFORMES					
	Auchenipteridae					
57	Glanidium ribeiroi Haseman, 1911	NUP 2443	Autochthonous*		Х	Х
58	Tatia jaracatia Pavanelli & Bifi, 2009	MZUSP 98248	Autochthonous*		Х	Х
	Callichthyidae					
59	Callichthys callichthys (Linnaeus, 1758)	NUP 5490	Nonnative	Baiting	Х	Х
60	Corydoras carlae Nijssen & Isbrücker, 1983	NUP 19034	Autochthonous*		Х	Х
61	Corydoras ehrhardti Steindachner, 1910	NUP 15802	Autochthonous		Х	Х
62	Corydoras cf. longipinnis Knaack, 2007	NUP 12809	Autochthonous		Х	
63	Corydoras sp.	NUP 709	Autochthonous		Х	Х
64	Hoplosternum littorale (Hancock, 1828)	NUP 11201	Nonnative	Baiting	Х	Х
	Clariidae					
65	Clarias gariepinus (Burchell, 1822)	NUP 3246	Nonnative [•]	Aquaculture	Х	
	Heptapteridae					
66	Heptapterus stewarti Haseman, 1911	MHNCI 10343	Autochthonous*			Х
67	Heptapterus sp.	NUP 15925	Autochthonous*		Х	Х
68	Imparfinis hollandi Haseman, 1911	NUP 2976	Autochthonous*		Х	Х
69	Rhamdia branneri Haseman, 1911	NUP 2448	Autochthonous*		Х	Х
70	Rhamdia voulezi Haseman, 1911	NUP 1659	Autochthonous*		Х	Х
71	<i>Rhamdia</i> sp.	NUP 5284	Autochthonous*		Х	Х
72	Rhamdiopsis moreirai Haseman, 1911	MHNCI 8929	Autochthonous		Х	Х
	Ictaluridae					
73	Ictalurus punctatus (Rafinesque, 1818)	NUP 584	Nonnative ■	Aquaculture	Х	Х
	Loricariidae					
74	Ancistrus abilhoai Bifi, Pavanelli & Zawadzki, 2009	MZUSP 104116	Autochthonous*		Х	Х
75	Ancistrus agostinhoi Bifi, Pavanelli & Zawadzki, 2009	MZUSP 104118	Autochthonous*		Х	
76	Ancistrus mullerae Bifi, Pavanelli & Zawadzki, 2009	MZUSP 104121	Autochthonous*		Х	
77	Hisonotus yasi (Almirón, Azpelicueta & Casciotta, 2004)	NUP 8720	Autochthonous*		Х	Х
78	Hypostomus agna (Miranda-Ribeiro, 1907)	NUP 21922	Autochthonous		Х	Х
79	Hypostomus albopunctatus (Regan, 1908)	NUP 593	Autochthonous		Х	Х
80	Hypostomus commersoni Valenciennes, 1836	NUP 552	Autochthonous		Х	Х
81	Hypostomus derbyi (Haseman, 1911)	NUP 677	Autochthonous		Х	Х
82	Hypostomus myersi (Gosline, 1947)	NUP 680	Autochthonous		Х	Х
83	Hypostomus nigropunctatus Garavello, Britski & Zawadzki, 2012	NUP 5082	Autochthonous*		Х	

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	Species	Voucher	Origin/Threat level	Introduction vector	Middle/ Lower Iguassu	Upper Iguassu
84	Loricariichthys cf. melanocheilus Reis & Pereira, 2000	NUP 10791	Nonnative	Aquarism	Х	
85	Loricariichthys cf. rostratus Reis & Pereira, 2000	MHNCI 11044	Nonnative	Aquarism	Х	
86	Neoplecostomus sp.	NUP 11087	Autochthonous*		Х	
87	Otothyropsis biamnicus Calegari, Lehmann A. & Reis, 2013	NUP 16004	Autochthonous		Х	
88	Pareiorhaphis parmula Pereira, 2005	NUP 15928	Autochthonous		Х	Х
89	Rineloricaria langei Ingenito, Ghazzi, Duboc & Abilhoa, 2008	MCP 42506	Autochthonous*			Х
90	Rineloricaria maacki Ingenito, Ghazzi, Duboc & Abilhoa, 2008	NUP 3059	Autochthonous*		Х	
	Pimelodidae					
91	Pimelodus britskii Garavello & Shibatta, 2007	NUP 1786	Autochthonous*		Х	Х
92	Pimelodus ortmanni Haseman, 1911	NUP 1664	Autochthonous*		Х	Х
93	Pseudoplatystoma corruscans (Spix & Agassiz, 1829)	NUP 11142	Nonnative	Aquaculture	Х	Х
94	Pseudoplatystoma reticulatum Eigenmann & Eigenmann, 1889	NUP 3247	Nonnative	Aquaculture	Х	Х
95	Steindachneridion melanodermatum Garavello, 2005	NUP 11903	Autochthonous*/EN		Х	
	Trichomycteridae					
96	Cambeva castroi (de Pinna, 1992)	NUP 3127	Autochthonous*		Х	Х
97	Cambeva crassicaudata (Wosiacki & de Pinna, 2008)	NUP 10827	Autochthonous*/EN		Х	
98	Cambeva davisi (Haseman, 1911)	NUP 19054	Autochthonous		Х	Х
99	Cambeva igobi (Wosiacki & de Pinna, 2008)	NUP 9866	Autochthonous*/EN		Х	
100	Cambeva mboycy (Wosiacki & Garavello, 2004)	NUP 19051	Autochthonous*/EN		Х	Х
101	Cambeva naipi (Wosiacki & Garavello, 2004)	MZUSP 38788	Autochthonous*			Х
102	Cambeva plumbea (Wosiacki & Garavello, 2004)	NUP 1614	Autochthonous*		Х	
103	Cambeva stawiarski (Miranda Ribeiro, 1968)	NUP 19049	Autochthonous		Х	Х
104	Cambeva taroba (Wosiacki & Garavello, 2004)	NUP 3125	Autochthonous*		Х	
105	<i>Cambeva</i> sp. 1	NUP 12660	Autochthonous*		Х	
106	<i>Cambeva</i> sp. 2	NUP 12661	Autochthonous*		Х	
107	Trichomycterus papilliferus Wosiacki & Garavello, 2004	NUP 17363	Autochthonous*/EN		Х	Х
	ATHERINIFORMES					
	Atherinopsidae					
108	Odontesthes bonariensis (Valenciennes, 1835)	NUP 1610	Nonnative	Reservoir	Х	
	CYPRINODONTIFORMES					
	Anablepidae					
109	Jenynsia diphyes Lucinda, Ghedotti & Graça, 2006	NUP 606	Autochthonous*/EN		Х	
110	Jenynsia eigenmanni (Haseman, 1911)	NUP 2862	Autochthonous*		Х	Х
	Poeciliidae					
111	Cnesterodon carnegiei Haseman, 1911	MHNCI 7609	Autochthonous*/VU			Х
112	Cnesterodon omorgmatos Lucinda & Garavello, 2001	MCP 22742	Autochthonous*/EN		Х	
113	Phalloceros harpagos Lucinda, 2008	NUP 19040	Autochthonous		Х	Х
114	Phalloceros spiloura Lucinda, 2008	MCP 27446	Autochthonous			Х
115	Poecilia reticulata Peters, 1859	NUP 19041	Nonnative	Aquarism	Х	
116	Xiphophorus hellerii Heckel, 1848	NUP 21119	Nonnative	Aquarism	Х	
	Rivulidae					
117	Austrolebias araucarianus Costa, 2014	MNRJ 9798	Autochthonous*		Х	
118	Austrolebias carvalhoi (Myers, 1947)	CAS 41178	Autochthonous*/CR			X

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	Species	Voucher	Origin/Threat level	Introduction vector	Middle/ Lower Iguassu	Upper Iguassu
	SYNBRANCHIFORMES					
	Synbranchidae					
119	Synbranchus marmoratus Bloch, 1795	NUP 19047	Nonnative	Baiting	Х	Х
	CICHLIFORMES					
	Cichlidae					
120	Australoheros angiru Říčan, Piálek, Almirón & Casciotta, 2011	NUP 11190	Autochthonous		Х	Х
121	Australoheros kaaygua Casciotta, Almirón & Gómez, 2006	NUP 1839	Autochthonous		Х	Х
122	Cichla kelberi Kullander & Ferreira, 2006	NUP 19171	Nonnative	Sport-fishing	Х	Х
123	Cichlasoma paranaense Kullander, 1983	NUP 9758	Nonnative	Aquarism	Х	
124	Coptodon rendalli (Boulenger, 1897)	NUP 3749	Nonnative [•]	Aquaculture	Х	Х
125	Crenicichla iguassuensis Haseman, 1911	FMNH 54159	Autochthonous*		Х	Х
126	<i>Crenicichla tapii</i> Piálek, Dragová, Casciotta, Almirón & Říčan, 2015	MLP 10560	Autochthonous*		Х	
127	Crenicichla tesay Casciotta & Almirón, 2009	NUP 3731	Autochthonous*		Х	Х
128	Crenicichla tuca Piálek, Dragová, Casciotta, Almirón & Říčan, 2015	MLP 10818	Autochthonous*		Х	
129	Crenicichla sp.	NUP 11288	Autochthonous*		Х	
130	Geophagus iporangensis Haseman, 1911	NUP 704	Autochthonous		Х	Х
131	Gymnogeophagus taroba Casciotta, Almirón, Piálek & Říčan, 2017	MLP 11258	Autochthonous*		Х	
132	Oreochromis niloticus (Linnaeus, 1758)	NUP 19048	Nonnative [•]	Aquaculture	Х	Х
	CENTRARCHIFORMES					
	Centrarchidae					
133	Micropterus salmoides (Lacepède, 1802)	NUP 11898	Nonnative [•]	Sport-fishing	Х	Х



Figure 2. Number of species per family of ichthyofauna recorded in the hydrographic basin of the Iguassu River. The colors indicate the order to which each family belongs.

Twenty-one species (52.5% of the total nonnative fish species) were introduced into the basin through this vector, with emphasis on alien species of Asian - *Ctenopharyngodon idella* (Valenciennes, 1844), *Cyprinus carpio* Linnaeus, 1758, *Hypophthalmichthys molitrix* (Valenciennes, 1844) and *Hypophthalmichthys nobilis*

(Richardson, 1845) – and African origin - *Oreochromis niloticus* (Linnaeus, 1758) and *Coptodon rendalli* (Boulenger, 1897). Fishing was another important vector of introduction of nonnative fish species since seven species (17.5% of the total nonnative fish species) were introduced as bait and three other species (7.5%) by sport fishing. Aquarium activities were responsible for the introduction of seven more species and stocking in reservoirs responsible for the introduction of two other species (5%) (Table 1, Figure 3).



Figure 3. Nonnative fish species according to their introduction vectors into the Iguassu River basin, Paraná State, Brazil.

Of the 93 autochthonous fish species of the Iguassu River, 64 were listed as endemic, which revealed an endemism rate of approximately 69%. Thirteen endemic fish species (approximately 10% of total species, 14% of total native fish species and 20% of endemic fish species) are listed as being under some level of threat (Table 1). Astyanax eremus and Austrolebias carvalhoi (Myers, 1947) were listed at the highest threat level (CR). Nine fish species, or approximately 69% of the species under some level of threat (Cambeva crassicaudata (Wosiacki & de Pinna, 2008); C. igobi (Wosiacki & de Pinna, 2008); C. mboycy (Wosiacki & Garavello, 2004); Cnesterodon omorgmatos Lucinda & Garavello, 2001; Glandulocauda caerulea; Jenynsia diphyes Lucinda, Ghedotti & Graça, 2006; Psalidodon gymnogenys (Eigenmann, 1911); Steindachneridion melanodermatum Garavello, 2005 and Trichomycterus papilliferus Wosiacki & Garavello, 2004), were listed in category EN. Astyanax jordanensis Vera Alcaraz, Pavanelli & Bertaco, 2009 and Cnesterodon carnegiei Haseman, 1911 were listed in the category VU.

Finally, we recorded the occurrence of at least 13 putatively undescribed species of autochthonous fish (listed as "sp." or with the suffix "aff.") for the Iguassu River basin above the Iguassu Falls (Table 1), which represented approximately 10% of the total number of fish species and 14% of the total number of native fish species. Of these species, all occurred in the middle/lower Iguassu River basin, and seven (*Cambeva* sp. 1, *Cambeva* sp. 2, *Crenicichla* sp., *Neoplecostomus* sp., *Astyanax* sp. 1, *Astyanax* sp. 2, and undescribed genus sp.) were considered exclusive to this stretch of the basin.

Discussion

Our compilation of data increased the number of fish species in the Iguassu River basin to 133 in the stretch above the Iguassu Falls. Our results revealed that 52 more species have been registered than mentioned by Ingenito et al. (2004) for the upper Iguassu River and 13 species more than recorded by Baumgartner et al. (2012) for the lower Iguassu River. It is important to highlight that most of the ichthyofaunal surveys available for the Iguassu River basin occurred in areas influenced by dams built on the main channel of the Iguassu River, since there is a greater financial incentive for research on this modality due to the need of hydroelectric companies to comply with environmental laws (Baumgartner et al. 2012, Frota et al. 2016a). However, in the last decade, the ichthyofauna in the Iguassu River basin has been increasingly studied for ecological and biogeographic purposes. This increase in sampling has revealed, especially at the headwaters of the basin, interesting or alarming new records of native and nonnative fish species (see Abilhoa et al. 2013, Frota et al. 2016a, Larentis et al. 2016, 2019, Delariva et al. 2018), promoting an increase in the number of fish species registered.

In comparing the species richness of the Iguassu River basin with that of other large basins in the Paraná State, it is noted that the hydrographic basins of the Piquiri, Tibagi and Paranapanema rivers, with 152 (Cavalli et al. 2018), 151 (Raio & Bennemann 2010) and 225 (Jarduli et al. 2020) fish species, respectively, exceeded the absolute species richness found in the Iguassu River basin. However, although it has numerically lower species richness, the high endemism rate of the ichthyofauna in the Iguassu River basin, which was estimated at 69% by this study, highlights the environmental importance of conservation of this basin, which is increasingly threatened by environmental degradation and by the introduction of nonnative fish species.

The Iguassu River ecoregion is known for the high rates of endemism among its ichthyofauna (Agostinho et al. 1997, Zawadzki et al. 1999, Baumgartner et al. 2012, Frota et al. 2016a, Daga et al. 2016, Delariva et al. 2018). In the 1990s, the rate of endemism was estimated by Agostinho et al. (1997) to be 80% and by Zawadzki et al. (1999) to be 75%. Our results show that there has been a decrease in the rate of endemism in the Iguassu River basin over the years. This fact is mainly due to the increase in collections in bordering basins, which has shown some cases of sharing of ichthyofaunal species previously considered endemic to the Iguassu River basin, for example, Psalidodon bifasciatus (see Frota et al. 2016a, 2019, 2020, Neves et al. 2020) and Cambeva stawiarski (see Cavalli et al. 2018, Morais-Silva et al. 2018). However, the rate of endemism in the Iguassu River basin, in the stretch above Iguassu Falls, remains an outlier when compared to those of other hydrographic basins that make up the Platina Basin system, for example, the Uruguay River basin (endemism rate estimated at 28%, Bertaco et al. 2016) and the Ivaí River basin (endemism rate estimated at 12%, Frota et al. 2016b).

In addition, 40 fish species (approximately 30%) were introduced into the Iguassu River basin, which is extremely worrying due to the possibility for future decline and potential extinction of autochthonous species (Daga et al. 2016, Ruaro et al. 2018), especially endemic species. The occurrence and establishment of nonnative fish species in aquatic environments often leads to their permanent presence, making subsequent eradication unlikely (Pérez et al. 1997). Representing serious risks to native fish species due to interspecific competition for resources and predation and potentially generating harmful hybridizations (Agostinho et al. 2007, Vitule et al. 2009), the introduced individuals may also contain intrinsic pathogens, larval phases of crustaceans and associated mollusks, which can also cause catastrophic effects on the native ichthyofauna (Casimiro et al. 2010, Vitule et al. 2009).

In Brazil, the introduction of nonnative fish species is common, and the only existing measure to solve this problem is the normative (laws and inspections) and educational approaches. However, this strategy has been failing due to the difficulty of enforcing the laws in a country with such extensive territory and with a society that lacks knowledge of the risks caused by these introductions (Azevedo-Santos et al. 2015). In general, the arrival of a new species in an aquatic environment due to anthropic action results from deliberate releases or escapes from confined environments due to the inefficiency of confinement or even due to accidents (Agostinho et al. 2007). Our compilation pointed to approximately 43% more nonnative fish species than recorded by Daga et al. (2016) for the Iguassu River basin. According to these authors, the Iguassu River basin has a history of species introduction since 1944, when the 'common carp' (Cyprinus carpio) was introduced in the region of the middle Iguassu River, possibly accidentally after the disruption of cultivation nurseries near to the river channel (Casimiro et al. 2018). Our survey corroborates the study of Daga et al. (2016) by revealing that the main vector of introduction of fish species in the Iguassu River was aquaculture, followed by the introduction from aquarism, fishing and stocking in reservoirs. The same vectors were also considered significant in the introduction of species in the hydrographic basin of the Piquiri River (Cavalli et al. 2018), although in smaller proportions than those reported here.

The main areas of introduction in the Iguassu River basin were concentrated in sites with high population density and high industrial activity, which requires the construction of dams and the establishment of aquaculture activities due to the greater need for electricity generation and food production (Daga et al. 2016). Although considered an important source of protein and income production worldwide, aquaculture is also one of the main vectors for the introduction of nonnative fish species in the Neotropical region and in the world (Gubiani et al. 2018, Lima et al. 2018). Considering that the cultivation of nonnative fish species in Brazil occurs mainly in cage nets (Lima et al. 2018), it is possible to infer that escapes are inevitable; therefore, each cage net is a continuous source of nonnative propagules for the environment (Azevedo-Santos et al. 2011). Damage such as alteration the diets of native fauna, alteration of the quality of the habitat due to eutrophication (Lima et al. 2018), invasion of genotypes, increased production of interspecific hybrids and the introduction and transmission of nonnative parasites (Nobile et al. 2020) are also reported to be due to the inopportune invasions associated with aquaculture.

Other activities, such as the release of aquarium fish and sport fishing, also stood out as important vectors of introduction into the Iguassu River basin. The ease of obtaining nonnative ornamental species from various parts of the world makes aquarium one of the main routes responsible for the introduction of these species into Brazilian watersheds (Agostinho et al. 2007, Azevedo-Santos et al. 2015). In general, individuals are introduced to natural or artificial environments by aquarists themselves, who give up this practice when they encounter some adversity, for example, with the excessive growth of individuals and the aggressiveness of some species (Magalhães & Jacobi 2013). Notably, aquarism was responsible for the introduction of the Palearctic fish, the 'dojo loach' (Misgurnus anguillicaudatus (Cantor, 1842)), which was released in the upper Iguassu River, probably unintentionally or deliberately (Abilhoa et al. 2013). Neotropical species have also been introduced due to this activity. This is the case of the 'dentudo' (Roeboides descalvadensis Fowler, 1932), of the 'espadinha' (Xiphophorus hellerii Heckel, 1848), of the 'cará' (Cichlasoma paranaense Kullander, 1983), of the 'barrigudinho' (Poecilia reticulata Peters, 1859), and of the 'cascudos-chinelo' (Loricariichthys cf. melanocheilus Reis & Pereira, 2000 and L. cf. rostratus Reis & Pereira, 2000). Two of the species introduced by aquarism belong to Poeciliidae (Poecilia reticulata and Xiphophorus hellerii), one of the main ornamental fish families marketed in Brazil (Magalhães & Jacobi 2013). Poecilids, in general, have a high invasion capacity and have caused different damages to local fauna (see Stockwell & Henkanaththegedara 2011). In addition to the fact that the species in this group are viviparous animals with high performance in urbanized environments (Ganassin et al. 2020), poecilids have clear advantages that are not observed in native fish (Deacon et al. 2011), representing a high risk to native and endemic populations, especially in aquatic environments in the vicinity of urban centers in the Iguassu River basin.

Sport fishing and the release of live bait are popular in Brazil and are practices that can stimulate species translocations between basins (Azevedo-Santos et al. 2015). In the Iguassu River hydrographic basin, sport fishing was responsible for the introduction of the 'tucunaréamarelo' (*Cichla kelberi* Kullander & Ferreira, 2006), which is native to the basins of the Araguaia and lower Tocantins rivers, and several other species, many of which are carnivorous and piscivorous (Agostinho et al. 2007), with high economic value (Britton & Orsi 2012) and whose life habits can destabilize the local ichthyofauna when they become established in the basin. Our results also revealed the presence of *Micropterus salmoides* (Lacepède, 1802) and *Salminus brasiliensis* (Cuvier, 1816), which were introduced in reservoirs to enhance sport fishing in the region (Daga et al. 2016; Ribeiro et al. 2017).

Hook escapes, as well as the intentional release of the remaining live bait at the end of the fishing trip, were possibly the mechanism for the introduction of some of the fish species found in the Iguassu River basin (Agostinho & Júlio Jr. 1996, Agostinho et al. 2007). *Gymnotus inaequilabiatus* (Valenciennes, 1839), *G. sylvius* Albert & Fernandes-Matioli, 1999, *Callichthys callichthys* (Linnaeus, 1758), and *Hoplosternum littorale* (Hancock, 1828) are widely used as bait in the capture of the 'Surubim-do-Iguaçu' (*Steindachneridion melanodermatum*), which is the largest species in the basin (Daga et al. 2016) and is currently threatened with extinction risk (ICMBio 2018).

The stocking of fish species in reservoirs, also called fishing or restocking, is a very common breeding and releasing practice (Casimiro et al. 2010). Mainly carried out by politicians and the hydroelectric sector (Vitule 2009, Agostinho et al. 2010), stocking can cause serious environmental damage, especially when carried out without adequate technical support and knowledge, which causes disregard for the environmental risks (Agostinho et al. 2010). Fingerlings of low genetic quality and often of nonnative fish species are deliberately introduced into the aquatic environment (Agostinho et al. 2007, Vitule 2009, Casimiro et al. 2010).

The several dams along the main course of the Iguassu River (Garavello et al. 1997, Baumgartner et al. 2012) and the fragmentation of habitats due to agricultural and urban activities (Baumgartner et al. 2012) add to the introductions of nonnative fish species, intensifying the threats to the endemic ichthyofauna in this ecoregion. According to the classification criteria of the IUCN, 20% of the endemic fish species of the Iguassu River basin are endangered. Among them, Astyanax eremus and Austrolebias carvalhoi need more attention because they are in the category of the greatest threat level (CR). Fragmentation and loss of habitat quality also threaten species with limited geographic distributions within the basin, especially those known only to their standard locations (ICMBio 2018). Populations of species that exhibit migratory behavior and that need stretches of rivers free of dams to complete their reproductive cycles, such as Steindachneridion melanodermatum, are also at serious risks due to the cascade of reservoirs along the Iguassu River and tributaries.

In summary, we recommend constant monitoring and increased collection efforts in the Iguassu River basin, especially in the regions that have not been sampled (Figure 1), which over the years have shown important contributions to the knowledge of their ichthyofauna. Our results highlight the gap between evolutionary knowledge and scientific knowledge of ichthyofauna in the Iguassu River basin, characterizing an expressive biodiversity deficit (see Hortal et al. 2015) in relation to possible new species and the accuracy of the geographic distributions of their species. Thus, efforts to apply management strategies to mitigate the negative effects of dam construction, loss of habitat quality and control of the spread of nonnative species must be better supported for this Neotropical hydrographic system with a high degree of endemism.

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Author Contributions

Luciano Mezzaroba: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Tiago Debona: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Augusto Frota: Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Weferson Júnio da Graça: Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Éder André Gubiani: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Conflict of Interest

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

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