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Inventory

Communities and occurrences of Squamata reptiles in different vegetation types of the Serra de São José, Minas Gerais, Brazil

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Abstract: The objective of the present study was to learn which species of Squamata reptiles occur in Protected Area São José, in Tiradentes, Minas Gerais, Brazil. Between November 2009 and December 2010 reptiles were captured. In total 157 specimens were recorded of 29 species, 16 snakes, 12 lizards and one amphisbaena. Among the snakes, Dipsadidae showed the greatest richness, with a total of nine species. The group of snakes had the highest number of species present in the community, but 79% of sampled specimens were lizards, *Enyalius bilineatus* being the most abundant species, with 21% of occurrence. The area with the highest richness was the Cerradão. The lower abundance was found in the Gallery Forest area (n=14), but it was the vegetation type with the highest equitability. Areas of Cerradão and Cerrado *sensu strictu* showed the most similarity. In these areas five species were recorded in common, *Bothrops neuwiedi* (n=3) being the only species of snake, and the two species of lizards most abundant in both environments were *Enyalius bilineatus* (n=32) and *Ameivula ocellifera* (n=19). *Ophiodes striatus* and *Xenodon merremii* were common to Cerradão and Dirty Field areas. There was no species recorded that were common to the environments of Cerrado and Dirty Field but two species not sympatric were found of the same genus, *Tropidurus torquatus,* which was found only in the Cerrado *sensu strictu* and *Tropidurus itambere* exclusively in Dirty Field. Since none of the rarefaction curves reached full asymptote, this highlights the need for further study due to the high probability of new species being recorded for the studied area.

Keywords: *Taxocenosis, Squamata, Campo das Vertentes, Inventory, Neotropical region.*

Comunidades e ocorrência de Répteis Squamata em diferentes tipos de vegetação da Serra de São José, Minas Gerais, Brasil

Resumo: O presente estudo teve como objetivo conhecer quais espécies de répteis Squamata ocorrem nas diferentes fitofisionomias da Área de Proteção Ambiental Serra de São José, Tiradentes, microrregião Campo das Vertentes, Sudeste de Minas Gerais, Brasil. Entre novembro de 2009 e dezembro de 2010 os répteis foram capturados utilizando-se de armadilhas de interceptação e queda e de funil instaladas em 10 pontos amostrais, sendo três em Mata de Galeria, três em Cerrado *sensu strictu*, três em Cerradão e um em Campo Sujo aberto. Durante um ano as armadilhas ficaram abertas ininterruptamente e foram amostradas 300 horas de procura limitada por tempo. Também foram realizados encontros ocasionais com registros fotográficos dos espécimes. Ao todo foram registrados 157 espécimes pertencentes a 29 espécies, sendo 16 de serpentes, 12 de lagartos e uma de anfisbena. Entre as serpentes, Dipsadidae apresentou a maior riqueza, com um total de nove espécies. O grupo das serpentes foi o com maior número de espécies presentes na comunidade, mas 79% dos espécimes amostrados eram lagartos, sendo *Enyalius bilineatus* a espécie mais abundante, com 21% de ocorrência. A menor abundância foi encontrada na área de Mata de Galeria (n=14), mas foi a fitofisiomia que apresentou a maior equitabilidade. Na área de Cerradão foi encontrada a maior riqueza, com 13 espécies registradas. As áreas de Cerradão e Cerrado *sensu strictu* foram as que apresentaram maior similaridade. Nestas áreas foram registradas cinco espécies em comum, sendo *Bothrops neuwiedi* (n=3) a única espécie de serpente, e as duas espécies de lagartos mais abundantes nos dois ambientes foram *Enyalius bilineatus* (n=32) e *Ameivula ocellifera* (n=19). *Ophiodes striatus* e *Xenodon merremii* foram comuns às áreas Cerradão e Campo Sujo. Não houve registro de espécie que fosse comum aos ambientes de Cerrado e Campo Sujo, mas foram encontradas duas espécies pertencentes ao mesmo gênero não simpátricas, como *Tropidurus torquatus* que foi encontrado apenas no Cerrado *sensu strictu* e *Tropidurus itambere* exclusivo do Campo sujo. *Notomabuya frenata* foi encontrada em todos os ambientes, exceto no Campo sujo, único local onde foi encontrado *Aspronema dorsivittatum*. Como nenhuma das curvas de rarefação atingiu a assíntota plena, evidencia-se a necessidade de mais estudos devido à alta probabilidade de registro de novas espécies para a área estudada.

Palavras-chaves: *Taxocenose, Squamata, Campo das Vertentes, Inventário, Região Neotropical.*

Introduction

Reptiles form a prominent group in almost all terrestrial assemblages, with more than 10,391 known species worldwide (Uetz 2016). In Brazil, there are 773 naturally occurring and reproducing species of reptiles (Costa & Bérnils 2015), which is the second largest number among all the countries of the world (Paglia et al. 2010). However, approximately one in five species of reptiles is endangered and, in the state of Minas Gerais, Brazil, the same proportion is still classified as data-deficient (Bérnils et al. 2009). These results reinforce the need for attention and research in tropical areas that exhibit the most dramatic rates of habitat loss (Böhm et al. 2013).

The Atlantic Forest and Cerrado biomes of Brazil have been recognized as "hotspots" for studying and conserving the world's diversity (Mittermeier et al. 2004). The greatest threats to Brazilian herpetofauna are concentrated in these phytogeographical domains and mainly result from habitat loss and degradation. These are critical situations for lizards and snakes in the remaining savanna and rocky fields of the states of São Paulo and Minas Gerais (Paglia et al. 2010). In some areas of Minas Gerais, these domains still require systematic sampling of herpetofauna with wide geographical coverage (Bérnils et al. 2009).

The remnants of the Atlantic Forest in Minas Gerais have been identified as being of interest for conservation because of high anthropogenic pressure caused by urbanization and deforestation (Drummond et al. 2005). Similarly, the Cerrado has suffered sharp changes due to its conversion to pasture, monocultures or other agricultural activities (Drummond et al. 2009; Marinho-Filho et al. 2010; Araújo & Almeida-Santos 2011). Studies on the herpetofauna of Minas Gerais have concentrated mainly on amphibians (Bérnils et al. 2009) and much of the knowledge about the reptile fauna of the Cerrado and Atlantic Forest of Minas Gerais is still at an initial limited stage.

Through expansion of reptiles inventories (Cruz et al. 2014; Lucas et al. 2016; Novelli et al. 2012; Sousa et al. 2010; São Pedro & Pires 2009; Silveira et al. 2010), it has been found that there is high diversity of reptiles in the Cerrado biome (Brites & Baub 1988; Recoder & Nogueira 2007). These studies have even shown that the distribution of some species is increasing (Santos et al 2009; Novelli et al. 2011). However, these studies are still scarce, considering the size and the variety of habitats in the Cerrado. This shortage becomes greater with regard to transition areas between the Cerrado and other biomes (Bertolucci et al. 2009; Sousa et al. 2010; Lucas et al. 2016).

The need for greater effort towards studying the diversity and distribution patterns of reptiles has been pointed out, given that there are still major gaps in knowledge regarding the composition of the fauna of Minas Gerais (Bérnils et al. 2009). Among the 112 priority areas for biodiversity conservation in Minas Gerais that have been defined (Drummond et al. 2005), the Serra de São José is regarded as one of the highest-priority areas for nature conservation within the state (Probio 2004). This area is located in a transition region between the savannas of central Brazil and the semi-deciduous forests of the southeast and south of the country (Oliveira-Filho & Machado 1993).

The aim of the present study was to make an inventory of the Squamata fauna and contribute information on the composition of species in four different vegetation types in the environmental protection area of Serra de São José, in the municipality of Tiradentes, and thus increase the knowledge of the herpetofauna of Minas Gerais, Brazil.

Methodology

Study área

The Serra de São José is located in the region of the Campo das Vertentes, in the basin of Rio das Mortes, in the southeast of the state of Minas Gerais. The Serra (mountain range) extends for about 12 km between the municipalities of Prados, Coronel Xavier Chaves and Santa Cruz de Minas, passing through São João del Rei and Tiradentes (21° 00'21° 02' S 44° 00'44° 15' W) (Oliveira-Filho & Machado 1993). The Serra region includes three protected areas administered by the State Forestry Institute: Environmental Protection Area (APA) of Serra de São José, Dragonflies State Wildlife Refuge of Serra de São José and Special Protection Area of Serra de São José.

The Serra de São José consists of a quartzite and sandstone massif at altitudes of 900-1430 m. The vegetation of the APA Serra de São José is quite diversified, with occurrence of semi-deciduous montane forest that belongs to the Atlantic Forest domain (Veloso et al. 1991) and also typical Cerrado vegetation (Silva et al. 2011). Above the altitude of 1250 m, the terrain consists predominantly of high meadows. The peaks of the range and a large part of its flanks are covered by rock fields vegetation, interrupted by gallery forest and small bodies of latosol with Cerrado vegetation (Silva et al. 2011). A belt of Cerrado dry forest of around 1 km in width extends along the southern border, while the northern border mainly comprises plantations in Cerrado areas (Alves & Kolbek 2009).

The average annual temperature is 22°C, and the average in the coldest months is 15°C. The area fits into the climatic type Cwb according to the Köppen scale, i.e. moderate subtropical humid, with two distinct seasons: one cold and dry (April to September) and another hot and rainy (October to May). The area forms part of the Andrelândia plateau, which is characterized by hills with convex and tabular summits and convex slopes (Carvalho 1987 Oliveira-Filho & Machado 1993; Alves & Kolbek 2009).

Sampled habitats

The sites selected for sampling were distributed into three areas of the APA Serra de São José: one in the north and two in the south. We sampled four types of vegetation: Cerrado *sensu strictu*, "Cerradão", gallery forest and fields with sparse shrubs. The northern region consists of a small area of Gallery forest at the Água Santa bathing area, one of Cerrado *sensu strictu* near to the Mangue trail entrance and another of Cerradão (large-vegetation Cerrado) near to the entrance to the head office of the bathing area. South of the mountains, traps were installed in two remote areas, 2.5 kilometers apart. One was located to the east of the forest called Bosque da Mãe D'Água (Alves 1991), and consisted of three parts: one in the Cerrado *sensu strictu* area and two in gallery forest. The other region was located at the western end of the mountain, near the Mangue trail. In this place, known as Coliseum, four plots were installed, one in the Cerrado *sensu strictu*, two in Cerradão and one in fields with sparse shrubs.

Sampling methods

The Squamata reptile sampling was conducted once a week between November 2009 and November 2010. Sets of pitfall traps were installed, with interception or guiding fences (AQs) as proposed by Greenberg et al. (1994) and Cechin & Martins (2000) and with funnel traps (FTs) as described by Hudson (2007), at 10 sampling points. Each sampling point consisted of 100 m of fence interception (1 m high), 10 funnel traps and five 20-liter buckets, in alternating funnel and bucket pairs. A total of 10 sets of traps were installed in the four different vegetation types, thus totaling 50 AQs, 100 FTs and 1000 m of guiding fence.

In addition to these sampling methods, trap specimens were also caught and collected through a limited-time search during the diurnal period (from 8:00 to 18:00), covering the various microhabitats. Specimens were sought on trees, in burlap, under fallen logs and in hollow trees, termite nests, burrows and rodents' galleries (Bernarde 2008). The specimens occasionally found by staff members or other people were used to supplement the list of species, and these were also considered to be among the four collection methods used by Zanella & Cechin (2006).

Specimens found during maintenance tasks on the farm known as Coliseum (weeding, maintenance of fence posts, planting and cleaning) and during other everyday jobs in the region were also registered in the present study. One snake specimen was registered near one of the capture and collection areas, at a location that a farm employee reported, and other specimens were photographed in the APA Serra de São José at locations outside of the sampling areas (BM Sousa, personal observation).

The specimens that were caught or collected alive were photographed and were mostly released near the capture site. No more than five specimens of each species were collected for identification and registration, and these were deposited as voucher specimens in the Herpetological Collection, Reptile Section, of the Federal University of Juiz de Fora (CHUFJF - Reptiles).

In this paper, the term "lizard" is used in the traditional ecological sense, i.e. Squamata with eyelids and at least traces of legs, which obviously do not constitute a monophyletic group. The taxonomy adopted was that of Frost et al. (2001), with some updates provided by Carrasco et al. (2012). In order to standardize the results, the taxonomy recommended by the Brazilian Society of Herpetology (Costa & Bérnils 2015) was also used. To identify specimens, field guides, keys and descriptions in papers (Peters et al. 1986, Rodrigues 1987; Ávila-Pires 1995; Doan & Arriaga 2002) were used. For Teiidae, a paper by Pyron et al. (2013) was used; and for Scincidae, the nomenclature proposed by Hedges & Conn (2012) was used. Representative specimens are listed in Appendix 1.

Data analysis

Statistical analyses were performed on species diversity and similarity between habitats. The richness of species of the four vegetation types sampled was compared by means of rarefaction curves in the EstimateS 9.1.0 software (Colwell 2013) with a thousand randomizations, through the Jackknife 1 estimator. The curves were generated through the bootstrap method, because this uses data on all species, i.e. not limited to rare species (Santos 2003), considering that snake fauna are often recorded with only one or two specimens of each species. The richness in the four vegetation types sampled was compared by means of species rarefaction curves. The similarity was studied through Jaccard's qualitative similarity index (Magurran 2004). The diversity was estimated using Margalef's index (DMg), which expresses richness weighted by the range of sample size; Simpson's index (1/D), which measures dominance influenced by the most common species, i.e. the likelihood that two individuals caught randomly would belong to the same species; and Shannon's index (H'), which places a value on the proportional abundance of species, emphasizing richness and homogeneity. The latter was used to calculate Shannon's equitability index (J).

Results

Overall, the sampling effort for this study lasted for 12 months, involving uninterrupted open traps and 52 field trips, totaling 8640 hours of bucket sampling and 300 hours of time-limited hunts. We recorded a total of 157 specimens belonging to 13 families and 29 species of Squamata reptiles: one species of amphisbaena (n = 3), 12 species of lizards (n = 123) and 16 snakes (n = 31) (Figures 1 to 3). Considering first-order jackknife estimators, the richness estimated for the study area, in the four vegetation types sampled was approximately 32 species (Figures 4 and 5). None of the species rarefaction curves reached the asymptote.

In this study, snakes contributed the largest number of species within the APA reptile community, but numerically, 79% of the individuals caught belonged to lizard families. Among the snakes, the family Dipsadidae was the richest, with a total of nine sampled species. For the lizards, the greatest richness was found in the family Teiidae (n = 3). The most abundant families were Teiidae (n = 35), Leiosauridae (n = 32) and Tropiduridae (n = 28), which accounted for 61% of all the Squamata reptiles studied (Table 1).

Although fields with sparse shrubs were only sampled at one point, this vegetation type presented important results because of the six species recorded at the sampling site, of which four were unique to this environment. The only way to compare all the capture sets in this study was by means of capture efficiency, i.e. the ratio between the number of animals caught and the number of days for which the traps remained in place (Table 2).

Table 1. Squamata Reptile species sampled in the Serra de São José, Tiradentes, Minas Gerais, and their abundances according to vegetation type. GF = Gallery Forest, CD = Cerradão, CE= Cerrado *sensu strictu*, CS = Dirty field, SI = no information about the location of collection / capture and ST = subtotal.

	GF	CD	CE	DF	SI	ST
Amphisbaenidae						
Leposternon microcephalum Wagler in Spix, 1824		1			2	3
Anguidae						
Ophiodes striatus (Spix, 1825)	1	1		2	1	5
Gekkonidae						
Hemidactylus mabouia (Moreau de Jonnès, 1818)					1	1
Gymnophthalmidae						
Ecpleopus gaudichaudi Duméril & Bibron, 1839	2	4				6
Heterodactylus imbricatus Spix, 1825	1	3				4
Leiosauridae						
Enyalius bilineatus Duméril & Bibron, 1837	5	10	17			32
Mabuyidae						
Aspronema dorsivittatum (Cope, 1862)				2		2
Notomabuya frenata (Cope, 1862)	1	1	8			10
Teiidae						
Ameiva ameiva (Linnaeus, 1758)				13		13
Ameivula ocellifera (Spix, 1825)		1	18			19
Salvator merianae (Duméril & Bibron, 1839)		1	2			3
Tropiduridae						
Tropidurus itambere Rodrigues, 1987				2		2
Tropidurus torquatus (Wied, 1820)			26			26
SQUAMATA: SERPENTES						126
Boidae						
Epicrates crassus Cope, 1862					1	1
Colubridae						
Chironius flavolineatus (Jan, 1863)		1				1
Chironius quadricarinatus (Boie, 1827)		1				1
Dipsadidae						
Atractus pantostictus Fernandes & Puorto, 1994					2	2
Echinanthera melanostigma (Wagler in Spix, 1824)	1					1
Erythrolamprus typhlus (Linnaeus, 1758)		4				4
Oxyrhopus guibei Hoge & Romano, 1978	1		2			3
Philodryas olfersii (Lichtenstein, 1823)				1		1
Sibynomorphus mikanii (Schlegel, 1837)					1	1
Sibynomorphus neuwiedi (Ihering, 1911)					1	1
Taeniophallus affinis (Günther, 1858)		1				1
Xenodon merremii (Wagler in Spix, 1824)		6		1		7
Elapidae						
Micrurus lemniscatus (Linnaeus, 1758)					1	1
Viperidae						
Bothrops jararaca (Wied, 1824)	2					2
Bothrops neuwiedi Wagler in Spix, 1824		1	2			3
Crotalus durissus (Linnaeus, 1758)			1			131
TOTAL	**14**	**36**	**76**	**21**	**10**	**157**

The Cerrado *sensu strictu* areas had highest abundance (n = 76), followed by the Cerradão area (n = 36). The lowest abundance was found

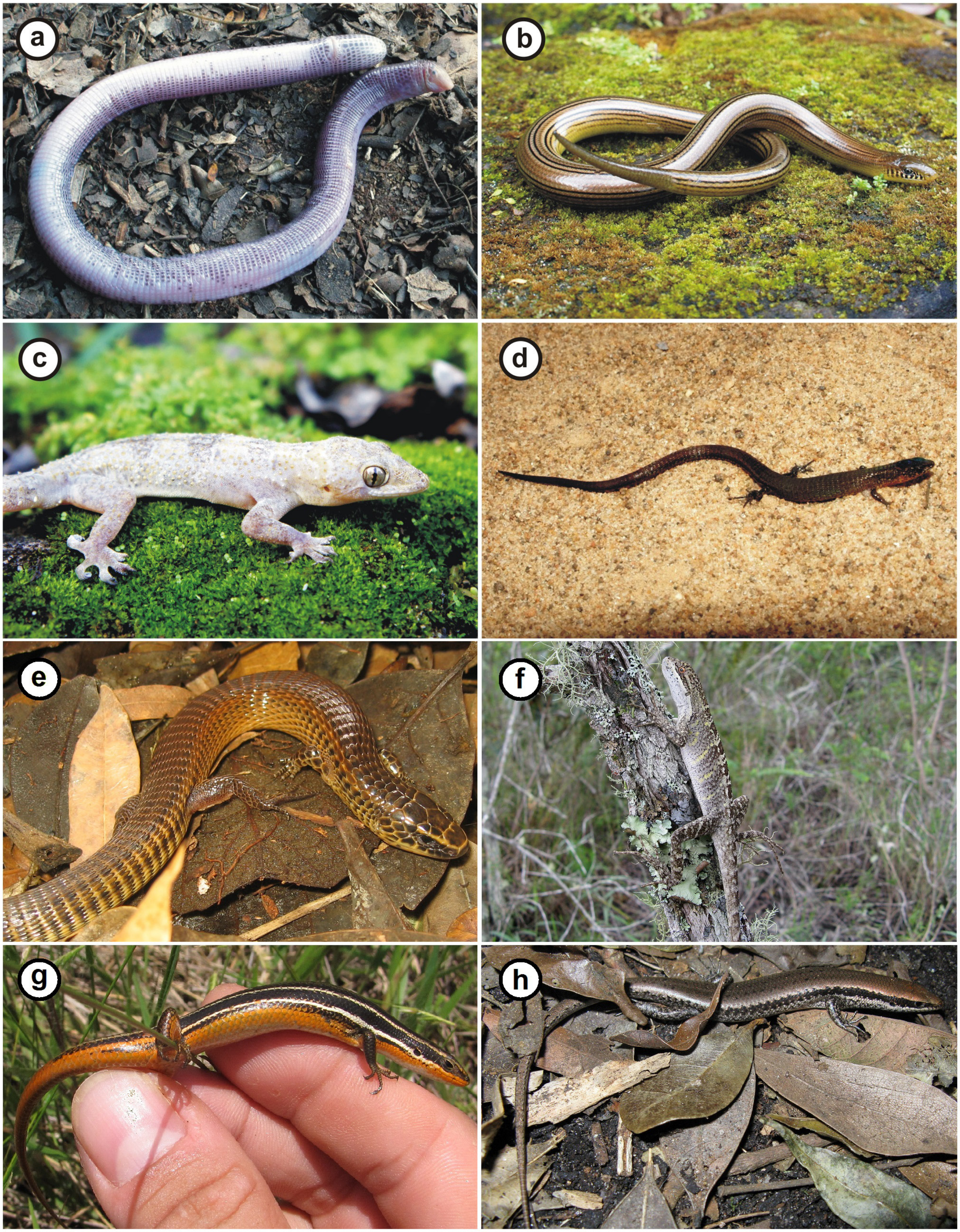

Figure 1. Squamata species recorded for the São José Mountains in Tiradentes, Minas Gerais. In a) *Leposternon microcephalum*, b) *Ophiodes striatus*, c) *Hemidactylus mabouia*, d) *Ecpleopus gaudichaudi*, e) *Heterodactylus imbricatus*, f) *Enyalius bilineatus*, g) *Aspronema dorsivittatum*, h) *Notomabuya frenata*.

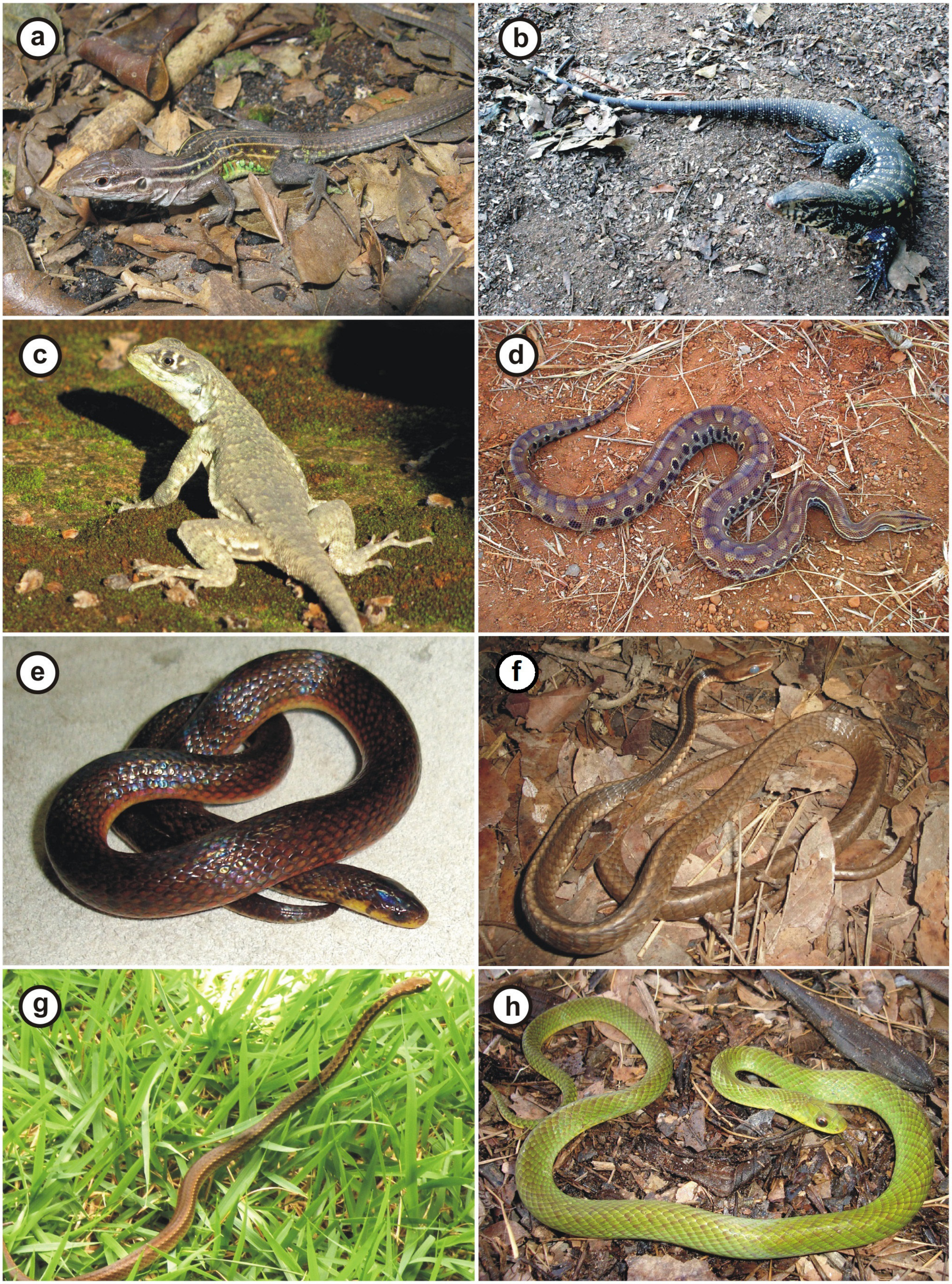

Figure 2. Squamata species recorded for the São José Mountains in Tiradentes, Minas Gerais. In a) *Ameivula ocellifera*, b) *Salvator merianae*, c) *Tropidurus torquatus*, d) *Epicrates crassus*, e) *Atractus pantostictus*, f) *Chironius flavolineatus,* g) *Echinanthera melanostigma*, h) *Erythrolamprus typhlus*.

Figure 3. Squamata species recorded for the São José Mountains in Tiradentes, Minas Gerais. In a) *Oxyrhopus guibei,* b) *Sibynomorphus mikanii,* c) *Sibynomorphus neuwiedi,* d) *Taeniophallus affinis,* e) *Xenodon merremii,* f) *Bothrops jararaca,* g) *Bothrops neuwiedi,* h) *Crotalus durissus*.

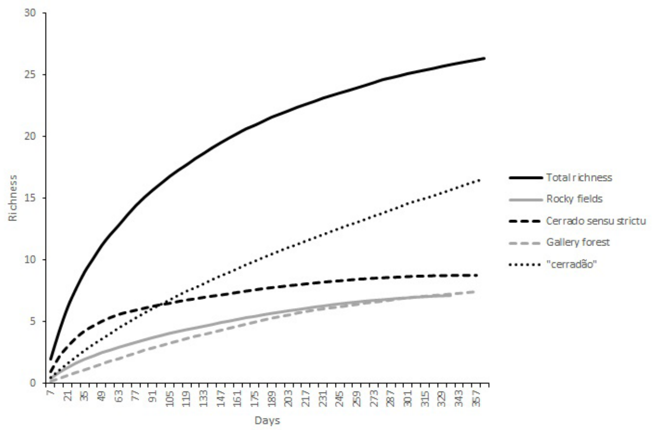

Figure 4. Accumulation curve of species of Reptilia in 1000 based randomization for each sampled vegetation type in the Conservation Area São José Mountains in Tiradentes, Minas Gerais between November 2009 and December 2010.

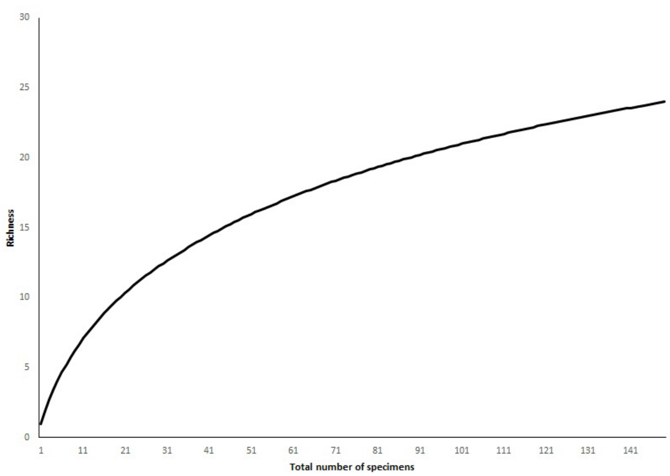

Figure 5. Accumulation curve of species of Reptilia in 1000 based randomization for total richness species in the Conservation Area São José Mountains in Tiradentes, Minas Gerais between November 2009 and December 2010.

in the gallery forest area (n = 14), but this was the vegetation type with the highest equitability. The greatest richness was found in the Cerradão area, with 13 species recorded. The areas that had highest diversity according to the three indexes were the Cerradão and gallery forest, respectively, as shown in Table 3.

The areas of Cerradão and Cerrado *sensu strictu* showed the greatest similarity (CJ = 0.31), with presence of five species in common recorded. *Bothrops neuwiedi* (n=3) was the only species of snake sampled, and two of the three most abundant species of lizards in both environments were *Enyalius bilineatus* (n = 32) and *Ameivula ocellifera* (n = 19). The other similarity values are shown in Table 4.

The species with fossorial and subfossorial habits such as *Leposternon microcephalum*, *Atractus pantostictus* and *Micrurus lemniscatus* were recorded only through the chance encounter method, as also were *Chironius quadricarinatus*, *Sibynomorphus mikanii* and *S. neuwiedi*. The only specimen of *S. neuwiedi* was found hurt near one of the sampling sites, while *Epicrates crassus* and *Hemidactylus mabouia* were photodocumented in the Serra de São José, in rock field areas. This method proved of highly important, especially with regard to snakes, because among the 16 species recorded, six were sampled only through chance encounters (Table 5).

Table 2. Capture efficiency of Squamata specimens for each line of Fall Trap and Funnel Trap, held from November 2009 to November 2010, in the São José Mountains, Tiradentes, Minas Gerais.

Vegetation type and Collection site	Collection days	Abundance	Richness	Eficiency
Gallery Forest - Águas Santas	244	1	1	0.004
Gallery Forest - Bosque Mãe D'Água 2	390	3	3	0.008
Cerrado sensu strictu - Águas Santas	192	2	1	0.010
Gallery Forest - Bosque Mãe D'Água 1	390	5	3	0.013
Cerradão - Águas Santas	275	4	2	0.015
Cerradão - Coliseum 1	390	10	8	0.026
Cerradão - Coliseum 2	390	15	9	0.039
Dirty Field – Coliseum	371	18	6	0.049
Cerrado sensu strictu - Bosque Mãe D'Água	390	29	7	0.074
Cerrado sensu strictu – Coliseum	390	40	6	0.103

Table 3. Richness, abundance, diversity and evenness in the vegetation types studied in the Serra de São José, Tiradentes, Minas Gerais.

Indices/Vegetation Type	Dirty Field	Cerrado	Gallery Forest	Cerradão
Margalef Diversity (Dmg)	1.64	1.36	3.06	2.28
Simpson Diversity (1/D)	2.41	4.04	5.40	6.33
Shannon Diversity (H')	1.26	1.55	1.74	2.06
Shannon Equitability(J)	0.70	0.80	0.97	0.89

Table 4. Qualitative similarity of Jaccard (CJ) between vegetation types Dirty Field, Cerrado sensu strictu and Riparian Forest, studied in the Serra de São José, Tiradentes, Minas Gerais.

	Dirty Field	Cerrado sensu strictu	Gallery Forest	Cerradão
Dirty Field	-	0	0.09	0.14
Cerrado sensu strictu	0	-	0.08	0.31
Gallery Forest	0.09	0.08	-	0.23

Table 5. Richness and abundance obtained according to each sampling methodology of reptiles in the Conservation Area São José Mountains in Tiradentes, Minas Gerais between November 2009 and December 2010. FT = Funnel Trap, PT = Pitfall Trap, SLT = Search limited by time, OE = Occasional Encounter.

	FT	PT	SLT	OE	Total
Richness	16	9	4	17	**29**
Abundance	66	62	8	21	**157**

Discussion

It can be seen that the composition of the communities varied according to the vegetation gradient (physiognomy), which is consistent with the pattern recorded by Nogueira et al. (2011). The vegetation type with the highest abundance recorded was the Cerrado *sensu strictu*, where about 50% of the specimens were caught and collected, while the Cerradão had the highest richness and diversity with overlapping between species of open areas and those of gallery forest areas. The greater heterogeneity of the Cerradão and the fact that the largest number of species was recorded in this area in the present study are consistent with the theory of MacArthur & MacArthur (1961), in which they stated that habitats with greater structural complexity tend to provide shelter for greater diversity. Although the traps used for sampling Squamata in this study are not ideal for tree-dwelling species and semi-arboreal species, the most abundant species in this study (*E. bilineatus*, which is semi-arboreal) was also more abundant in the Cerrado *sensu strictu* than in the gallery forest.

According to Nogueira et al. (2005) in a study in Cerrado areas, the lizard fauna is distributed in a mosaic, consisting of species with strict and predictable requirements as to the type of environment, which follow the habitat spots available. These data corroborate those found in the present study. However, one anthropic factor affecting the size of the populations of Squamata in the APA Serra de São José is fires. Large-scale fires occur frequently, and this was observed on the north side of the Sierra de São José and in the woods south of the mountain, during the period when this study was being developed.

Silva & Bates (2002) considered that these forested areas play an important role in biodiversity conservation, through maintaining viable populations of typical forest species in Cerrado *sensu strictu* environments. Therefore, the greater similarity between the sampled areas of Cerradão and Cerrado *sensu strictu* in the present study can be explained through formation of mosaics of Cerradão forested areas with Cerrado *sensu strictu* areas and fields with sparse shrubs, thus forming environments that are more heterogeneous than the areas of gallery forest. This makes it possible both for animals typical of forest environments and for animals typical of open areas to occupy this type of habitat. In a study on herpetofauna associated with gallery forests in the Federal District, Brandão & Araújo (2001) highlight the importance of forested environments in Cerrado areas, for maintaining local diversity.

According to Nogueira et al. (2005), the Cerrado provides great number of replacement species for nearby locations because of the mosaic of vegetation types. These authors studied the richness, fauna composition and local distribution of lizards in a 6,000 ha fragment in the Cerrado of central Brazil. They found that the richness values, with 17 sympatric species belonging to seven families outweighed most of the data from previous studies in Cerrado localities.

Representatives of the Squamata fauna studied here have been registered in other areas of the Cerrado, including *Bothrops neuwiedi*, *Crotalus durissus*, *Chironius flavolineatus*, *Ameivula ocellifera*, *Enyalius bilineatus*, *Oxyrhopus guibei* and *Xenodon merremii* (Bailey 1955, Teixeira-Filho et al. 1995; Nogueira 2001; France et al. 2008, Pinto et al. 2008; Sawaya et al. 2008); and in the Atlantic Forest, including *Bothrops jararaca*, *Ecpleopus gaudichaudi*, *Erythrolamprus typhlus* and *Taeniophallus affinis* (Di-Bernardo 1992; Marques et al. 2001; Feio & Caramaschi 2002; São-Pedro Pires & 2009; Condez et al. 2009). However, *B. neuwiedi* was also recorded in the Atlantic Forest area by França et al. (2012).

Among the lizards caught in the present study, only *Salvator merianae*, *Ecpleopus gaudichaudii* and *H. imbricatus* were not recorded by Nogueira et al. (2009) in Cerrado areas in central Brazil. However, Novelli et al. (2011, 2012) recorded *H. imbricatus* in gallery forest of this biome in the municipality of Ingaí in southern Minas Gerais. In the study by Cruz et al. (2014) in the Ouro Branco mountains, the species *E. gaudichaudii* was recorded, while *H. imbricatus* was recorded in riparian forest. These authors considered that these two species were endemic to the Atlantic Forest biome.

The genus *Ecpleopus* is currently considered to be monotypic, consequent to its last review by Uzzell (1969). However, recent molecular studies have indicated that it has greater taxonomic complexity. The evidence suggests that the name *E. gaudichaudi* may not apply to individuals in Minas Gerais and that there could soon be a new revision of the genus (Carolino 2010). This species was considered rare until a few years ago (Eisemberg et al. 2004), but expansion of the collection efforts has shown that this species is common, as reported by Dixo & Verdade (2006) in forested areas of the Morro Grande Forest Reserve in Cotia, São Paulo, in which 54% of the lizards caught belonged to this species.

Other records of reptile fauna in transition regions between the Atlantic Forest and Cerrado in Minas Gerais, near the study area, were also consulted. Sousa et al. (2010) found 29 species in the municipality of Ritápolis, of which 16 were also recorded in the present study. In Ouro Branco, out of the 28 species of snakes registered by São-Pedro & Pires (2009), 10 were also found in the present study and by Silveira et al. (2010) in the municipalities of Ouro Preto, Mariana and Itabirito.

The richness of 29 species that was recorded in the present study was greater than in two other Squamata reptile surveys in similar environments of the Atlantic Forest, conducted by Ulisses & Caramaschi (2002) , who found richness of 11 species, and Gomides (2010), who found richness of 14 species. However, in most studies in similar areas, the species richness was greater than that presented in this survey (Silveira et al, 2010; Costa et al, 2010; Moura et al, 2012; Cross et al., 2014). This highlights the need for greater effort towards sampling of Squamata reptiles in the APA Serra de São José.

None of the rarefaction curves reached the full asymptote (Figure 4), which highlights the need for more studies and new data analysis methods, as seen in the study by Lopez et al. (2012), because of the high likelihood that new species may be recorded in the area studied. It is necessary to invest greater efforts in unsampled environments such as waterlogged fields and rock fields at the top of the São José mountains. Other important factors that influence the sampled data include the methods used for sample capture and collection. The methods that were used in the present study are the ones that are most used. However, further investments in searching for arboreal species are still needed, because this group was considered to be undersampled in this study.

The invasive species *Hemidactylus mabouia* is related to the urban environment, but has colonized rock areas near the study sites and presents high potential for colonization through occupying both preserved and degraded habitats, as previously also observed by Sousa & Freire (2010). In the rock field area of the APA Serra de São José, one specimen of *Epicrates* was photographed in the vicinity of the municipalities of Tiradentes and Ritápolis. The species of *Epicrates* occurring in the collection area of the present study (Souza, 2011) may have been *E. crassus*, given that this species had been recorded by Sousa et al. (2010) in the municipality of Ritápolis. According to Passos & Fernandes (2008), *E. crassus* is a species in the family Boidae, which is found in open areas, mainly in the southeastern and central-western regions of Brazil.

Among the snakes found, five have medical importance: the viperids *Bothrops jararaca*, *Bothrops neuwiedi* and *Crotalus durissus*, the elapid *Micrurus lemniscatus* and the dipsadid *Philodryas olfersii*. Accidents involving these snakes can be considered serious (Cardoso et al. 2003; Salomão et al. 2003).

None of the species recorded in this study present any level of threat on the IUCN list (2015), at national level (Machado et al. 2008) or at state level (COPAM 2010). However, *H. imbricatus* is classified as a "presumably endangered species" for the state of Minas Gerais (Biodiversitas 2007).

During this study, threats to the reptile fauna were detected at the sampling sites. The north face of the mountain range is subject to strong anthropic pressure, such as wood-cutting activities and the presence of tracks and garbage resulting from tourism. Next to the region chosen for evaluation on the north side of the mountain range is the entrance to the Mangue trail, which is greatly used for crossing the mountain range. Although the environments studied in the south of the APA are little affected by tourism, mainly because they are private areas, there is some pressure from hunters and there have been some reports of consumption of meat from *Salvator merianae*. Another threat to the local fauna that was noted is fires. Large fires have occurred in several consecutive years, including to the north of the study area during the experiment; and on September 9, 2012, when 480 hectares of forest were burned (Gazette 2012) on the south side of the Serra de São José. According to reports from residents, such occurrences are common in the APA. The number of plant and animal species registered, including new species, highlights the importance of the protected areas of the Serra de São José for maintenance of species diversity regionally and in the whole of the state of Minas Gerais. The most recent data available for the entire state come from the study by Bérnils et al. (2009). This area requires continual monitoring by the proper bodies, given its importance for the survival of several species that have been little studied or are unknown.

Appendix

Appendix 1. List of specimens collected and deposited at the Herpetological Collection-Reptiles of Universidade Federal de Juiz de Fora, Minas Gerais.

Ameiva ameiva, 788, 789, 790; *Ameivula ocellifera*, 786, 787, 798, 799, 801; *Leposternon microcephalum*, 782, 783, 813; *Aspronema dorsivittatum*, 802; *Atractus pantostictus*, 780, 781; *Bothrops jararaca*, 773, 775; *Bothrops neuwiedi*, 829; *Chironius flavolineatus*, 772; *Chironius quadricarinatus* 807; *Echinanthera melanostigma*, 797; *Ecpleopus gaudichaudi*, 793, 794, 814, 815; *Enyalius bilineatus*, 784, 777; *Oxyrhopus guibei*, 795, 806; *Philodryas olfersii*, 778; *Sibynomorphus mikanii*, 812; *Sibynomorphus neuwiedi*, 791; *Tropidurus itambere*, 796; *Tropidurus torquatus*, 774, 821, 822; *Salvator merianae*, 678; *Xenodon merremii*, 677, 805.

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inventory

Inventory of mollusks from the estuary of the Paraíba River in northeastern Brazil

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Abstract*:* Coastal ecosystems of northeastern Brazil have important biodiversity with regard to marine mollusks, which are insufficiently studied. Here we provide an inventory of mollusks from two sites in the estuary of the Paraíba River. Mollusks were collected in 2014 and 2016 on the coast and sandbanks located on the properties of Treze de Maio and Costinha de Santo Antônio. The malacofaunal survey identified 12 families, 20 genera and 21 species of bivalves, 17 families, 19 genera and 20 species of gastropods and one species of cephalopod. Bivalves of the family Veneridae Rafinesque, 1815 were the most representative, with a total of five species. Gastropods of the family Littorinidae Children, 1834 had the greatest species richness. The most abundant species were: *Neritina virginea* (Linnaeus, 1758), *Brachidontes exustus* (Linnaeus, 1758), *Crassostrea brasiliana* (Lamarck, 1819), *Cerithium atratum* (Born, 1778), *Anomalocardia brasiliana* (Gmelin, 1791), *Parvanachis obesa* (C. B. Adams, 1845), *Phrontis polygonata* (Lamarck, 1822), *Littoraria angulifera* (Lamarck, 1822), *L. flava* (King, 1832), *Tagelus plebeius* (Lightfoot, 1786), *Echinolittorina lineolata* (d'Orbigny, 1840) and *Iphigenia brasiliensis* (Lamarck, 1818). The results show that the study area has considerable species richness of Mollusca, requiring environmental monitoring in the region mainly due to the economic importance of some species to the local population.

Keywords***:*** *Biodiversity, Mollusca, Gastropoda, Bivalvia, Cephalopoda, tropical coastal ecosystems.*

Inventário de moluscos do estuário do Rio Paraíba no nordeste do Brasil

Resumo*:* Os ecossistemas costeiros do nordeste do Brasil têm uma importante biodiversidade de moluscos marinhos, a qual ainda é insuficientemente estudada. Este trabalho representa um inventário dos moluscos em duas localidades no estuário do Rio Paraíba. Moluscos foram coletados entre 2014 e 2016 na costa e área de restinga localizadas nas propriedades de Treze de Maio e Costinha de Santo Antônio. Este levantamento malacofaunístico identificou 12 famílias, 20 gêneros e 21 espécies de bivalves, 17 famílias, 19 gêneros e 20 espécies de gastrópodes e uma espécie de cefalópode. Os bivalves da família Veneridae Rafinesque, 1815 foram os mais representativos com um total de cinco espécies. Os gastrópodes da família Littorinidae Children, 1834 apresentaram a mais alta riqueza de espécies. As espécies mais abundantes foram: *Neritina virginea* (Linnaeus, 1758), *Brachidontes exustus* (Linnaeus, 1758), *Crassostrea brasiliana* (Lamarck, 1819), *Cerithium atratum* (Born, 1778), *Anomalocardia brasiliana* (Gmelin, 1791), *Parvanachis obesa* (C. B. Adams, 1845), *Phrontis polygonata* (Lamarck, 1822), *Littoraria angulifera* (Lamarck, 1822), *L. flava* (King, 1832), *Tagelus plebeius* (Lightfoot, 1786), *Echinolittorina lineolata* (d'Orbigny, 1840) e *Iphigenia brasiliensis* (Lamarck, 1818). Os resultados mostram que a área estudada tem uma considerável riqueza de espécies de Mollusca, necessitando de monitoramento ambiental, principalmente devido à importância econômica de algumas espécies para a população local.

Palavras-chave***:*** *Biodiversidade, Mollusca, Gastropoda, Bivalvia, Cephalopoda, ecossistemas costeiros tropicais.*

Introduction

The coast of Brazil has a considerable diversity of marine ecosystems (Amaral & Jablonski 2005, MMA 2010) and significant species richness with regard to invertebrates living in a wide variety of intertidal and sublittoral environments (Amaral & Jablonski 2005, Amaral et al. 2006, 2006-2012, Rios 2009). However, the biodiversity of invertebrates, especially mollusks remains poorly sampled and insufficiently researched in shallow waters of northeastern Brazil (Barroso et al. 2013, Cunha et al. 2016). Underestimated knowledge on the aspects of the biodiversity of Mollusca in northeastern Brazil has been demonstrated based on the discovery of ecological interactions (Queiroz et al. 2011, 2013, Lima et al. 2016a), records of occurrence and the description of new species (Barroso et al. 2013, Barros et al. 2015, Lima & Guimarães 2015, Cunha et al. 2016, Lima et al. 2013, 2016b,c Lima & Christoffersen 2016).

The state of Paraíba (northeastern Brazil) has approximately 138 km of coastline (De Assis et al. 2012) and a considerable variety of marine ecosystems and environments (e.g., mangroves, estuarine habitats, diverse reef formations, rocky beaches, sandy beaches and tide pools) (Melo et al. 2006, Araújo et al. 2008, De Assis et al. 2012, Duarte et al. 2014, 2015, Lima et al. 2014, Medeiros et al. 2016). The biodiversity of marine invertebrates (mainly mollusks and polychaetes) in this coastal area has been increasingly studied (Duarte et al. 2014, 2015, Santos et al. 2010, 2014, Lomônaco et al. 2011, De Assis et al. 2012, Brito et al. 2013, Fukuda et al. 2013, Paresque & Nogueira 2014, Lima et al. 2014, Prata et al. 2014, Lucena et al. 2015, Lima & Christoffersen 2016, Paresque et al. 2014, 2016), however, the mollusk diversity of large areas, such as basin of the Paraiba River remains unknown.

The marine zone surrounding the Treze de Maio and Costinha de Santo Antônio properties in the municipality of Lucena (state of Paraíba) is under the influence of abiotic and biotic factors of the Atlantic Ocean as well as the estuary of the Paraíba River. Part of the area located on these properties will be allocated to the implementation of a shipyard, so it is of fundamental importance to generate knowledge on the composition of marine mollusks in the area for environmental management and conservation strategies, including monitoring purposes.

The aim of this study was to provide a malacofaunistic survey from the estuary of the Paraíba River in the state of Paraíba (northeastern Brazil).

Material and Methods

1. Study site

The studied area is located on the properties of Treze de Maio and Costinha de Santo Antônio (06°58’17.59”S, 34°51’47.19”W), which are within the area of influence of the estuary of the Paraíba River in the city of Lucena, state of Paraíba, northeastern Brazil. The surrounding coastal environment is characterized by mangrove forests (Sassi 1991) in non-urbanized areas and a large sand bank that is exposed at low tide (personal observations). The study area is under the influence of the Atlantic Ocean (Medeiros et al. 2016) and the main tributaries on the right (Sanhauá, Tambiá and Mandacaru Rivers) and left (Paroeira, Tiririm, Ribeira and Guia Rivers) margins, which carry sediment, nutrients, domestic sewage and industrial waste into the region (Sassi 1991, Marcelino et al. 2005). The area is not under the direct influence of wave impacts and has a beach with flat to slightly steep areas (personal observations), with predominantly sandy-muddy (Sassi 1991) (Figure 1).

1.1. Data collection and analysis

Mollusks were collected in two campaigns (June 2014 and February 2016) in a region planned for the implementation of a shipyard on the Treze de Maio and Costinha de Santo Antônio properties and adjacent areas. Collections were carried out at low tide through active searches lasting approximately two hours per day. During the first campaign, trawls were also employed for the collection of cephalopods. During the second campaign (February 15th to 19th, 2016), mollusks were manually collected at low tide in the supratidal to subtidal regions located along the coastal area and adjacent sandbanks of the properties. A total of eight samples of unconsolidated substrates were randomly collected from the shallow subtidal region in these areas at a depth of about 1 m: four collection sites along the coastal area of the properties (Figure 1B - dashed yellow line of

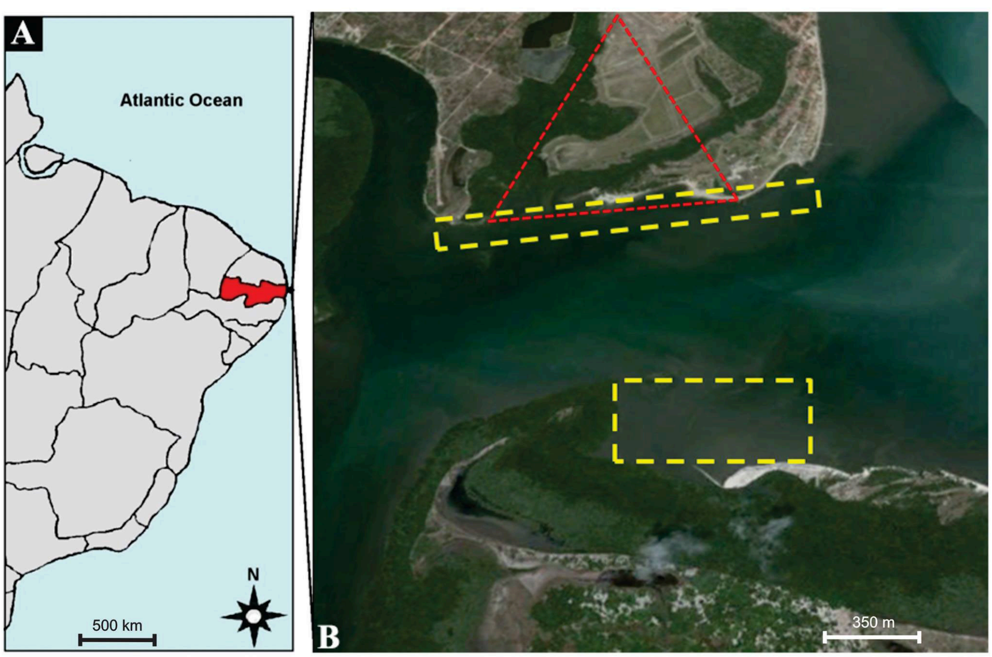

Figure 1. (A) Map of Brazilian coast and state of Paraíba (highlighted in red); **(B)** Photo of estuary of Paraíba River showing Treze de Maio and Costinha de Santo Antônio properties (upper area) and adjacent region (dashed red: construction area of shipyard; dashed yellow: collection area).

upper area) and four collection sites on the adjacent sandbanks (Figure 1B - dashed yellow line of lower area).

All mollusks and substrates collected were fixed in 5% formalin. In the laboratory, the substrates were processed through sieves (5 to 2 mm in mesh size) to separate mollusks. All specimens were preserved in 70% ethanol. The classification of Mollusca was based on the World Register of Marine Species (WORMS). The identification at the specific level was mainly based on Rios (2009), Tunnell Jr. et al. (2010) and Redfern (2013).

Most of the material analyzed is deposited and available for study at the "Coleção de Invertebrados Paulo Young, Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba (UFPB MOLL), João Pessoa, Paraíba, Brazil".

Results

A total of 1978 specimens from three classes, 14 orders, 31 families, 39 genera and 40 species were recorded for the estuarine region surrounding the Treze de Maio and Costinha de Santo Antônio properties (Table 1). This total includes one family and one species of cephalopod, 12 families, 20 genera and 21 species of bivalve and 18 families, 19 genera and 20 species of gastropod (Table 1; Figures 2–13).

Carditida, Myida, Anaspidea, Cephalaspidea, Cycloneritimorpha and Myopsida were each represented by one species, whereas Arcida, Lucinida and Mytilida were each represented by two species. Cardiida, Venerida, Littorinimorpha and Neogastropoda were the most representative groups,

Table 1. Checklist of Mollusca from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties. (-) species addressed in another study; (--) species observed in the environment.

Class	Order	Family	Species	Spec.	Voucher
	Arcida	Arcidae	*Anadara chemnitzi* (Philippi, 1851)	01	3553
	Arcida	Arcidae	*Lunarca ovalis* (Bruguière, 1789)	18	3554
	Cardiida	Donacidae	*Iphigenia brasiliensis* (Lamarck, 1818)	26	3555
	Cardiida	Cardiidae	*Dallocardia muricata* (Linnaeus, 1758)	06	3556
	Cardiida	Tellinidae	*Macoma constricta* (Bruguière, 1792)	17	3557
	Cardiida	Tellinidae	*Eurytellina lineata* (Turton, 1819)	12	3558
	Cardiida	Solecurtidae	*Tagelus plebeius* (Lightfoot, 1786)	52	3559
	Carditida	Crassatellidae	*Crassinella* sp.	01	3560
	Lucinida	Lucinidae	*Divalinga quadrisulcata* (d'Orbigny, 1846)	03	3561
	Lucinida	Lucinidae	*Phacoides pectinatus* (Gmelin, 1791)	11	3562
Bivalvia	Myida	Myidae	*Sphenia fragilis* (H. Adams & A. Adams, 1854)	01	3563
	Mytilida	Mytilidae	*Mytella charruana* (d'Orbigny, 1842)	12	3564
	Mytilida	Mytilidae	*Brachidontes exustus* (Linnaeus, 1758)	238	3565
	Ostreida	Ostreidae	*Crassostrea brasiliana* (Lamarck, 1819)	207	3566
	Ostreida	Ostreidae	*Ostrea* cf. *puelchana* d'Orbigny, 1842	01	-
	Venerida	Veneridae	*Anomalocardia brasiliana* (Gmelin, 1791)	149	3567
	Venerida	Veneridae	*Chione cancellata* (Linnaeus, 1767)	01	3624
	Venerida	Veneridae	*Chione subrostrata* (Lamarck, 1818)	18	3625
	Venerida	Veneridae	*Lamelliconcha circinata* (Born, 1778)	03	3626
	Venerida	Veneridae	*Tivela mactroides* (Born, 1778)	06	3627
	Unassigned	Mactridae	*Mulinia cleryana* (d'Orbigny, 1846)	08	3628
	Anaspidea	Aplysiidae	*Aplysia dactylomela* Rang, 1828	03	--
	Cephalaspidea	Bullidae	*Bulla striata* Bruguière, 1792	12	3630
	Cycloneritimorpha	Neritidae	*Neritina virginea* (Linnaeus, 1758)	610	3631
	Littorinimorpha	Cassidae	*Semicassis granulata* (Born, 1778)	01	3632
	Littorinimorpha	Littorinidae	*Littoraria angulifera* (Lamarck, 1822)	54	3633
	Littorinimorpha	Littorinidae	*Littoraria flava* (King, 1832)	37	3634
	Littorinimorpha	Littorinidae	*Echinolittorina lineolata* (d'Orbigny, 1840)	36	3635
	Littorinimorpha	Naticidae	*Stigmaulax cayennensis* (Récluz, 1850)	02	3636
	Neogastropoda	Columbellidae	*Parvanachis obesa* (C. B. Adams, 1845)	128	3637
	Neogastropoda	Melongenidae	*Pugilina tupiniquim* Abbate & Simone, 2015	15	3638
Gastropoda	Neogastropoda	Muricidae	*Stramonita brasiliensis* Claremont & D. G. Reid, 2011	09	3639
	Neogastropoda	Muricidae	*Chicoreus brevifrons* (Lamarck, 1822)	01	3640
	Neogastropoda	Nassariidae	*Phrontis polygonata* (Lamarck, 1822)	86	3641
	Neogastropoda	Olivellidae	*Olivella minuta* (Link, 1807)	02	3642
	Neogastropoda	Turbinellidae	*Turbinella laevigata* Anton, 1838	02	3643
	Unassigned	Calliostomatidae	*Calliostoma adspersum* (Philippi, 1851)	01	3644
	Unassigned	Cerithiidae	*Cerithium atratum* (Born, 1778)	169	3645
	Unassigned	Cerithiidae	*Bittiolum varium* (Pfeiffer, 1840)	01	3646
	Unassigned	Ellobiidae	*Melampus coffea* (Linnaeus, 1758)	15	3647
	Unassigned	Epitoniidae	not identified	01	-
	Unassigned	Trochidae	*Tegula viridula* (Gmelin, 1791)	01	3648
Cephalopoda	Myopsida	Loliginidae	*Lolliguncula brevis* (Blainville, 1823)	01	-

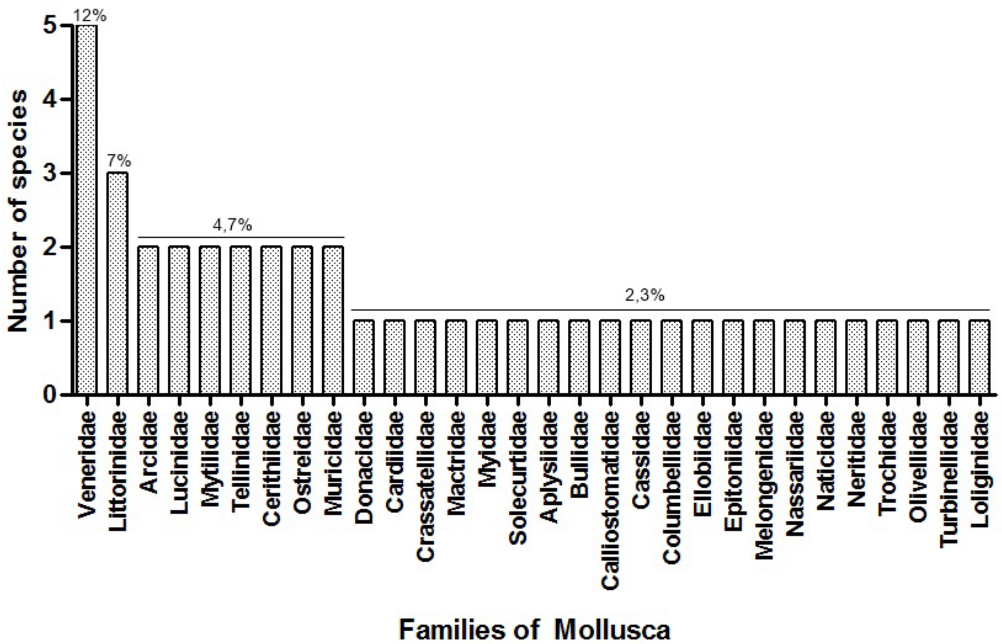

Figure 2. Number of species and relative frequency of mollusk families collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties.

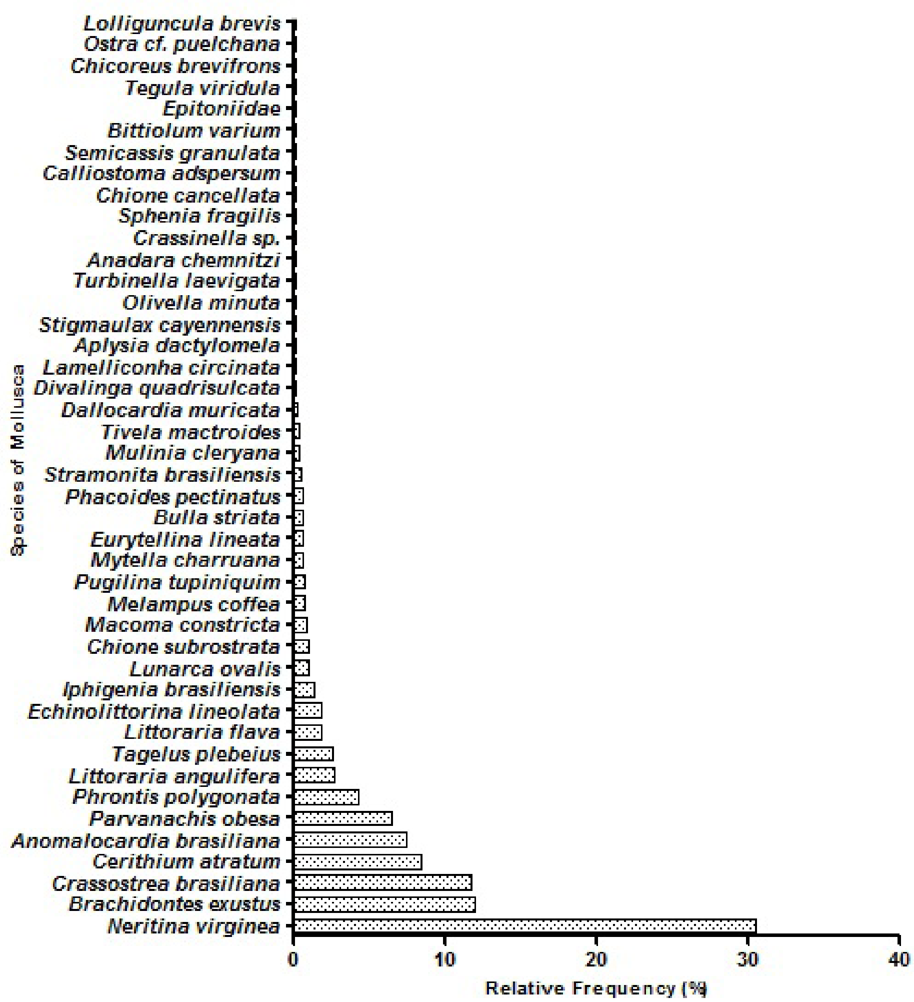

Figure 3. Species and relative frequency of mollusks collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties.

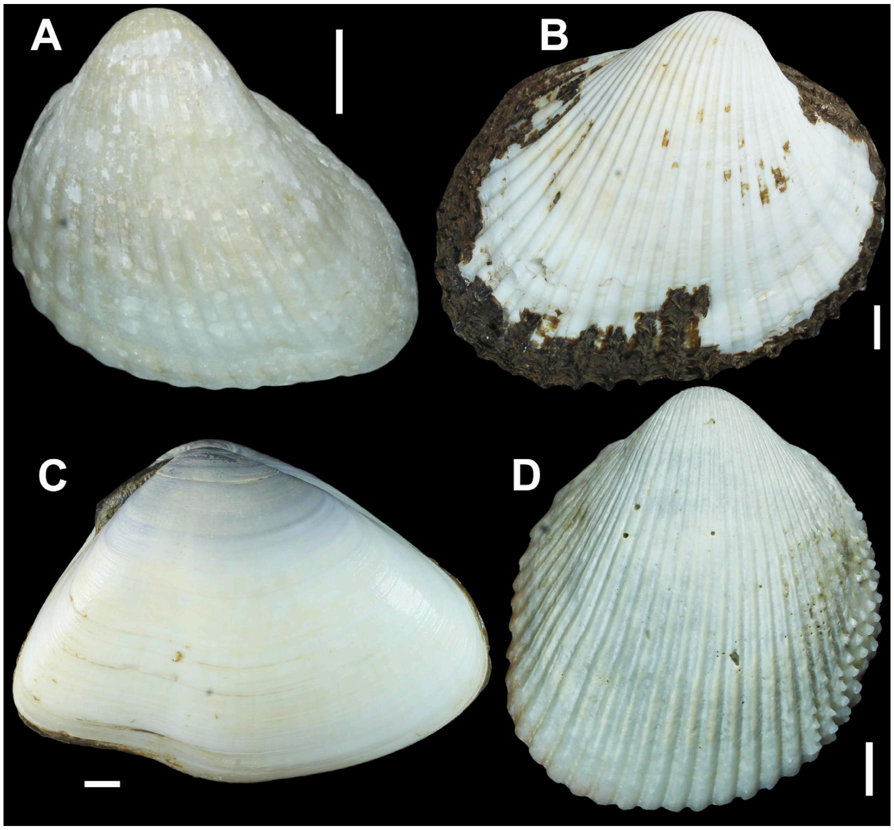

Figure 4. External view of shell of the bivalves collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Anadara chemnitzi*, (B) *Lunarca ovalis*, (C) *Iphigenia brasiliana*, (D) *Dallocardia muricata*. Scale bars: 5 mm.

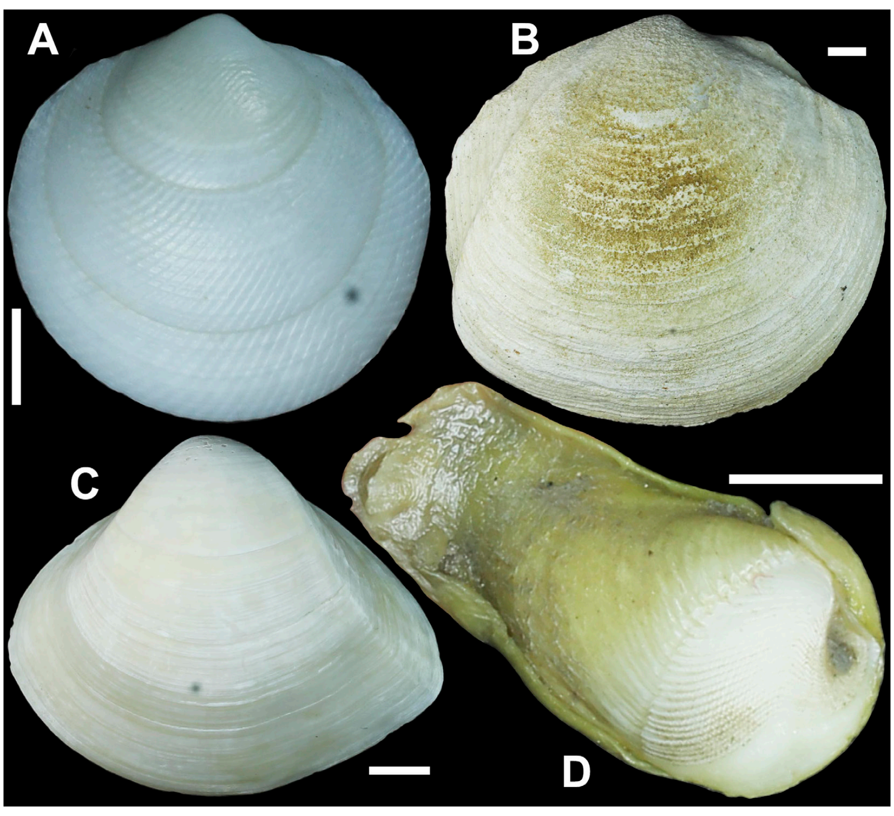

Figure 5. External view of shell of the bivalves collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Divalinga quadrisulcata*, (B) *Phacoides pectinatus*, (C) *Mulinia cleryana*, (D) *Sphenia fragilis*. Scale bars: 5 mm.

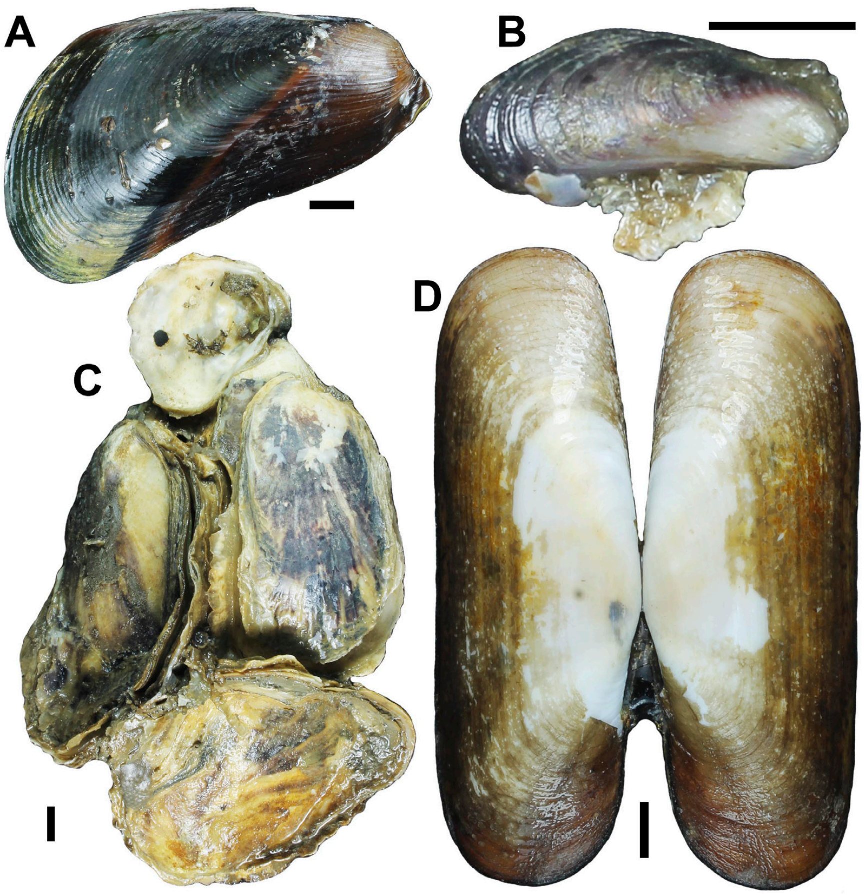

Figure 6. External view of shell of the bivalves collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Mytella charruana*, (B) *Brachidontes exustus*, (C) *Crassostrea brasiliana*, (D) *Tagelus plebeius*. Scale bars: 5 mm.

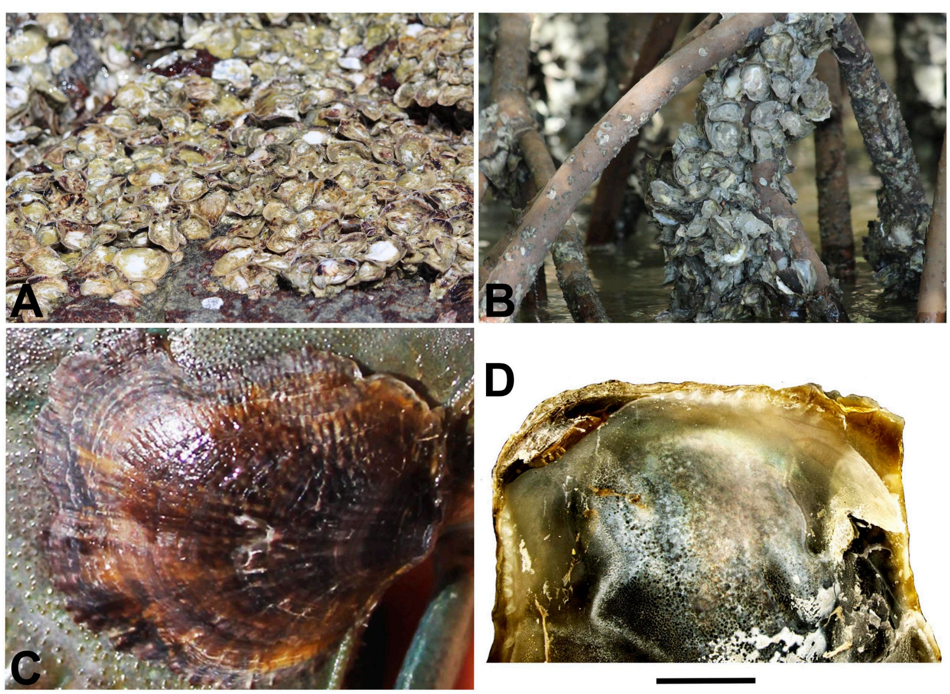

Figure 7. *Crassostrea brasiliana* on rock bottoms (A) and mangrove roots (B) and *Ostrea* cf. *puelchana* in external view of shell, left valve (C) and internal view of shell, left valve (D) from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties. Scale bars: C. 1 cm, D. 5 mm.

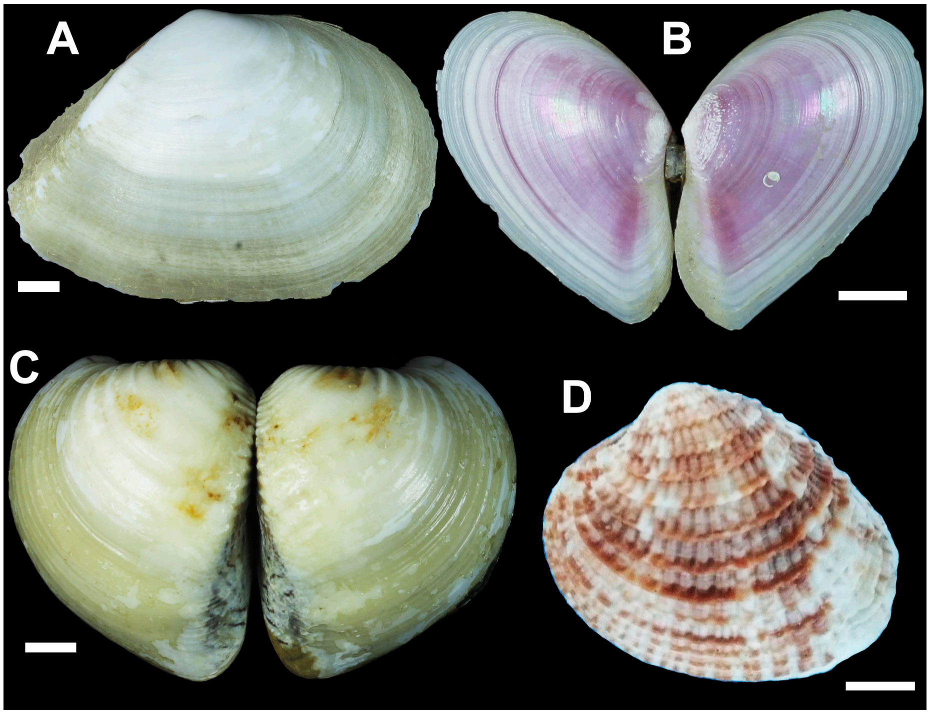

Figure 8. External view of shell of the bivalves collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Macoma constricta*, (B) *Eurytellina lineata*, (C) *Anomalocardia brasiliana*, (D) *Chione cancellata*. Scale bars: 5 mm.

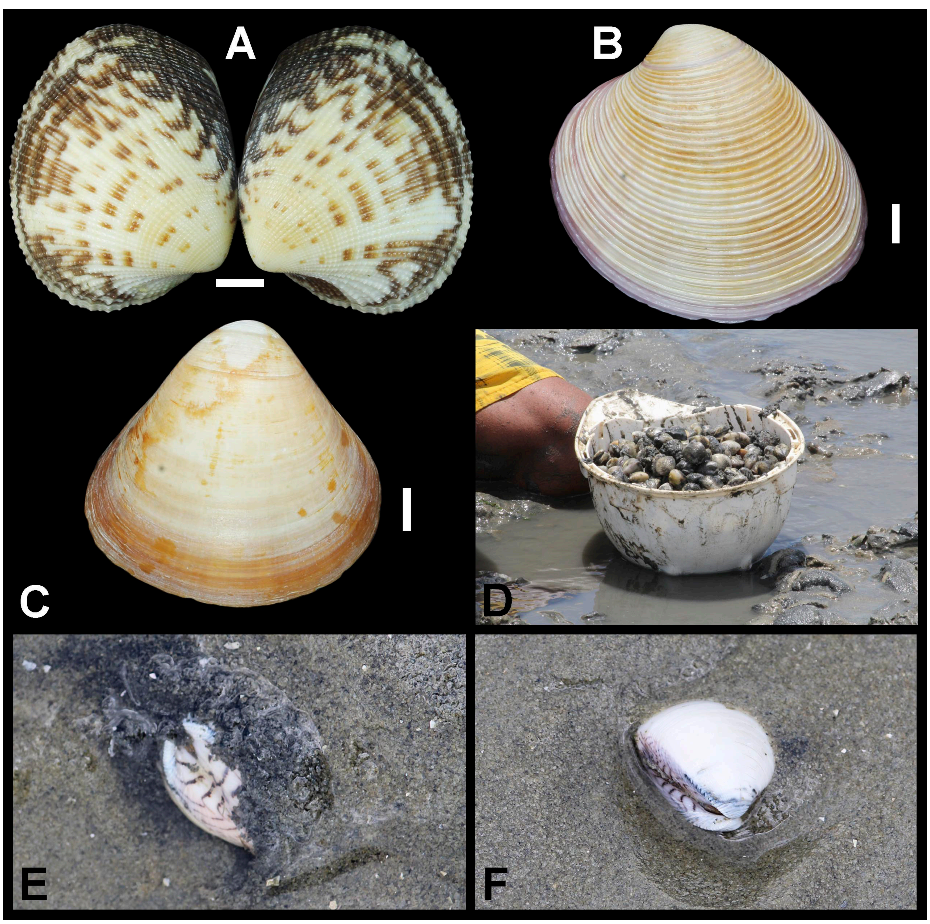

Figure 9. External view of shell of the bivalves collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Chione subrostrata*, (B) *Lamelliconcha circinata*, (C) *Tivela mactroides*, (D–F) *Anomalocardia brasiliana* (length: about 31 mm). Scale bars: A–C. 5 mm.

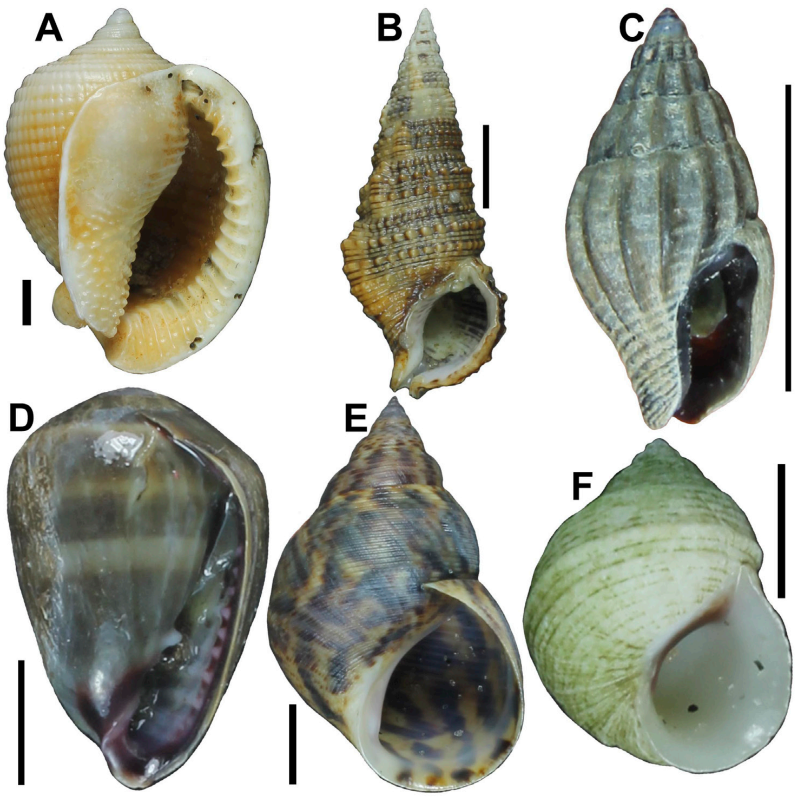

Figure 10. Ventral view of shell of the gastropods collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Semicassis granulata*, (B) *Cerithium atratum*, (C) *Parvanachis obesa*, (D) *Melampus coffea*, (E) *Littoraria angulifera*, (F) *L. flava*. Scale bars: 5 mm.

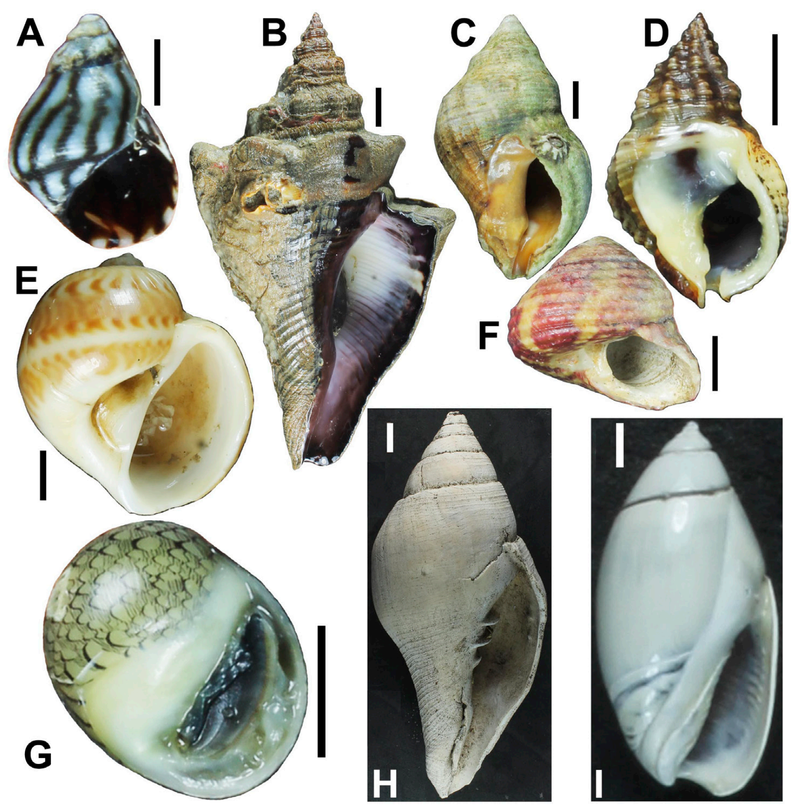

Figure 11. Ventral view of shell of the gastropods collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Echinolittorina lineolata*, (B) *Pugilina tupiniquim*, (C) *Stramonita brasiliensis*, (D) *Phrontis polygonata*, (E) *Stigmaulax cayennensis*, (F) *Tegula viridula*, (G) *Neritina virginea*, (H) *Turbinella laevigata*, (I) *Olivella minuta*. Scale bars: 5 mm.

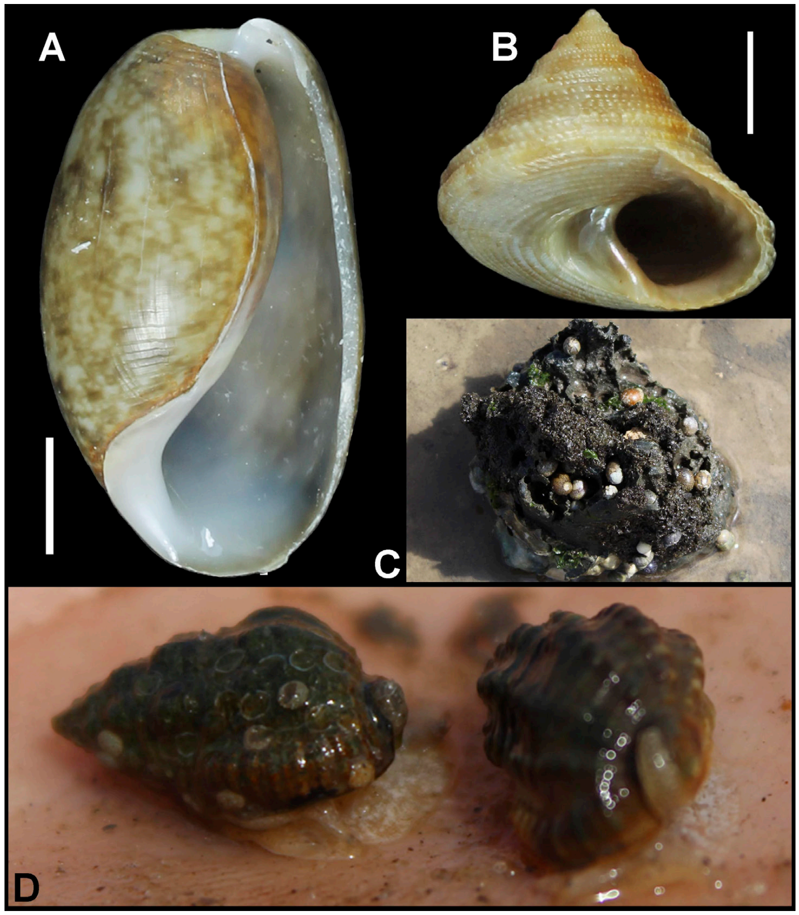

Figure 12. Ventral view of shell of the gastropods collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties: (A) *Bulla striata*, (B) *Calliostoma adspersum*, (C) *Neritina virginea* (length: about 10 mm), (D) *Phrontis polygonata* (length: about 11 mm). Scale bars: A–B. 5 mm.

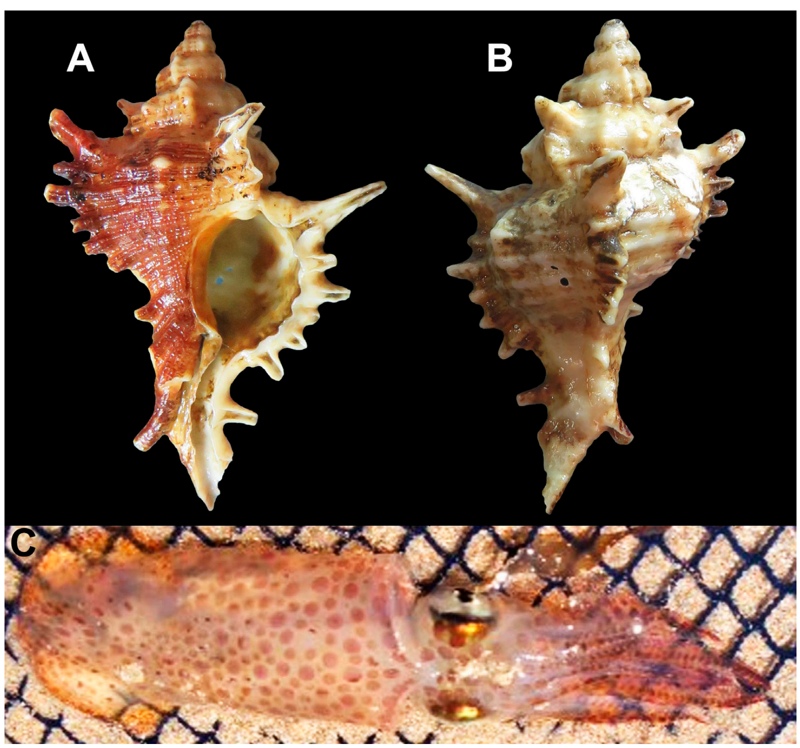

Figure 13. Ventral (A) and dorsal (B) view of shell of the gastropod *Chicoreus brevifrons* (length: 4.7 cm) and dorsal view (C) of the cephalopod *Lolliguncula brevis* (length: about 10 cm) collected from estuary of Paraíba River on surrounding Treze de Maio and Costinha de Santo Antônio properties.

each with five to seven species and representing 35% of the mollusks recorded (Table 1; Figure 2). The family Mactridae and another six families of gastropods were not attributed to the order level based on the WORMS classification (Table 1). Veneridae (Venerida) showed the highest number of species (12%), followed by Littorinidae (Littorinimorpha) (7.1%) (Figure 2). Seven families were each represented by two species (Arcidae, Cerithiidae, Lucinidae, Muricidae, Mytilidae, Ostreidae and Tellinidae) (Table 1; Figure 2). The other families recognized in this study (e.g., Donacidae, Columbellidae and Loliginidae) were each represented by only one species (Table 1; Figure 2).

Except for the single specimen from Epitoniidae, all mollusks were identified at the specific level. The epitoniid sampled in this study is probably an undescribed species and will be addressed in another paper. *Calliostoma adspersum* (Philippi, 1851) and *Chicoreus brevifrons* (Lamarck, 1822) are formally recorded for the first time for the coast of Paraíba. Other species are not duly reported in the literature from the region, but are very abundant along the coast of Brazil to be considered new records for the state. No introduced or exotic mollusks were found in the area sampled.

In terms of abundance, bivalves Mytilidae [*Brachidontes exustus* (Linnaeus, 1758)], Ostreidae [*Crassostrea brasiliana* (Lamarck, 1819)] and Veneridae [*Anomalocardia brasiliana* (Gmelin, 1791)] as well as gastropods Neritidae [*Neritina virginea* (Linnaeus, 1758)], Columbellidae [*Parvanachis obesa* (C. B. Adams, 1845)] and Cerithiidae [*Cerithium atratum* (Born, 1778)] were the most numerous, together representing 76% of the total number of individuals (Table 1; Figures 2–3).

Discussion

This survey is the first attempt to know the biodiversity of marine mollusks of the estuary of the Paraíba River. The species richness and composition of the region, including many species of economic value show the importance of this transitional aquatic environment in the northeastern Brazil. Information on the shell morphology, geographical distribution and ecological aspects of the species listed here were described by Rios (2009), Tunnell Jr. et al. (2010) and Redfern (2013).

Previous malacofaunal surveys have been conducted in coastal ecosystems of Brazil. Ourives et al. (2011) listed 94 gastropod species for Camamu Bay in the state of Bahia (northeastern Brazil). Barroso et al. (2013) found 47 species of mollusks from the Grande Island estuarine complex in the state of Ceará (northeastern Brazil). Longo et al. (2014) recognized 62 gastropod species associated with the brown algae *Sargassum* sp. in the São Sebastião Channel on the northern coast of the state of São Paulo (southeastern Brazil). Tallarico et al. (2014) provide a list of 52 bivalve species inhabiting the intertidal and subtidal zones of the São Sebastião Channel. Duarte et al. (2014) found 65 mollusk species in three shallow-water reef habitats along the coast of the state of Paraíba. Duarte et al. (2015) identified 18 species of gastropods associated with macroalgae in Cabo Branco on the coast of the same state. The results of these studies show that mollusks need to be investigated further in many coastal regions of the country and require better alpha taxonomic and ecological knowledge.

Knowledge on malacofauna in coastal ecosystems of the state of Paraíba has become increasingly more accurate in recent years due to sampling efforts and studies conducted by local researchers (Duarte et al. 2014, 2015). However, several shallow-water habitats in the region require inventories to gain a better understanding of the diversity of marine invertebrates, mainly Mollusca, which is the second most diversified phylum in the world.

The families Veneridae and Littorinidae had the largest number of known species in the present study. In contrast, the families Neritidae, Columbellidae and Cerithiidae were recognized with only a single species, but as the most abundant mollusks in the study area. Other families (e.g., Solecurtidae, Crassatellidae, Myidae, Ellobiidae, Naticidae, Trochidae, Turbinellidae and Loliginidae) were also commonly represented by only a few specimens of a single species. In the present investigation, the number of species was similar to that found by Barroso et al. (2013). In both studies, six families of gastropods were recognized (Aplysiidae, Bullidae, Cerithiidae, Neritidae and Olivellidae, Turbinellidae), with a total of six species in common, and seven families of bivalves were found (Cardiidae, Donacidae, Lucinidae, Myidae, Solecurtidae, Tellinidae and Veneridae), represented by 10 species collected from both estuarine regions. The considerable difference in the composition between the two studies may be the result of factors such as state of conservation, sampling effort and environmental complexity. The study area in the present investigation is near a port and affected by both domestic sewage and industrial waste (Sassi 1991, Marcelino et al. 2005). In contrast, Grande Island is located in an estuarine complex with the best conserved mangrove areas in northeastern Brazil (Carlos et al. 2010, Barroso et al. 2013). Although not addressing the malacofauna of estuarine regions, other taxonomic studies (e.g., Longo et al. 2014, Tallarico et al. 2014, Duarte et al. 2014, 2015) report somewhat similar compositions and abundances for the taxonomic groups found in the present investigation.

There is an interesting diversity of feeding habits among the mollusks studied, especially when considering gastropods. All bivalve species identified are filter feeders (see Rios 2009). Cerithiidae and Neritidae are among the most abundant families of herbivorous caenogastropods sampled from coastal environments of Brazil (Rios 2009, Ourives et al. 2011, Duarte et al. 2014, Longo et al. 2014). *Neritina virginea* and *Cerithium atratum* were the most abundant herbivorous gastropods collected in the present study, both of which are commonly found associated with seagrass (see Longo et al. 2014, Duarte et al. 2015). On the other hand, neogastropods of the families Columbellidae, Melongenidae, Muricidae, Nassariidae and Olivellidae are among the main carnivorous and/or scavenger gastropod groups living in estuarine regions (Rios 2009, Ourives et al. 2011). Only one species of each family was found in the material studied, although these groups are usually more numerous in other Brazilian estuarine ecosystems (see Rios 2009, Ourives et al. 2011). The columbellid *Parvanachis obesa* was the most abundant neogastropod collected from the estuary of the Paraíba River. Ourives et al. (2011) identified six columbellids from Camamu Bay (in the state of Bahia), demonstrating that the species richness of this family is even greater in estuarine regions of northeastern Brazil. Although the present study does not allow an inference about potential anthropic impacts on mollusc diversity, there is likely a decline in species richness of the aforementioned families as a possible direct consequence of pollution and environmental changes in the region, making the invertebrate assemblage increasingly susceptible to a reduction of biodiversity.

The possible presence of an undescribed species of Epitoniidae in the study area will be investigated further. Microgastropods of this family are known to be free-living predators of other invertebrates or often living on stony and soft corals, hydroids, hydrocorals, discophores, siphonophores, gorgonians, zoanthids and sea anemones, feeding on living cnidarian tissues (Robertson 1963, 1970, Bouchet & Warén 1986, Lima et al. 2012). More studies involving micromollusks of the estuary of the Paraíba River are needed, mainly considering the possibility of new species in the region.

Although widely distributed along the Brazilian coast, *Calliostoma adspersum* and *Chicoreus brevifrons* are the only species identified in this study that can be considered of uncommon occurrence in estuarine regions. The calliostomatid lives usually on muddy, sandy bottoms at depths of approximately 25 m, while the muricid lives under rocks commonly feeding on barnacles and oysters (Rios 2009). Both species are reported herein for an estuarine area with many oyster beds, which is the preferred food of muricid gastropods (D'assaro 1966, Ponder 1998, Herbert et al. 2007, Rossato et al. 2014, Lima et al. 2016a). On the other hand, all species found are commonly distributed in estuarine ecosystems of Brazil, such as the bivalves *Anomalocardia brasiliana* and *Iphigenia brasiliana*, which have great economic importance to fishing communities.

Coastal communities of invertebrates have been drastically affected by multiple anthropogenic impacts (Migotto & Marques 2006). The fragmentation and destruction of habitats constitute the most serious threat to marine biodiversity (Gomes et al. 2000, Amaral & Jablonski 2005, Migotto & Marques 2006). The construction of shipyards causes profound changes in coastal habitats, directly and indirectly affecting the invertebrate assemblage. This habitat change disrupts the physicochemical environment, leading to the loss of local biodiversity. Furthermore, a number of chemical compounds and materials used in shipyard activities may have a significant impact on marine benthic fauna. Indeed, benthic invertebrates are efficient organisms for the evaluation and monitoring of such anthropogenic disturbances in coastal ecosystems (Chiarelli & Roccheri 2014). Thus, surveys of benthic invertebrate assemblages in marine habitats, such as the estuarine region studied herein, are of fundamental importance to biomonitoring and the development of conservation strategies. Marine gastropods and bivalves have been successfully employed in environmental monitoring studies worldwide (Oehlmann & Schulte-Oehlmann 2002). In many habitats, oysters and clams are the indicators most commonly used to reflect the impact of pollution/contamination (Oehlmann & Schulte-Oehlmann 2002). Among the bivalves in the study area, *Anomalocardia brasiliana*, *Crassostrea brasiliana*, *Iphigenia brasiliana*, *Macoma constricta* and *Tagelus plebeius* are the main species with potential as bioindicators, especially of domestic, industrial and agricultural waste, due to their relatively large size, abundance, limited mobility (commonly sedentary/sessile as adults) and probably sensitivity to chemicals in the marine environment.

Thus, surveys of benthic invertebrate assemblages in marine habitats, such as the estuarine region studied herein, are of fundamental importance to biomonitoring and the development of conservation strategies.

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Amphibians of Vassununga State Park, one of the last remnants of semideciduous Atlantic Forest and Cerrado in northeastern São Paulo state, Brazil

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Abstract: Although São Paulo state has one of the best known amphibian fauna in Brazil, there are still protected areas for which the species composition remains unknown. Here, we present the first species list of anuran amphibians in Vassununga State Park. This area is one of the last remnants of semideciduous Atlantic Forest and Cerrado in the northeastern region of São Paulo state, southeastern Brazil. To survey species, we visited six sites (two ponds, two streams, and two transects) in December, January, and February of 2014-2015 and 2015-2016, totalizing 18 days of field samplings at each site. We recorded 24 anuran species belonging to four families: Bufonidae (2 species), Hylidae (11 species), Leptodactylidae (10 species), and Microhylidae (1 species). Anurans consist mainly of generalist and widely distributed species. Although none of the species recorded are threatened with extinction according to the International Red List of Endangered Species (IUCN), four species have declining population and another three species have unknown population trends.

Keywords**:** *Anurans, Biodiversity, Cerrado, Atlantic Forest, Species list, Conservation.*

Anfíbios do Parque Estadual de Vassununga, um dos últimos remanescentes de Mata Atlântica Semidecidual e Cerrado no nordeste do estado de São Paulo, Brasil

Resumo: Embora o estado de São Paulo tenha uma das faunas de anfíbios mais bem conhecidos do Brasil, existem áreas protegidas cuja composição de espécies ainda é desconhecida. Aqui, nós apresentamos a primeira lista de espécies de anfíbios anuros para o Parque Estadual de Vassununga. A área é um dos últimos remanescentes de Mata Atlântica Semidecidual e Cerrado no nordeste do estado de São Paulo, sudeste do Brasil. Para o inventário das espécies nós visitamos seis locais (duas lagoas, dois córregos, e duas trilhas) em dezembro, janeiro e fevereiro de 2014-2015 e 2015-2016, totalizando 18 dias de amostragens de campo em cada local. Nós registramos 24 espécies de anuros pertencentes a quatro famílias: Bufonidae (2 espécies), Hylidae (11 espécies), Leptodactylidae (10 espécies) e Microhylidae (1 espécie). A anurofauna consistiu principalmente de espécies generalistas e amplamente distribuídas. Embora nenhuma das espécies registradas esteja ameaçada de extinção segundo a IUCN, quatro espécies estão em declínio populacional e outras três espécies têm tendência populacional desconhecida.

Palavras-chave: *Anuros, Biodiversidade, Cerrado, Mata Atlântica, Lista de espécies, Conservação.*

Introduction

Knowing the species composition of a region is important because it is the basic dataset for ecology, systematics, biogeography, and conservation biology (e.g., Collen et al. 2008; Da Silva et al. 2012, 2014). It is recognized that Brazil has an amazing amphibian diversity (1080 species; Segalla et al. 2016), with the São Paulo state harboring 22% of known species (Rossa-Feres et al. 2011). Although, the amphibian fauna of São Paulo state is one of the best known in the country, there are still considerable herpetological survey gaps in some regions (Rossa-Feres et al. 2011). These gaps are more evident inland (i.e., northwestern and northeastern regions of the state) than in the coastal region of the state, where most surveys have been conducted (Rossa-Feres et al. 2011). For example, the increased number of anuran surveys in the last decade has contributed to records of new species in the inland areas of the state (Vasconcelos et al. 2006, Prado et al. 2008, Da Silva et al. 2009, 2010). Even protected areas such as the Vassununga State Park (created in 1970) still have not been subject to a complete faunal survey. This is worrisome because this protected area harbors one of the last remnants of Cerrado and semideciduous Atlantic Forest in the region, two biomes that are considered global hotspots for biodiversity conservation (Myers et al. 2000, Mittermeier et al. 2004). The Vassununga State Park is located in the northeastern region of the São Paulo state, which is considered one of the most deforested and fragmented regions in the state (Rodrigues et al. 2008). The vegetation cover has been removed for the establishment of agricultural crops, pastures and urban areas, which reduced the vegetation to 9% of its original extent (Kronka et al. 1993). Here, we provide the first list of anuran species of the Vassununga State Park, a region considered to be a geographical gap in the inventories of species in the Sao Paulo state, Brazil.

Material and Methods

1. Study area

The Vassununga State Park (PEV; 21°43’05”S, 47°35’49”W; 553 m above sea level) is located in the municipality of Santa Rita do Passa Quatro, northeastern São Paulo state (Figure 1). It is a Protected Area with 2071.42 ha created on October 26, 1970. The region holds different physiognomies of two Brazilian biomes, Atlantic Forest and Cerrado, which are considered global hotspots for biodiversity conservation (Myers et al. 2000, Mittermeier et al. 2004). In the PEV, the Atlantic Forest is represented by the Seasonal Semideciduous Forest *sensu stricto* (Veloso et al. 1991, Pennington et al. 2006), which is characterized by having 20-50% of tree species that lose part or all their leaves in the winter or during the dry season. The Cerrado is represented by three formations (Oliveira-Filho & Ratter 2002): (i) “campo cerrado”, which is formed by dry grassland scattered with shrubs and small trees; (ii) “cerrado sensu stricto”, which is dominated by trees and shrubs that are often 3-8 m tall but still with a fair amount of herbaceous vegetation among them; and (iii) “cerradão”, which is an almost closed woodland with crown cover of 50% to 90% that is made up of trees, often of 8–12 m or even taller, that cast considerable shade so that the ground layer is greatly reduced. The climate of this region is characterized by two well-defined seasons during the year: hot and wet (September to April), during which approximately 85% of the annual rainfall occurs, and a pronounced dry season (May to August), with average precipitation of only 15% of the annual rainfall. The average annual precipitation is 1,427 mm (± 246.83 SD, http://www.ciiagro.sp.gov.br/ciiagroonline/).

2. Research Design and Field Methods

We sampled anurans in two ponds (TP1 = 21°43’9.83”S, 47°38’44.5”W; TP2 = 21°43’52.6”S, 47°35’3.6”W), two streams (S1 = 21°38’59.3”S, 47°38’23.1”W; S2 = 21°43’14.6”S, 47°35’45.4”W), and two transects (T1 = 21°36’35.3”S, 47°37’22.2”W; T2 = 21°43’29.8”S, 47°35’42.7”W; Figure 2) in December, January, and February of 2014-2015 and 2015-2016 totalizing 18 days of field samplings in each site. The surveys were concentrated in this period because most anuran species from this region has reproductive activity during the rainy season (Vasconcelos & Rossa-Feres 2005, Provete et al. 2011). We used surveys at breeding sites (Scott Jr. & Woodward 1994) and visual encounters (Crump & Scott Jr. 1994), two methods that are considered to be complementary (Crump & Scott Jr. 1994, Zimmerman 1994), to record the presence of anuran at these sites. For the surveys at breeding sites, we recorded calling males from

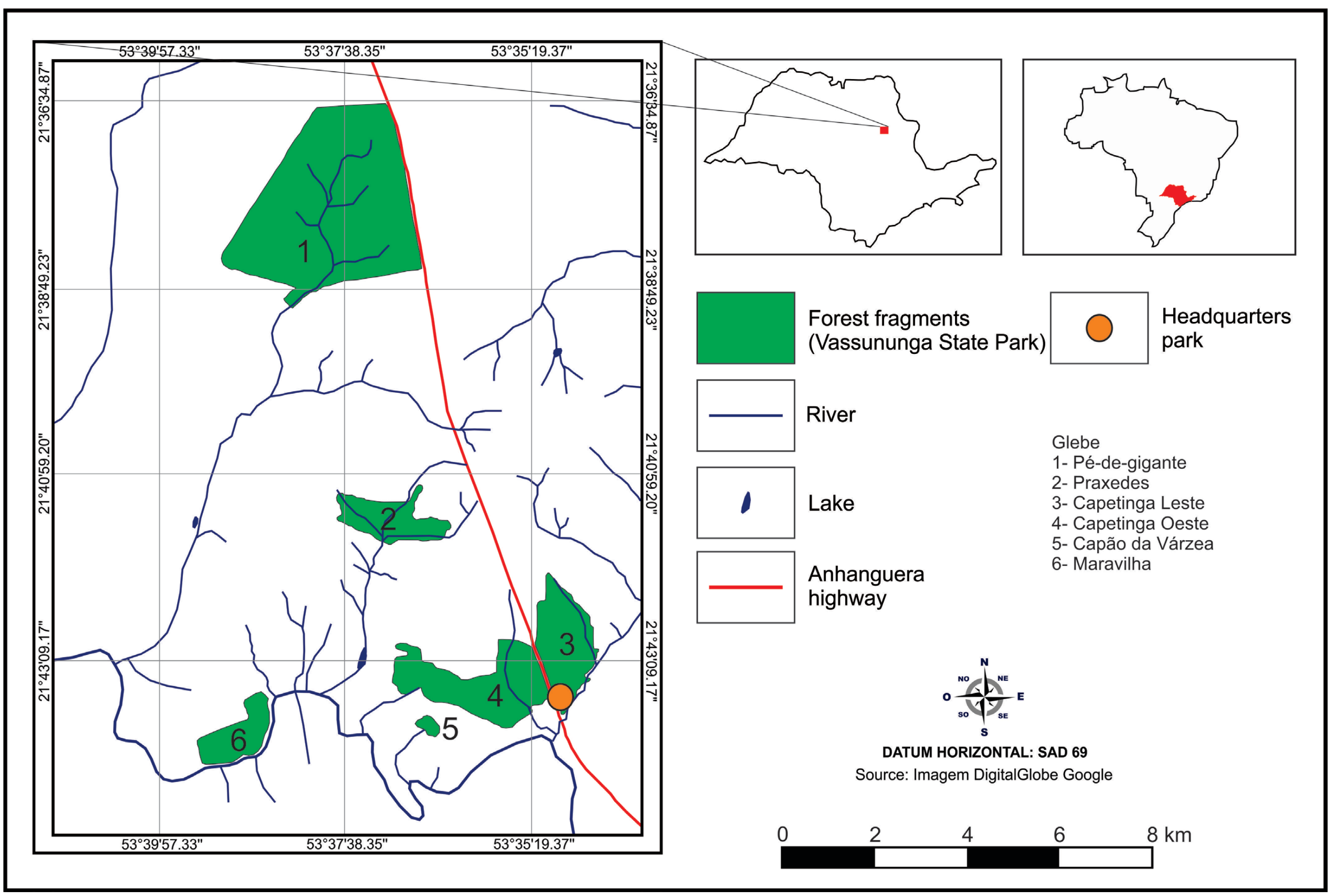

Figure 1. Maps showing the São Paulo state highlighted in Brazil (at right) and the study area highlighted in northeastern São Paulo state (at center). In detail (at left), the six isolated forest fragments (named Glebe) that comprise the total area of the Vassununga State Park.

Figure 2. Sites sampled in the Vassununga State Park: (a) temporary pond in open area (TP1; 21°43'9.83"S, 47°38'44.5"W), (b) temporary pond within gallery forest (TP2; 21°43'52.6"S, 47°35'3.6"W), (c) stream within gallery forest (S1; 21°38'59.3"S, 47°38'23.1"W), (d) stream within gallery forest (S2; 21°43'14.6"S, 47°35'45.4"W), (e) transect in the Cerradão formations (T1; 21°36'35.3"S, 47°37'22.2"W), and (f) transect in Cerradão formations (T2; 21°43'29.8"S, 47°35'42.7"W).

19 h to 24 h, while for visual encounter survey, we walked slowly inside the forest fragment (transects) or around ponds and streams for 30 min looking for individuals hidden under trunks, bromeliads, stones, branches, and leaf litter. All collected specimens were anesthetized and killed with 10% lidocaine (spray solution), fixed in 10% formaldehyde, and stored in 70% ethanol. All specimens are housed in the Coleção de Anfíbios do Departamento de Zoologia da Universidade Estadual Paulista (CFBH), municipality of Rio Claro, São Paulo state, Brazil.

3. Data Analysis

To assess the sampling efficiency of the surveys, we used a species accumulation curve (Gotelli & Colwell 2001) generated from the data of incidence of anurans during the inventory period. The total number of species recorded each day was considered as a sample, totaling 18 samples. Considering the diverse richness estimators available, we chose to use the first-order Jackknife algorithm based on its performance when compared to other estimators (Magurran 2004, Walther & Moore 2005, Hortal et al. 2006). All analyzes were performed in R v 3.2.2 (R Core Team, 2016) using the *vegan* (Oksanen et al. 2016) and *BiodiversityR* (Kindt & Coe 2015) packages with 1,000 randomizations. Taxonomic nomenclature follows Frost (2016). The conservation status of species was obtained from the International Red List of Endangered Species (IUCN 2015).

Results and Discussion

We recorded 24 species of anuran amphibians (Table 1, Figure 3) belonging to four families: Bufonidae (2 species), Hylidae (11 species), Leptodactylidae (10 species), and Microhylidae (1 species). Although the

Table 1. Anuran species recorded at the Vassununga State Park. Sites: TP1 = temporary pond 1, TP2 = temporary pond 2, S1 = stream 1, S2 = stream 2, T1 = transect 1, T2 = transect 2. Formations: CE = "Cerradão", GF = Gallery Forest, OA = open area. *Species with declining population according to IUCN (2015).

Family / Species	Sites	Formations
Family Bufonidae		
Rhinella ornata (Spix, 1824)*	T1	CE
Rhinella schneideri (Werner, 1894)	TP1, S1, T1	OA, GF, CE
Family Hylidae		
Dendropsophus elianeae (Napoli & Caramaschi, 2000)	TP1	OA
Dendropsophus jimi (Napoli & Caramaschi, 1999)	TP1	OA
Dendropsophus minutus (Peters, 1872)	TP1	OA
Dendropsophus nanus (Boulenger, 1889)	TP1, TP2	OA, GF
Hypsiboas albopunctatus (Spix, 1824)	TP1, TP2, S2	OA, GF
Hypsiboas faber (Wied-Neuwied, 1821)	TP1	OA
Hypsiboas lundii (Burmeister, 1856)*	T1, S1	CE, GF
Itapotihyla langsdorffii (Duméril & Bibron, 1841)*	TP2	GF
Scinax fuscovarius (A. Lutz, 1925)	TP1, S2	OA, GF
Scinax similis (Cochran, 1952)	TP1, S2	OA, GF
Trachycephalus typhonius (Linnaeus, 1758)	TP1, TP2	OA, GF
Family Leptodactylidae		
Leptodactylus fuscus (Schneider, 1799)	TP1, T2	OA, CE
Leptodactylus labyrinthicus (Spix, 1824)	S2	GF
Leptodactylus latrans (Steffen, 1815)	TP1	OA
Leptodactylus mystaceus (Spix, 1824)	TP2	GF
Leptodactylus mystacinus (Burmeister, 1861)	TP1	OA
Leptodactylus podicipinus (Cope, 1862)	TP1	OA
Physalaemus centralis Bokermann, 1962	TP1	OA
Physalaemus cuvieri Fitzinger, 1826	TP1	OA
Physalaemus marmoratus (Reinhardt & Lütken, 1862)	TP1	OA
Physalaemus nattereri (Steindachner, 1863)*	TP1	OA
Família Microhylidae		
Elachistocleis cesarii (Miranda-Ribeiro, 1920)	TP1	OA

species accumulation curve is close to achieving asymptote, the observed species richness was smaller than the estimated species richness, indicating that more species could be recorded if we increase the sampling effort or utilize other sampling methods (e.g., pitfall traps, Figure 4).

Since Vizotto (1967), 37 anuran species have been recorded in the northwestern region of the São Paulo state (Provete et al. 2011). In this study, we recorded approximately 65% of all species that occur in the region. Only *Hypsiboas albopunctatus* had already been recorded at the study site (Toledo et al. 2007). The species richness observed in this study was similar to those found both in areas with predominance of Cerrado formations such as Estação Ecológica Assis (23 species; Ribeiro-Júnior & Bertoluci 2009), E. E. Itirapina (28 species; Brasileiro et al. 2005), E. E. Jataí (21 species; Prado et al. 2009), and E. E. Santa Bárbara (33 species; Araujo et al. 2013), and areas with a predominance of seasonal semideciduous forest such as Floresta Estadual Edmundo Navarro (21 species; Toledo et al. 2003), E. E. Caetetus (24 species; Bertoluci et al. 2007; 34 species; Brassaloti et al. 2010), and Parque Estadual Morro do Diabo (28 species; Santos et al. 2009). Although none of the recorded species is currently threatened with extinction, three species (*Dendropsophus elianeae*, *Physalaemus centralis*, and *P. marmoratus*) have unknown population trends, and four species (*Rhinella ornata*, *Hypsiboas lundii*, *Itapotihyla langsdorffii*, and *Physalaemus nattereri*) have declining population (IUCN 2015). Furthermore, Vasconcelos & Doro (2016) showed that habitat loss has negative impacts in the geographic ranges of *R. ornata*, *H. lundii*, and *I. langsdorffii*. This fact becomes even more worrying considering that northeastern region of the São Paulo state has one of highest deforestation and fragmentation rates in the state (Rodrigues et al. 2008).

Approximately 50% of the species (*Dendropsophus elianeae*, *D. jimi*, *D. minutus*, *Hypsiboas faber*, *Leptodactylus latrans*, *L. mystacinus*, *L. podicipinus*, *Physalaemus centralis*, *P. cuvieri*, *P. marmoratus*, *P. nattereri*, and *Elachistocleis cesarii*) occurred exclusively in ponds in open areas, while 10 species (*Rhinella schneideri*, *Dendropsophus nanus*, *Hypsiboas albopunctatus*, *H. lundii*, *Itapotihyla langsdorffii*, *Scinax fuscovarius*, *S. similis*, *Trachycephalus typhonius*, *Leptodactylus labyrinthicus*, and *L. mystaceus*) occurred in streams and ponds. *Hypsiboas lundii* was the only species found vocalizing exclusively on streams inside gallery forest (Table 1). The large number of anuran species associated with water bodies in Cerrado and semideciduous forest fragments during the reproductive period has been demonstrated in other studies (Brasileiro et al. 2005, Bertoluci et al. 2007, Araujo et al. 2009, Santos et al. 2009, Araujo & Almeida-Santos 2011, 2013, Valdujo et al. 2012). According to Santos et al. (2009), anuran communities from Semideciduous Atlantic Forest and Cerrado environments are more similar to each other than they are to those of Ombrophilous Forest environments. This pattern of similarity in anuran communities can be interpreted as a result of the seasonally dry climate that most likely limits the occurrence of anuran species typical of the Ombrophilous Atlantic Forest in Semideciduous Atlantic Forest and Cerrado areas (Santos et al. 2009, Da Silva et al. 2012).

Although several authors have shown that the anuran species of the Cerrado and semideciduous forest fragments are mostly associated with open areas (e.g., Brasileiro et al. 2005, Araujo et al. 2009, Santos et al. 2009, Araujo & Almeida-Santos 2011, 2013, Valdujo et al. 2012), here we recorded 50% of species (12 species) occurring in forest areas. It should be emphasized that forests are important mesic environments, being used by adults and juveniles as refuge sites and for foraging, hibernation, and

Figure 3. Anuran species recorded in the Vassununga State Park, northeastern São Paulo state, Brazil. In brackets, for each vouchered specimen we provide the acronym of the Coleção de Anfíbios do Departamento de Zoologia da Universidade Estadual Paulista, municipality of Rio Claro (CFBH) followed by the registration number and the snout-vent length (SVL). a = *Rhinella ornata* (CFBH 40455; SVL 38.18 mm), b = *R. schneideri* (CFBH 38871; SVL 124.39 mm), c = *Dendropsophus elianeae*, d = *D. jimi* (CFBH 40456; SVL 24.03 mm), e = *D. minutus*, f = *D. nanus* (CFBH 38872; SVL 19.07 mm), g = *Hypsiboas albopunctatus* (CFBH 38856; SVL 52.74 mm), h = *H. faber*, i = *H. lundii* (CFBH 38870; SVL 64.8 mm), j = *Itapotihyla langsdorffii*, k = *Scinax fuscovarius* (CFBH 38862; SVL 39.23 mm), l = *S. similis* (CFBH 40455; SVL 38.18 mm), m = *Trachycephalus typhonius* (CFBH 38866; SVL 31.87 mm), n = *Leptodactylus fuscus* (CFBH 38869; SVL 33.98 mm), o = *L. labyrinthicus*, p = *L. latrans*, q = *L. mystaceus*, r = *L. mystacinus* (CFBH 38859; SVL 55.95 mm), s = *L. podicipinus*, t = *Physalaemus centralis* (CFBH 40457; SVL 33.68 mm), u = *P. cuvieri* (CFBH 38912; SVL 28.71 mm), v = *P. marmoratus* (CFBH 38865; SVL 37.05 mm), w = *P. nattereri* (CFBH 38867; SVL 41.48 mm), x = *Elachistocleis cesarii*. Photos: Ronildo Alves Benício.

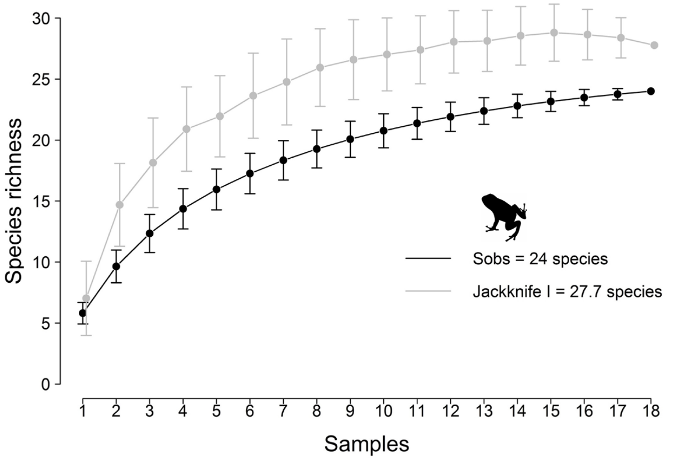

Figure 4. Species accumulation curve (black line) and Jackknife algorithm first order (gray line) representing the anuran species richness observed and estimated, respectively, based on 18 samples from December 2014 to February 2016. The dots show the mean generated by 1000 randomizations and the vertical bars indicate the standard deviation.

migration, especially during dry periods (Da Silva & Rossa-Feres 2007). This study area is one of the most deforested and fragmented in the state, and because of the lack of biological knowledge, it is classified as a priority for fauna and flora inventories (Rodrigues et al. 2008, Rossa-Feres et al. 2011). In this sense, the species list of anurans for Vassununga State Park represents an important step in increasing the knowledge about the distribution of diversity of São Paulo state.

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Author Contributions

RAB and FRS contributed in the acquisition, analysis, data interpretation and writing of the work.

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editorial

Brief history of the Brazilian Platform on Biodiversity and Ecosystem Services/BPBES

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It all started during the First Authors Meeting of the IPBES AMERICAS Regional Assessment in Bogotá (July 19 to July 23, 2015). The Brazilian group of experts assembled there saw an opportunity to think about producing a Brazilian Assessment, following the same concepts and structure established by IPBES for the AMERICAS. As a result, Carlos A. Joly (IPBES/MEP Member), Cristiana S. Seixas (Co-chair of the AMERICAS Regional Assessment), Fabio Scarano (CLA of Chapter 6 of the AMERICAS Regional Assessment), Jean P.B. Ometto (CLA of Chapter 5 of the AMERICAS Regional Assessment), Mercedes Bustamante (CLA of Chapter 4 of the AMERICAS Regional Assessment) decided to organize a meeting of Brazilian researchers already engaged on IPBES to discuss the viability of producing the First Assessment on Brazilian Biodiversity and Ecosystem Services under the premises and the Conceptual Framework of IPBES.

On November 2015, in Indaiatuba/São Paulo, the first meeting was organized with a group of 26 researchers (later named the core Group) already engaged with IPBES and with a representative of the Ministry of Environment. This meeting was supported by the BIOTA/FAPESP Program (São Paulo Research Foundation/FAPESP Research Program on Biodiversity[1]) and the Brazilian Foundation for Sustainable Development/FBDS[2] (NGO) and resulted in the establishment of a Steering Committee as well as an outline of the proposed assessment, based on the chapter and content structure of the Americas Regional Assessment.

These consolidated results were presented and discussed with the Secretary of Programs and Policies Development from the Ministry of Science, Technology and Innovation and the unfolding was the submission, by Fabio Scarano and Carlos A. Joly, of a pledge for resources to develop the Brazilian Assessment on Biodiversity and Ecosystem Services. This pledge was approved in early 2016 and consisted of: 6 24 months post-doc scholarships, 6 24 month technical scholarships, plus money for organizing meetings (transport, lodging and food), development of a communication strategy and cost of diagramming, revision and printing the assessment to be delivered in June/2018. It also included the commitment of inserting all results of the assessment in the Brazilian Information System on Biodiversity/SiBBr[3]. With the support of one post-doc (Young Fellows) each member of the Steering Committee became the CLA of one of the chapters and the work of preparing the assessment started immediately.

In parallel, Carlos Joly and Fabio Scarano had a meeting with the President of the Brazilian Society for the Progress of Science/SBPC[4] (the Brazilian equivalent of the American AAAS) to discuss ways and means of establishing a permanent structure that could be recognized and embraced by Brazilian Researchers from all over the country. Previous experiences have shown that sometimes good proposals were dead at birth because they started under the umbrella of one or few States, and the support of such a traditional and respected organization was important to the success of the assessment. SBPC is an organization established in all Brazilian States, with regional secretaries, had the experience of leading a Working Group of Experts to discuss with the Brazilian Parliament changes in the Brazilian Forest Code (a battle that was lost to the interests of the agribusiness), and covers all areas of research, including natural and social sciences, humanities and economics, and thus important to have a balanced approach to the Brazilian Assessment on BES.

During 2016, the core Group wrote the white paper: "*Contribution to an intersectoral dialogue: building a Brazilian Assessment on Biodiversity and Ecosystem Services*" to be used as the basis for discussions with different sectors of society. With this first product, meetings were organized with *Focal Groups*. These groups represents different stakeholder: representatives of Non-Governmental Organizations, of different federal government organizations (Ministries of Environment; Science and Technology; Agriculture; Brazilian Foundation for Indigenous People), with ILK holders (indigenous students from a MSc Program focused on indigenous knowledge and rights), with representatives of the Private Sector, and others are still being organized. A common demand of all groups is the continuity of post-assessments activities, that is, that the assessment does not end in itself.

Once established as a Working Group of SBPC, and having the support of both Ministries, Environment and Science and Technology, but not directly subordinated to governmental bureaucracy, the Core Group started to invite researchers from all regions of Brazil and from different areas of knowledge, to work on the Brazilian Assessment. With the approval of the financial support by the Brazilian National Research Council (CNPq in Portuguese) until June 2019, the group decided to move on step forward and create the Brazilian Platform on Biodiversity and Ecosystem Services (BPBES).

Launched on February 2017 in São Paulo city, the **Brazilian Platform on Biodiversity and Ecosystem Services/BPBES**[5] has the mission of producing regular assessments of the best available knowledge, by science and other knowledge systems, on biodiversity and ecosystem services issues, in a permanent dialogue with different sectors within society, and to be used to improve the interface between science and policy. Under the overall coordination of Carlos A. Joly and Fabio R. Scarano, BPBES now has six full-time postdoctoral fellows, six part-time technical fellows and 35 collaborator authors of the Assessment, in addition to the core Group. There are approximately 70 experts working in the five chapters of the Brazilian Assessment, since in the first authors meeting it was decided to merge chapters 3 and 4 (Chapter 3: Status, trends and future dynamics of biodiversity and ecosystems; Chapter 4: Direct and indirect drivers of change in biodiversity and nature's contributions to people).

[1] www.biota.org.br
[2] www.fbds.org.br
[3] www.sibbr.gov.br
[4] http://portal.sbpcnet.org.br
[5] www.bpbes.net.br/en

At the same time, specific follow ups are being discussed in partnership with other Brazilian initiatives. The first of this kind, and following the same premises of IPBES, will be the topic Pollination, Pollinators and Food Production in Brazil, developed in partnership with the Brazilian Plant-Pollinator Interaction Network[6] (REBIPP in Portuguese). This report will be delivered on July 2018 along with the first Brazilian Assessment on Biodiversity and Ecosystem Services. Two other similar initiatives are already under discussion: one for Inland Water (in partnership with the Brazilian Limnology Association[7]) and another specific for the private sector (through the Brazilian Business Council for Sustainable Development – CEBDS[8]).

Maintaining current rates of greenhouse gas emissions, projected effects of climate change on biodiversity and ecosystem services in Brazil by 2100 include loss of species in the order of 25%, savannization of the Amazon, expansion of forests on the Brazilian Pampas, and impoverishment of the Caatinga, Cerrado and Pantanal. In addition, ocean acidification, coral whitening and sea level rise pose a risk to coastal and marine environments. Brazil has the opportunity to consolidate itself as the main model for sustainable development through the integrated vision of biodiversity and ecosystem services with socioeconomic and human development and the consequent incorporation into public policies in the country. BPBES wants to be a reference in the insertion of such themes as a strategic element of public policy, contributing to an innovative and sustainable model for socioeconomic development in Brazil.

[6] www.rebipp.org.br
[7] www.ablimno.org.br
[8] http://cebds.org/en/

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Drosophilidae (Insecta, Diptera) in the state of Pará (Brazil)

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***Abstract*:** This list contains information on the Drosophilidae that occur in the Brazilian state of Pará, Amazon biome, and an analysis of the current knowledge of Drosophilidae based on museum material and literature records. This list includes a detailed account of the material deposited in the entomological collections of the Museu Paraense Emílio Goeldi and Museu de Zoologia da Universidade de São Paulo, up to 2015. In total, 122 species of Drosophilidae were registered, including 27 new records for the state of Pará and 22 are new records for the Amazon; for instance, the genera *Diathoneura* and *Rhinoleucophenga*, and three new records for Brazil, (*Drosophila fasciola*, *Diathoneura flavolineata* and *Drosophila neochracea*). The state of Pará is the third state in Brazil in terms of numbers of species of Drosophilidae, with 17% locally native species. Despite the high species richness, there is still a lot to be known about the states's Drosophilidae fauna. This study highlights the importance of scientific collections, particularly as an aid to study regional biodiversity.

Keywords*:* *species composition, Amazon, Diathoneura, Rhinoleucophenga.*

Drosophilidae (Insecta, Diptera) no estado do Pará (Brasil)

Resumo: As informações reunidas nesta lista referem-se à ocorrência de Drosophilidae no estado do Pará, bioma Amazônia com uma análise do estado de conhecimento do grupo. Este documento contém uma revisão detalhada do material depositado na Coleção Entomológica do Museu Paraense Emílio Goeldi e Museu de Zoologia da Universidade de São Paulo até 2015, bem como a análise de toda a literatura relevante. Um total de 122 espécies de Drosophilidae foi registrado, com 27 novos registros para o estado do Pará e 22 são novas ocorrências para a Amazônia. Entre estes o primeiro registro para os gêneros *Diathoneura* e *Rhinoleucophenga*, além de três registros novos para o Brasil (*Drosophila fasciola*, *Diathoneura flavolineata* e *Drosophila neochracea*). O estado do Pará é o terceiro maior estado brasileiro em termos de número de espécies registradas, com 17% das espécies com ocorrência exclusiva no Estado. Ainda assim há grandes lacunas para o conhecimento da fauna dos Drosophilidae do estado. Este estudo destaca a importância das coleções científicas, acima de tudo, como um elemento crucial para compreensão da biodiversidade regional.

Palavras-chave: *Composição de espécies, Amazônia, Diathoneura, Rhinoleucophenga.*

Introduction

There are more than 4,200 Drosophilidae species in the world (Bächli 2015). This number will most likely increase as new species are continuously being described, particularly in the Neotropical region (Silva & Martins 2004, Vilela & Bächli 2005, Ratcov & Vilela 2007, Culik & Ventura 2009, Schmitz et al. 2009, Figuero & Rafael 2011, Figuero et al. 2012, Gottschalk et al. 2012, Acurio et al. 2013, Poppe et al. 2014, Junges & Gottschalk 2014, Guillín & Rafael 2015, Junges et al. 2016, Vidal & Vilela 2015). In Brazil, the Drosophilidae family is represented by 16 genera and 305 species (Tidon et al. 2015). Taxonomic inventories are needed to we fill the knowledge gaps on biodiversity (Hortal et al. 2015). Althought the first collections for the Amazon dating from 1920-1959 period (Duda 1927; Hendel 1936; Dobzhansky & Pavan 1943; Pavan 1950; Pavan 1959), the richness of Drosophilidae fauna of the brazilian Amazon is still little known, states such as Acre, Amapá, Roraima, Rondônia e Tocantins have less than 12 recorded species (Gottschalk et al. 2008). Among the Amazonian states, only Amazonas and Pará have more consistent inventory Drosophilid (Bächli 2015). As the result, the scenario of the distribution of species for this region is still very incomplete.

The Drosophilidae fauna of the state of Pará has been sporadically studied since 1920, by pioneer systematists (Duda 1927; Hendel 1936; Burla et al. 1949), evolutionary biologists (Pavan 1950) and more recently by ecologists (De Toni et al. 2005; Martins & Oliveira 2007;

Martins & Santos 2007; Silva & Martins 2009; Praxedes & Martins 2014 and Robe et al. 2014). Currently, in Pará there are 75 Drosophilidae species from nine genera that are from two subfamilies Steganinae and Drosophilinae (Bächli 2015).

Throughout the years a great volume of material has accumulated in scientific collections. Material from the state of Pará is, for the most part, deposited in the collections Museu Paraense Emílio Goeldi (MPEG) and Museu de Zoologia da Universidade de São Paulo (MZUSP). The Drosophilidae species in the MPEG deposit were mostly collected in the Amazon, from expeditions dating back to the 1970s. At MZUSP it is known to hold Brazil's largest Diptera collection, with specimens from large expeditions during the 1940s and 1950s, particularly in the Amazon, coordinated by Dr. Crodowaldo Pavan (Magalhães 2010). We analyzed material from these two collections and using literature data to complement our survey. Our goal was to list the species of Drosophilidae that are found in the state of Pará, with the aim to fill a knowledge gap of Drosophilae found in the Amazon.

Material and Methods

The state of Pará, Northern Brazil, has an area of 1,248,000 km^2, is completely immersed in the Amazon Biome (IBGE 2010). The climate is Equatorial (Am) and Tropical (As), according to Köppen classification, with a mean temperature of 25 °C and minimum precipitation of 1,300 mm per year and maximum of 2,400 mm per year (Moraes et al. 2005).

Occurrence data for Drosophilidae was compiled based on literature research, and the examination of material deposited in the entomological collection of the MPEG and MZUSP up to 2015. Data was plotted on a map of Brazil's municipalities. The identification of all Drosophilidae at MPEG was rechecked. For this, we dissected the genitalia of, and examined, up to five males of each species (following the procedure described by Bächli et al. 2004). The identity of species represented only by females was checked using external morphological characters whenever possible. Drosophilidae specimens that were in 70% ethanol and from the wet collection at MPEG, were mounted on entomological pins after being dissected. The specimen terminalia were stored in microtubes containing glycerin and pinned together with the specimen. Specimens deposited in the MZUSP had already been dissected and their terminalia were stored in microtubes containing glycerin, thus facilitating confirmation. The individual number of each species deposited in the MPEG collection was recorded. The total number of individual specimens of each species found in MZUSP was not accounted.

We searched Drosophilidae literature using the website compiled by Gerhard Bächli (Bächli 2015). We also verified and analyzed each literature reference for the state of Pará.

Results and Discussion

In total, 122 species of Drosophilidae in 11 genera were found, distributed in 24 of the 144 municipalities of the state of Pará (Figure 1). Nine genera belong to the subfamily Drosophilidae (*Chymomyza* Czerny,

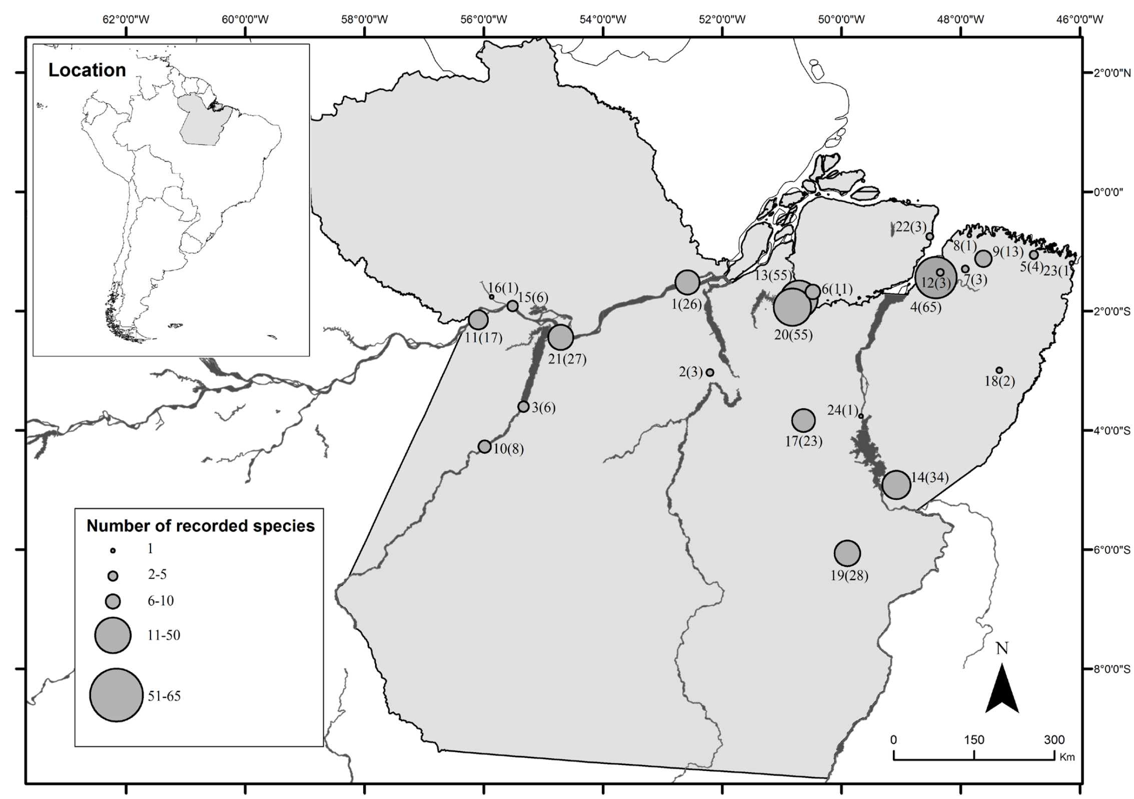

Figure 1. Distribution of Drosophilidae in Pará, indicating the known species richness of each municipality. Map of South America at the upper left with the state of Pará highlighted in gray. The numbers on the map correspond to the following municipalities. In parenthesis correspond to the number of species for each locality. 1. Almeirim (1°31'22" S, 52°34'55" W); 2. Altamira (3°2'10" S, 52°12'21" W); 3. Aveiro (3°36'21" S, 55°19'55" W); 4. Belém (1°26'27" S, 48°24'50" W); 5. Bragança (1°3'46" S, 46°46'22" W); 6. Breves (1°40'55" S, 50°28'48" W); 7. Castanhal (1°17'49"S, 47°55'19" W); 8. Curuça (0°44'2" S, 47°51'18" W); 9. Igarapé-Açu (1°7'37" S, 47°37'4" W); 10. Itaituba (4°16'33" S, 55°59'2" W); 11. Juruti (2°9'7" S, 56°5'31" W); 12. Marituba (1°21'18" S, 48°20'31" W); 13. Melgaço (1°48'14" S, 50°42'43" W); 14. Nova Ipixuna (4°55'15" S, 49°4'37" W); 15. Óbidos (1°55'4" S, 55°31'4" W); 16. Oriximiná (1°45'57" S, 55°51'57" W); 17. Pacajá (3°50'16" S, 50°38'16" W); 18. Paragominas (2°59'42" S, 47°21'10" W); 19. Parauapebas (6°4'4" S, 49°54'7" W); 20- Portel (1°56'9" S, 50°49'15" W); 21. Santarém (2°26'34" S, 54°42'28" W); 22. Salvaterra (0°45'10" S, 48°31'1" W); 23. Viseu (1°11'49" S, 46°8'24" W); 24. Tucuruí (3°46'4" S, 49°40' 22" W).

1903, *Diathoneura* Duda, 1924, *Drosophila* Fallén, 1823, *Hirtodrosophila* Duda, 1923, *Mycodrosophila* Oldenberg, 1914, *Neotanygastrella* Duda, 1925, *Scaptodrosophila* Duda, 1923, *Zaprionus* Coquillett, 1901 and *Zygothrica* Wiedemann, 1830) and two belong to Steganinae (*Leucophenga* Mik, 1886 and *Rhinoleucophenga* Hendel, 1917). The actualized list of all species can be found in Table 1. References in the column 'record' of Table 1 represent the oldest record for the species. The table also shows the municipalities of Pará where each species was recorded from, and the total number of specimens deposited in the collection of the Museu Paraense Emílio Goeldi. The map (Figure 1) shows the distribution of Drosophilidae in Pará, indicating the known species richness of each municipality. The municipality of Belém had the greatest number of species, 65, followed by Melgaço (MEL) and Portel (POR), with 55 species, and Santarém (SAN), with 27. More than half of the species (66) occurred in less than three municipalities. *Drosophila malerkotliana,* an invasive species present in the Amazon since the 1980s, was the most widespread species, occurring in 15 municipalities. The second most abundant species was the native species *Drosophila nebulosa*, *Drosophila sturtevanti* and *Drosophila willistoni,* which were found in 13 of the 24 municipalities.

Table 1. Drosophilidae recorded in the state of Pará, Brazil. Municipalities: ALM = Almeirim, ALT = Altamira, AVE = Aveiro, BEL = Belém, BRA = Bragança, BRE = Breves, CAS = Castanhal, CUR = Curuça, IGA = Igarapé-Açu, ITA = Itaituba, JUR = Juruti, MAR = Marituba, MEL= Melgaço, NIP = Nova Ipixuna, OBI = Óbidos, ORI = Oriximiná, PAC = Pacajá, PARG = Paragominas, PARP = Parauapebas, POR = Portel, SAN= Santarém, SAL= Salvaterra, TUC= Tucuruí and VIS= Viseu. Records: MPEG= Museu Paraense Emílio Goeldi and MZUSP= Museu de Zoologia da Universidade de São Paulo collections. # in col: Total Drosophilidae specimens deposited in the MPEG.

Species	Municipalities	Records	# in col
Subfamily Drosophilinae			
Genus *Chymomyza*			
***aldrichii* group**			
[3]*Chymomyza bicoloripes* (Malloch, 1926)	ALM and SAN	Hendel (1936)	
Chymomyza diatropa Grimaldi, 1986	MEL and POR	MPEG and Schmitz et al. (2013)	23
Genus *Diathoneura*			
[1,2,4]*Diathoneura flavolineata* Duda, 1927	BEL	MZUSP	
Genus *Drosophila*			
Subgenus *Drosophila*			
***annulimana* group**			
[1]*Drosophila annulimana* Duda, 1927	BEL, MEL and POR	MPEG	5
Drosophila araicas Pavan & Nacrur, 1950	ALM, BEL,MEL and POR	MPEG and Val (1982)	7
[1,4]*Drosophila arapuan* Da Cunha & Pavan, in Pavan & Da Cunha, 1947	PARP	MPEG	1
Drosophila ararama Pavan & Da Cunha, 1947	BEL and NIP	MPEG, Pavan & Cunha (1947)	14
[3]*Drosophila caxiuana* Gottshalk et al., 2012	MEL and POR	MPEG and Gottschalk et al. (2012)	5
***bromeliae* group**			
[3]*Drosophila speciosa* Silva & Martins, 2004	BEL,CUR, MEL and POR	MPEG and Silva & Martins (2004)	429
***calloptera* group**			
Drosophila atrata Burla & Pavan, 1953	AVE and PARP	MPEG and Burla & Pavan (1953)	2
Drosophila calloptera Schiner, 1868	BEL, MEL, POR and SAN	MPEG and Burla & Pavan (1953)	5
[1,4]*Drosophila quadrum* (Wiedemann, 1830)	OBI	MZUSP	
***canalinea* group**			
[3]*Drosophila annularis* Sturtevant, 1916	SAN	Hendel (1936)	
[1,4]*Drosophila annulosa* Duda, 1929	MEL and POR	MPEG	2
Drosophila canalinea Patterson & Mainland, 1944	BEL, MEL, PARP and POR	MPEG and Patterson & Stone (1952)	1
Drosophila davidgrimaldii Vilela & Bächli, 1990	MEL, SAN and POR	MPEG and Vilela & Bächli (1990)	1
[3]*Drosophila hendeli* Vilela & Bächli, 1990	SAN	Vilela & Bächli (1990)	
***caponei* group**			
[1,4]*Drosophila caponei* Pavan & Cunha, 1947	BEL	MPEG	9
[1,2,4]*Drosophila neochracea* Wheeler, 1959	BEL and TUC	MPEG	5
***cardini* group**			
***cardini* subgroup**			
Drosophila cardini Sturtevant, 1916	BEL, NIP, PAC and PARP	MPEG and Heed & Russell (1971)	17
Drosophila cardinoides Dobzhansky & Pavan, 1943	AVE, BEL, IGA, MAR, NIP and PARP	MPEG and Streisinger (1946)	9
Drosophila neocardini Streisinger, 1946	MEL, NIP, PAC and POR	MPEG and Silva & Martins (2009)	2
Drosophila neomorpha Heed & Wheeler, 1957	ALM, BEL, JUR, MEL, NIP, PAC, PARP and POR	MPEG and De Toni et al. (2005)	11
Drosophila parthenogenetica Stalker, 1953	MEL, NIP, PAC and POR	MPEG and De Toni et al. (2005)	6
Drosophila polymorpha Dobzhansky & Pavan, 1943	BEL and BRE	Dobzhansky & Da Cunha (1955)	
***coffeata* group**			
Drosophila coffeata Williston, 1896	ALM, BEL, MEL, PARP, POR and SAN	MPEG and Hendel (1936)	91
Drosophila fuscolineata Duda, 1925	BEL	MPEG and Dobzhansky & Pavan (1950)	2

[1]New records for the state of Pará; [2]New records for Brazil; [3]Species that are recorded in Brazil only in the state of Pará; [4]New records for Amazon

Table 1. Continued...

Species	Municipalities	Records	# in col
***dreyfusi* group**			
Drosophila camargoi Dobzhansky & Pavan in Pavan, 1950	BEL BRE, MEL, NIP and POR	MPEG and Pavan (1959)	71
[3]*Drosophila decemseriata* Hendel, 1936	SAN	Hendel (1936)	
***flavopilosa* group**			
[3]*Drosophila melina* Wheeler, 1962	BEL	MPEG and Santos & Martins 2000	256
***guarani* group**			
***guaramunu* subgroup**			
[1,4]*Drosophila griseolineata* Duda, 1927	PARP	MPEG	1
***guarani* subgroup**			
Drosophila ornatifrons Duda, 1927	BEL	MPEG and Pavan (1959)	1
***pallidipennis* subgroup**			
Drosophila pallidipennis Dobzhansky & Pavan, 1943	BEL	Dobzhansky & Pavan (1950)	
***peruensis* subgroup**			
Drosophila peruviana Duda, 1927	AVE and BEL	MZSP and Pavan (1959)	
***repleta* group**			
***fasciola* subgroup**			
Drosophila ellisoni Vilela, 1983	ALM, BEL, IGA, JUR, NIP and PAC	MPEG and Vilela (1983)	11
[1,2,3,4]*Drosophila fasciola* Williston, 1896	NIP, PAC and PARP	MPEG	7
Drosophila fascioloides Dobzansky & Pavan, 1943	BEL	Wasserman (1962)	
[1,4]*Drosophila ivai* Vilela, 1983	ALM, NIP, PAC, PARP and POR	MPEG	19
[1,4]*Drosophila mapiriensis* Vilela & Bächli, 1990	BEL, MEL, NIP and POR	MPEG	6
Drosophila moju Pavan, 1950	BEL, JUR, NIP and PARP	MEPG and Vilela (1983)	11
[3]*Drosophila mojuoides* Wasserman, 1962	BEL	MPEG and Vilela (1983)	1
[1,4]*Drosophila papei* Bächli & Vilela, 2002	NIP and PARP	MPEG	12
[1,4]*Drosophila querubimae* Vilela, 1983	MEL, NIP, PAC and POR	MPEG	3
***hydei* subgroup**			
Drosophila hydei Sturtevant, 1921	BEL	MPEG and Martins & Oliveira (2007)	6
***mercatorum* subgroup**			
[1]*Drosophila mercatorum* Patterson & Wheeler, 1942	BEL	MPEG	1
[1]*Drosophila paranaensis* Barros, 1950	NIP and PARP	MPEG	7
***mulleri* group**			
[1]*Drosophila aldrichi* Patterson, in Patterson & Crow, 1940	ALT, MEL and POR	MPEG	10
***repleta* subgroup**			
Drosophila eleonorae Tosi *et al*., 1990	ALT	MPEG and Tosi et al. (1990)	41
Drosophila fulvimacula Patterson & Mainland, 1944	ALM, BEL, JUR, MEL, NIP, PAC, PARP, POR	MPEG and Dobzhansky & Da Cunha (1955)	52
[1,4]*Drosophila limensis* Pavan & Patterson in Pavan & Da Cunha, 1947	MEL and POR	MPEG	2
[1]*Drosophila repleta* Wollaston, 1858	MEL and POR	MPEG	11
***tripunctata* group**			
I subgroup			
Drosophila mediocris Frota-Pessoa, 1954	MEL and POR	MPEG and Praxedes & Martins (2014)	1
Drosophila neoguaramunu Frydenberg, 1956	BEL	MPEG and Martins & Santos (2007)	1
[1,4]*Drosophila setula* Heed & Wheeler, 1957	PAC	MPEG	3
II subgroup			
[1,4]*Drosophila cuaso* Bächli, Vilela & Ratcov, 2000	ALM, BEL, MEL, NIP, PARP and POR	MPEG	9
III subgroup			
Drosophila addisoni Pavan, 1950	BEL	Pavan (1959)	
Drosophila frotapessoai Vilela & Bächli, 1990	BEL, MEL and POR	MPEG and Martins & Oliveira (2007)	1
Drosophila medioimpressa Frota-Pessoa, 1954	MEL and POR	MPEG and Praxedes & Martins (2014)	1
Drosophila mediopicta Frota-Pessoa, 1954	MEL and POR	MPEG and Praxedes & Martins (2014)	1
Drosophila mediostriata Duda, 1925	BEL, MEL and POR	Martins & Oliveira (2007)	
Drosophila mesostigma Frota-Pessoa, 1954	BEL	Val (1982)	
[1,4]*Drosophila paramediostriata* Townsend & Wheeler, 1955	BEL and PARP	MPEG	4
[1,4]*Drosophila trapeza* Heed & Wheeler, 1957	PARP	MPEG	1
IV subgroup			
[1,4]*Drosophila albicans* Frota-Pessoa, 1954	PARP	MPEG	4

[1]New records for the state of Pará; [2]New records for Brazil; [3]Species that are recorded in Brazil only in the state of Pará; [4]New records for Amazon

Table 1. Continued...

Species	Municipalities	Records	# in col
Without subgroup			
[3]*Drosophila argenteifrons* Wheeler, 1954	CAS	Wheeler (1954)	
Subgenus *Phloridosa*			
[1,4]*Drosophila lutzii* Sturtevanti, 1916	BEL	MPEG	6
Subgenus *Sophophora*			
***melanogaster* group**			
***ananassae* subgroup**			
Drosophila ananassae Doleschall, 1858	BEL, BRA, IGA, MEL, NIP, PAC, PRAP and POR	MPEG and Praxedes & Martins (2014)	107
Drosophila malerkotliana Parshad & Paika, 1964	ALM, BEL, BRA, BRE, IGA, JUR, MEL, NIP, PAC, PARG, PARP, POR, SAN, SAL and VIS	MPEG and Martins (2001)	533
***melanogaster* subgroup**			
Drosophila melanogaster Meigen, 1830	BEL, MEL and POR	MPEG and David et al. (2006)	15
Drosophila simulans Sturtevant, 1919	ALM, BEL, IGA, JUR, MEL, NIP and POR	MPEG and Dobzhansky & Pavan (1950)	44
***montium* subgroup**			
Drosophila kikkawai Burla, 1954	BEL, IGA, MEL, NIP, PAC, PARP and POR	MPEG and Praxedes & Martins (2014)	88
***saltans* group**			
***cordata* subgroup**			
Drosophila neocordata Magalhães, 1956	ALM, BEL, MEL, NIP and POR	MPEG and Praxedes & Martins (2014)	9
***elliptica* subgrupo**			
Drososphila neoelliptica Pavan & Magalhães in Pavan, 1950		MPEG and Praxedes & Martins (2014)	1
***parasaltans* subgroup**			
Drosophila parasaltans Magalhães, 1956	ALM, JUT, MEL and POR	MPEG and Praxedes & Martins (2014)	2
Drosophila subsaltans Magalhães, 1956	ALM, BEL, JUR, MEL, NIP, PAC and POR	MPEG and Magalhães (1956)	2
***saltans* subgroup**			
Drosophila austrosaltans Spassky, 1957	BEL, JUR, MEL and POR	MPEG and Praxedes & Martins (2014)	2
Drosophila prosaltans Duda, 1927	ALM, ALT, AVE, BEL, BRA, BRE, JUR, MEL, NIP, PAC, PARP and POR	MPEG and Dobzhansky & Streisinger (1944)	3
Drosophila pseudosaltans Magalhães, 1956	MEL and POR	MPEG and Bicudo (1973)	16
Drosophila saltans Sturtevant, 1916	ALM, MEL and POR	MPEG and De Toni et al. (2005)	1
***sturtevanti* subgroup**			
Drosophila dacunhai Mourão & Bicudo, 1967	MEL and POR	MPEG and Martins et at. (2008)	5
[3]*Drosophila milleri* Magalhães, 1962	MEL and POR	MPEG and Praxedes & Martins (2014)	5
Drosophila magalhaesi Mourão & Bicudo, 1967	ALM, BEL, JUR, MEL and POR	MPEG and Martins et at. (2008)	18
Drosophila sturtevanti Duda, 1927	ALM, AVE, BEL, BRE, IGA, ITA, JUR, MEL, NIP, PAC, PARP, POR and SAN	MEPG and Dobzhansky (1944)	132
***willistoni* group**			
***bocainensis* subgroup**			
Drosophila capricorni Dobzhansky & Pavan, 1943	BEL, MEL and POR	MPEG and Dobzhansky & Pavan (1950)	1
Drosophila fumipennis Duda, 1925	BEL, BRE, JUR, MEL and POR	MPEG and Pavan (1959)	35
Drosophila nebulosa Sturtevant, 1916	ALM, AVE, BEL, BRE, IGA, JUR, MEL, NIP, PAC, PARG, PARP, POR and SAL	MEPG and Dobzhansky & Pavan (1950)	63
***willistoni* subgroup**			
Drosophila equinoxialis Dobzhansky, 1946	ALM, BEL, BRE, IGA, MEL, NIP, PAC, PARP, POR and SAN	MPEG and Burla et al. (1949)	23
Drosophila paulistorum Dobzhansky & Pavan in Burla *et al*., 1949	ALM, BEL, BRE, IGA, JUR, MEL, NIP, PAC, PRAP, POR and SAN	MPEG and Townsend (1954)	105
Drosophila tropicalis Burla *et al*., 1949	BEL, MEL, POR, SAN and SAL	MPEG and Burla et al. (1949)	26
Drosophila willistoni Sturtevant, 1916	ALM, BEL, BRA, BRE, IGA, ITA, JUR, MEL, NIP, PAC, PARP, POR and SAN	MPEG and Dobzhansky & Mayr (1944)	104
Ungrouped species			
Drosophila impudica Duda, 1927	BEL, JUR, NIP, PAC and PARP	MPEG, MZSP and Pavan (1959)	8
Drosophila tuchaua Pavan, 1950	BEL, MEL and POR	MPEG and Pavan (1950)	55
Subgenus *Siphlodora*			
[1,4]*Drosophila flexa* Loew, 1866	NIP	MPEG	2

[1]New records for the state of Pará; [2]New records for Brazil; [3]Species that are recorded in Brazil only in the state of Pará; [4]New records for Amazon

Table 1. Continued...

Species	Municipalities	Records	# in col
Hirtodrosophila			
Ungrouped species			
[3]*Hirtodrosophila pictiventris* Duda, 1925	Localities not mentioned	Vilela & Bächli (2004)	
Hirtodrosophila subflavohalterata Burla, 1956	MEL and POR	Robe et al. 2014	
Genus *Mycodrosophila*			
[3]*Mycodrosophila brunnescens* Wheeler & Takada, 1963	BEL	Wheeler &Takada (1963)	
[3]*Mycodrosophila elegans* Wheeler & Takada, 1963	CAS	Wheeler &Takada (1963)	
Mycodrosphila projetans Wheeler & Takada, 1963	MEL and POR	Robe et al.(2014)	
Genus *Neotanygastrella*			
[3]*Neotanygastrella chymomyzoides* Duda, 1927	BEL	Hendel (1936)	
Neotanygastrella tricoloripes Duda, 1925	SAN	Hendel (1936)	
Genus *Paraliodrosophila*			
Paraliodrosophila antennata Wheeler, 1957		Wheeler (1954)	
***latifasciaeformis* group**			
Scaptodrosophila latifasciaeformis (Duda, 1940)	BEL, BRE, IGA, JUR, MEL, NIP, PAC, PARP and POR	MPEG and Pavan (1959)	50
Genus *Zaprionus*			
***vittiger* group**			
Zaprionus indianus Gupta, 1970	ALM, BEL, MEL, NIP, PAC, PARP, POR and SAN	MEPG and David et al. (2006)	8
Genus *Zygothrica*			
***dispar* group**			
***aldrichii* subgroup**			
[3]*Zygothrica aldrichi* Sturtevant, 1920	BEL	Hendel (1936)	
[3]*Zygothrica mediovitta* Grimaldi, 1987	SAN	Grimaldi (1987)	
Zygothrica microeristes Grimaldi, 1987	ALM, BEL, ITA, OBI and ORI	MZSP and Grimaldi (1987)	
Zygothrica paraldrichi Burla, 1956	ALM, BEL, ITA, MAR, OBI and SAN	MZSP and Grimaldi (1987)	
Zygothrica pilipes Hendel, 1936	ITA, OBI and SAN	MZSP and Hendel (1936)	
[3]*Zygothrica radialis* Grimaldi, 1987	ALM, BEL and ITA	MZSP and Grimaldi (1987)	
[3]*Zygothrica somatia* Grimaldi, 1987	ALM, OBI and SAN	MZSP and Grimaldi (1987)	
Zygothrica zygia Grimaldi, 1987	OBI and SAN	Grimaldi (1987)	
***caudata* subgroup**			
Zygothrica caudata (Hendel, 1913)	SAN	Hendel (1936)	
***dispar* subgroup**			
Zygothrica dispar (Wiedmann, 1830)	SAN	MZUSP and Hendel (1936)	
Zygothrica prodispar Duda, 1925	BEL, ITA, MAR and SAN	MPEG and Grimaldi (1987)	3
***atriangula* group**			
Zygothrica atriangula Duda, 1927	SAN	Burla (1956)	
atriangulata group			
Zygothrica virgatinigra Burla, 1956	MEL and POR	Robe et al. (2014)	
***bilineata* group**			
Zygothrica bilineata (Williston, 1896)	ITA	Grimaldi (1990)	
***orbitalis* group**			
Zygothrica orbitalis (Sturtevant, 1916)	SAN	Hendel (1936)	
***vittinubila* group**			
[1,4]*Zygothrica vittinubila* Burla, 1956	NIP	MPEG and MZUSP	1
Ungrouped species			
[3]*Zygothrica laevifrons* Duda, 1927	SAN	Hendel (1936)	
Subfamily Steganinae			
Genus *Leucophenga*			
[3]*Leucophenga argenteofasciata* Kahl, 1917	SAN	Duda (1927)	
Genus *Rhinoleucophenga*			
[1,4]*Rhinoleucophenga punctulata* Duda, 1929	CAS	MPEG	5

[1]New records for the state of Pará; [2]New records for Brazil; [3]Species that are recorded in Brazil only in the state of Pará; [4]New records for Amazon

The scientific collections contributed 27 new records for Pará and 22 for the Amazon. Of these, 24 were in the MPEG collection, two in the MZUSP collection and one recorded in both collections. Among the new records, *Drosophila fasciola* (Williston, 1896), *Diathoneura flavolineata* Duda, 1927 and *Drosophila neochracea* Wheeler, 1959 are new records for Brazil. *D. fasciola* Williston, 1896 had been recorded from the United States, Mexico, El Salvador, Caribbean, Panama, Colombia and Guyana; *Drosophila flavolineata* (Duda, 1927) only from Costa Rica; and *Drosophila neochracea* (Wheeler, 1959) from Bolivia and Ecuador (Bächli 2015). The *vittimaculosa* group of *Zygothrica* (*Z. vittinubila* Burla, 1956), the subgenus *Siphlodora* of *Drosophila* (*D. flexa*), the genera *Rhinoleucophenga* (*R. punctulata*) and *Diathoneura* (*D. flavolineata*) are new records for Pará. Seven cosmopolitan or semi-cosmopolitan species were found in the state; *Drosophila ananassae*, *D. kikkawai*, *Drosophila malerkotliana* Parshad & Paika, 1964, *Drosophila melanogaster* Meigen, 1830, *D. simulans* Sturtevant, 1919, *Scaptodrosophila latifasciaeformis* (Duda, 1940) and *Zaprionus indianus* Gupta, 1970.

Of the 29 species listed in Table 1 and which were found in neither collections, only 23 of the species are only known from their original descriptions. Three were recently collected in Pará, but the reference specimens were not preserved (*Drosophila pallidipennis* Dobzhansky & Pavan, 1943, *Zygothrica aldrichi* Sturtevant, 1920 and *Zygothrica dispar* Wiedemann, 1830). Three species, *Drosophila polymorpha* Dobzhansky & Pavan, 1943, *Drosophila mesostigma* Frota-Pessoa, 1954 and *Drosophila mediostriata* Duda, 1925 were listed as being in the MPEG collection, but upon re-examination of the respective material, their identification was not confirmed. In the case of *D. polymorpha,* the males were identified as *Drosophila neomorpha* Heed & Wheeler, 1957. The similarity between their terminalia and the possibility of introgression between these two species was discussed by De Toni et al. (2005). Upon re-examination of material identified as *D. mediostriata*, we found *Drosophila paramediostriata* Townsend & Wheeler, 1955 and *Drosophila frotapessoai* Vilela & Bächli, in addition to underscribed species.

The species *Drosophila mediocris* Frota-Pessoa, 1954, *Drosophila medioimpressa* Frota-Pessoa, 1954, *Drosophila neoelliptica* Pavan & Magalhães, 1950, *Drosophila pseudosaltans* Magalhães, 1956 and *D. milleri* Magalhães, 1962, mentioned in the literature, are represented in the MPEG collection. However, only by females preserved in ethanol, precluding detailed examination of the parts that are necessary to confirm identification. Among these, only *D. milleri* was not known from other parts of Brazil, being historically restricted to Puerto Rico.

Wheller (1957), suggests that the presence of *Hirtodrosophila thoracis* (Williston, 1896) in Pará, based on Burla (1956). However, here, we considered this record as belonging to *Paraliodrosophila antennata* Wheeler, 1957, following Vilela & Bächli (2007), according to whom the genitalia of the holotype of *P. antennata* was illustrated by Burla as if it was *H. thoracis*.

Among the species recorded from Pará, 21 species have been nowhere else in Brazil, but have been recorded or observed in other countries (Table 1). Most of those species had only been previously found in Northern localities of the Neotropical region, particularly Colombia, Central America and Caribbean (Bächli 2015). This finding suggests an affinity between the Amazon fauna and the fauna of those other regions.

Additionally, six species occur only in Pará: *D. caxiuana, D. speciosa*, *Z. somatia, D. hendeli, D. decemseriata* and *M. brunnescens*. The last three are known only from their original descriptions; *D. hendeli* and *D. decemseriata* were collected more than 85 years ago, by the Austrian entomologist Hans Zerny in 1927, during an expedition to Fazenda Taperinha, in Santarém (Zerny 1929). At that time, the specimens were deposited at the Naturhistorisches Museum Wien (NMW). There, in 1936, the entomologist Friedrich Hendel determined 11 specimens as *D. annularis* Sturtevant, 1916 and nine as *D. decemseriata* (Hendel 1936). In 1990 Vilela and Bächli described *D. hendeli*, based on one of the specimens identified as *D. annularis* by Hendel (Vilela & Bächili 1990). *D. decemseriata* which was described in 1936 by Hendel has not been found anywhere else. The type of *M. brunnescens* was collected by the geneticist Theodosius Dobzhansky in Belém-PA, in 1952 (Wheeler & Takada 1963), and was apparently never collected again. *D. caxiuana* and *D. speciosa*, in contrast, have been recorded more recently. In 2001, *D. speciosa* was recorded from cacauí flowers (*Thebroma speciosum* Willd. Ex Spreng) (Silva & Martins 2004) and was thus collected from flowers in various municipalities across the state, as observed in the MPEG (Table 1). *D. caxiuana* was collected in 2008 in banana traps (Gottschalk et al. 2012). It is possible that these taxa are locally endemic to the Amazon Biome, however, it is possible that their rarity is due to collecting limitations.

There is still a lot to be learnt about the richness and composition of Drosophilidae species in the Amazon biome. Despite being the second largest state of Brazil, and being within the Amazon biome, Pará comes third in Drosophilidae species richness in the country, with 17% of locally native species, behind the states of São Paulo and Santa Catarina (Gottschalk et al. 2008). Considering the geographic coverage of the records in the collections and literature records, it is obvious that there is still much to be learned about the region's biodiversity. The known distribution of poorly sampled, diverse groups such as the Drosophilidae often reflects where past collecting efforts were more concentrated, rather than the real local diversity. Only 24% of the species known to occur in the state were not represented in the collections studied and those, in turn, have contributed to widen the known geographic distribution of 21% of the species in the list. This result highlights the importance of scientific collections as a source of information on local biodiversity. Most records, either in the literature or in the collections, are from material collected with banana baits. It is possible that the list presented here can be enriched not only by collecting in places that have not been sampled, but also by using other sources, such as flowers, fungi, decomposing leaves and other attractants.

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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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Short Communication

Emergence and establishment of native and non-native species in soils of remnant and converted highland grasslands – southern Brazil

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Abstract: Native grasslands in the Campos de Cima da Serra, Brazil, are being converted at speed for exotic tree plantations and cropland. The impact of modified and novel soil conditions on the establishment of native grassland species is unknown; establishment of non-native species, deliberately or accidentally introduced, could be favoured. In a common garden composed of fully randomized replicate samples of soils collected from remnant grassland, former cropland and pine plantations, we tested emergence and establishment of five cold-season species: Native low-tussock grass *Piptochaetium montevidense* (Spreng.) Parodi; native legume *Trifolium riograndense* Burkart; naturalized low-tussock grass *Vulpia bromoides* (L.) Gray; low-tussock grass *Holcus lanatus L.,* cultivated and naturalized in Brazil; and a cultivar of non-native *Trifolium repens*. Other than expected, soil type and species*soil type interactions had no significant effect on seedling emergence after 132 days in the field. Species effect on seedling emergence, however, was highly significant. *Vulpia bromoides* emergence was significantly highest in all soil types. *Holcus lanatus* and *Trifolium riograndense* both achieved second highest emergence rates and did not differ significantly from each other. Lowest overall emergence rates were found in the non-native clover cultivar. Lab germination tests failed for *Piptochaetium*, although it showed reasonable emergence in the field. Good performance of the native clover is encouraging for future grassland restoration, but the value of highly germinable *Vulpia* as a forage remains to be tested. *Holcus* tolerates a wide range of soil conditions and its life history traits may promote naturalization, or even invasiveness. Native grasslands of the region should be monitored for this species. Studies like these, but set up on a larger geographical scale and with a wider array of native species, will be essential in developing ecological restoration methods for southern Brazilian grasslands.

Keywords: *Seedling emergence, degraded soil, native forage species, invasive species.*

Emergência e estabelecimento de espécies nativas e exóticas em solos de campos convertidos e nativos remanescentes do Planalto - Sul do Brasil

Resumo: Uma acelerada conversão do campo nativo em plantações de espécies florestais exóticas e lavouras tem ocorrido nos Campos de Cima da Serra, Sul do Brasil. Ainda é desconhecido o impacto que as condições edáficas, em solos alterados ou preservados, exercem no estabelecimento de espécies nativas do campo; as espécies exóticas, introduzidas deliberada ou acidentalmente, talvez sejam favorecidas. Em um *common garden* composto por repetições aleatórias de amostras de solo, coletadas em áreas de antigas plantações de pinus, de antigas lavouras ou em áreas com campo nativo preservado, foram testados a emergência e o estabelecimento de cinco espécies hibernais: *Piptochaetium montevidense* (Spreng.) Parodi, gramínea cespitosa nativa; *Trifolium riograndense* Burkart, leguminosa nativa; *Vulpia bromoides* (L.) Gray, gramínea cespitosa naturalizada no sul do Brasil; *Holcus lanatus L.,* gramínea cespitosa exótica, cultivada e disseminada no Brasil; e um cultivar de *Trifolium repens,* leguminosa exótica largamente utilizada. Diferentemente do esperado, o tipo de solo e interações espécie*tipo de solo não tiveram efeito significativo na emergência de plântulas após 132 dias de teste a campo. O efeito da espécie na emergência das plântulas, entretanto, foi altamente significativo. A emergência de *Vulpia bromoides* foi significativamente superior em qualquer tipo de solo. Ambos *Holcus lanatus* e *Trifolium riograndense* apresentaram as segundas maiores taxas de emergência, não diferindo significativamente entre si; as taxas mais baixas foram apresentadas pelo cultivar de trevo não nativo. O Teste de Germinação em laboratório falhou para o *Piptochaetium*, apesar de este ter demonstrado razoável emergência a campo. O bom desempenho do trevo nativo é encorajador para futuras restaurações de pastagens nativas; o valor forrageiro de *Vulpia*, que apresentou alta germinação, ainda precisa ser testado. *Holcus* tolera uma ampla faixa de condições de solo, e suas características adaptativas podem vir a torná-lo naturalizado ou mesmo invasivo. Os campos da região devem ser monitorados em função dessa espécie. Estudos como esse, mas configurados em escala geográfica maior e com maior variedade de espécies nativas, serão essenciais no desenvolvimento de métodos de restauração para os Campos Sulinos Brasileiros.

Palavras-chave: *Emergência de plântulas, solo degradado, espécie campestre nativa, espécie invasora.*

Introduction

In the past decade, considerable changes in the landscape and in land use have occurred in the highlands of southern Brazil. Agricultural and silvicultural practices modify biotic and abiotic ecosystem components through liming, application of N-P-K fertilizer, deliberate and inadvertent introduction of non-native species. There is an increasing need for restoration of native grasslands after grassland conversion even inside designated conservation units (Hermann et al. 2016). However, for most native forages, routines for seed sourcing, testing and propagation have not been established. Revegetation of these areas relies on colonization from the regional species pool which contains both native and non-native species.

A major concern is that non-native taxa might turn invasive in these modified areas as "passengers of change" (see e.g. MacDougall & Turkington 2009). The Eurasian-origin tussock grass *Holcus lanatus*, for example, is being deliberately seeded in winter pastures of the *Campos de Cima da Serra* - which are often established in rotation with intensely cultivated crops such as potato and maize - and has expanded outside these areas in recent years. Cultivars of Eurasian-origin legumes *Trifolium pratense* and *Trifolium repens* are also used for winter forage and establish spontaneously, principally in roadsides and lawns in settlement areas. Native grassland species are presumably well adapted to the highly acidic grassland soils, but their performance in altered soil, relative to that of non-native species, is unknown.

The aim of this pilot-project is to determine the effect of original and novel soil conditions, modified by the expansion of agriculture and silviculture in the region, on the emergence of native and non-native plant species. Consequences for grassland restoration seeding are discussed.

Experimental setup and monitoring

The soils to be tested are sourced from a subset of areas investigated within the research project KO1741/3-1: 5 remnant grazed and burnt native grasslands, 5 pine plantations on former grassland, logged and re-grazed ("Ex-pine-plantation"), and 5 croplands on former grassland, re-grazed after cessation of arable land use ("Ex-Cropland"; see Table 1 and Koch et al. 2016 for further information on land use history and vegetation composition). All are located in the municipalities of São Francisco de Paula and Cambará do Sul, between 50°31'25.18"W and 50° 6'57.74"W, 29° 4'45.88"S and 29°23'2.54"S, Rio Grande do Sul state. From 24^{th} to 27^{th} January 2015 soil samples were taken from 0 – 10 cm depth in random locations on each site. We used this depth instead of the usual 0 – 20 cm because this superficial soil layer has most impact on the germination of the seeded seeds, and the principal aim of this study was not to observe the seed bank of the soil.

Five cold-season species were tested: Native low-tussock grass *Piptochaetium montevidense* (Spreng.) Parodi; native legume *Trifolium riograndense* Burkart; naturalized low-tussock grass *Vulpia bromoides* (L.) Gray; low-tussock grass *Holcus lanatus L.,* cultivated and naturalized in Brazil; and a cultivar of non-native *Trifolium repens*. Seeds of the first four were collected in the study region in mid-summer 2013 and 2014. *T. repens* did not produce sufficient seeds in the field and the seeds were store-bought. For details on harvest, seed cleaning and storage see Table S1, Appendix. This species set does not optimally represent grasslands of the region as it does not contain, for example, characteristic large-tussock species of the genera *Andropogon* and *Sorghastrum*. Unfortunately, these species often produce infertile seed (see also Overbeck et al. 2006) and in spite of repeated harvests across the region, not enough viable seeds could be obtained.

In March 2015, the seeds were subjected to germination tests in the Seed Analysis Laboratory of the Faculty of Agriculture at UFRGS – Porto Alegre according to the Rules for Seed Testing (Brasil 2009). The treatment to overcome seed dormancy was manual scarification with sandpaper number 180 for 20 seconds for both *Trifolium* species; for the other species we used KNO_3 (0.2%) solution. For the germination test, we used 4 repetitions with 100 seeds for each species. *Piptochaetium montevidense*, *Trifolium riograndense* and *Vulpia bromoides*, as many other native species, are not included in these Rules. Thus, we decided to use similar parameters for them as are given for other species from the same genus by various authors (Brasil 2009; Suñé & Franke 2006; Fochesato et al. 2000). Seeds were tested in a germination chamber with 8 hours light and 16 hours dark cycle. Type and duration of treatments are detailed in Table S2, Appendix. 1,000 seeds of each species were weighed at UERGS - São Francisco de Paula (more results, see Table 2). Median germination percentages per species are given in Table 2.

The field experiment was performed in a common garden in an open area (park lawn) belonging to the Public Agency of Transport (DAER), São Francisco de Paula. Soil was filled into in perforated 20 ^{x} 13 ^{x} 5 cm aluminium trays. There were 9 trays for each donor site: 8 repetitions of untreated soil and one tray with sterilized soil in order to assess and delete from analyses emergence from soil bank and seed rain. Land use type and donor site were noted on the bottom of the trays to enable a blinded monitoring. Trays were placed in a fully randomized setup on white shading fabric to reduce weed growth, with sufficient space between them to reduce contamination by soil splashing, and covered with tulle to minimize contamination by seed rain.

Table 1. Characterisation of soil source sites: Median time of conversion and of recovery; four indicators of soil fertility (median (min-max)); per-tray biomass produced in 4.25 months in the common garden (median (min-max)) as indicator of productivity. Same letter: no significant difference between soil types. Detailed statistics results for soil in Table S4, Appendix, and for biomass in Table S5, Appendix.

	Converted	In recovery	pH_{H2O}	Base sat. [%]	Al [cmolc/dm³]	P [mg/dm³]	Total dry biomass [g]
Native pasture	Never	-	4.3 (4.2 - 4.8) ^{a}	10 (7-38) ^{a}	4.8 (1.2 - 7.6) ^{ab}	2.1 (1.8 - 7.3) ^{a}	6.9 (4.8-10.2) ^{a}
Ex-Pine plantation	20 ys ago	3 ys	4.1 (3.8 - 4.3) ^{b}	6 (3-12) ^{a}	6.2 (4.0 - 10.7) ^{a}	4.0 (2.0 - 7.3) ^{a}	5.8 (5 - 15.5) ^{a}
Ex-Cropland	5.5. ys ago	5 ys	5.3 (4.5 - 5.4) ^{c}	64 (16-69) ^{b}	0.3 (0.1 - 4.5) ^{b}	12 (2.6 - 41.0) ^{a}	13 (7.4 - 20.1) ^{a}

Table 2. Thousand-seed-weight, lab germination and field emergence across all soil types of tested species. Median (min-max); emergence column, same letter: no significant difference between species. Detailed statistics results in Table S3, Appendix.

	TSW [g]	Lab germin. [%]	Germinable/sown	Emergence[1] [%]	Soil effect on emergence
Trifolium riograndense	0.5399	35	18/50	29 (9-40) [a]	n.s.
Vulpia bromoides	0.6691	79	20/25	100 (60-128*) [b]	n.s.
Holcus lanatus	0.2630	79	40/50	30 (18-49) [a]	n.s.
Trifolium repens	0.6145	78	39/50	6 (1-14) [c]	n.s.
				Emergence[2] [%]	
Piptochaetium montevidense	0.5403	0.75	n.a./50	13 (4-21)	n.s.

Emergence[1]: Median (min-max) % of germinable seed; Emergence [2]: Median (min-max) % of sown seed; *Values >100 due to higher germination success in the field than in the lab.

On 9th February 2015, each tray was subdivided in 6 squares (subplots): one unseeded control, five sown with seeds of one species (25 seeds of *Vulpia*, 50 seeds of the other species; *Vulpia* seeds were too large, including awns, to seed the same amount), randomly assigned to subplots. A thin soil layer was spread over the seeds and lightly pressed down. Trays were watered in the first week after setup and afterwards exposed to ambient climate.

In five of eight trays, all emerged seedlings, seeded and spontaneous, were counted and harvested once per month after sowing, on 11th March, 10th April, 8th May and 21st June (29, 60, 88 and 132 days after setup). Three of eight trays were left to grow undisturbed, and censused and harvested on 21st June; these were originally intended to monitor establishment and biomass production of seeded species. In counts we distinguished between the five seeded species, unseeded graminoids, and unseeded herbs. Biomass of each tray was dried at 105°C for 1-2 days and weighed in the lab of UERGS-São Francisco de Paula; at the final harvest, biomass was separated into seeded and unseeded grasses and herbs. In order to facilitate identification of seedlings, study species had also been seeded into two trays with commercial potting soil where their development could be observed. Pictures of each tray were taken every month for additional control and monitoring.

In the second half of June 2015, the common garden site was vandalized by stray dogs and the experiment closed after a total of 132 days. However, emergence of all species in all soils peaked between day 60 and day 88; we are therefore confident to have captured most of the potential total emergence.

Soil chemical data presented in Table 1 were analysed by the Soil Analysis Laboratory of the Faculty of Agriculture at UFRGS - Porto Alegre from one composite sample per site, collected in southern winters 2013/14 and 2014/15.

Analysis

For analysis, for each species and tray, we subtracted background emergence in unseeded subplots from number of seedlings in seeded subplots (two seedlings total in controls in April and May, 16 total in June, not more than 5% of total emergence of a species in a given tray; zero emergence in sterilized trays). For all species except *Piptochaetium*, we expressed emergence as number of seedlings related to number of germinable sown seed per subplot; *Piptochaetium* emergence is number of seedlings related to number of sown seed per subplot (see Table 2).

Due to partial destruction of the experiment, not enough replicate trays remained to analyse cumulative emergence (five seeded trays per donor site) and undisturbed establishment (three seeded trays per donor site) as originally planned. Instead, we analysed overall emergence by pooling emergence data from undisturbed and regularly harvested trays, summing data from all four surveys for the latter, in order to obtain a minimum of five replicate trays per donor site. A given site is represented by the median of these replicates in the analyses.

Due to the low number of replicate source sites and heterogeneous variance between them, we calculated and graphed medians of emergence, and we employed nonparametric univariate tests to test for significant differences in soil chemical parameters and in biomass production between soil types. We ran a generalised linear mixed model with emergence as dependent variable, soil type as fixed and species as random factor. In a second step, we again employed nonparametric univariate tests to test for significant differences in seedling emergence between species across soil types and per soil type (Kruskal-Wallis-test, Mann-Whitney U-test for pairwise post-hoc comparisons). Soil impact on *Piptochaetium* emergence was analysed separately. The data were analyzed and graphed with SPSS 24.0.

Results

Viable seeds were obtained for all five species, although with varying success. More than three quarters of *Holcus*, *Vulpia* and *T. repens* seeds and one third of *T. riograndense* seeds germinated in two weeks under laboratory conditions. For *Piptochaetium* this test failed. Lab germination was practically zero (Table 2), while median 13% of sown seeds emerged in 132 days in the field.

With five replicate sites per soil type, we were able to represent the original grassland soil conditions as well as significant alterations by land use change (Table 1 and Table S4, Appendix). pH and base saturation of grasslands are low (median 4.3, and 10%), and are further lowered by conversion to pine plantation, significantly in the case of pH. Liming and fertilization for agriculture significantly increase pH and also reduce aluminium toxicity, and this effect persisted up to five years after areas were taken out of agricultural use. Higher bioavailability of nutrients of former cropland soils was reflected by ca. two times higher total biomass production than in grassland and ex-pine plantations (Table 1), although only herb biomass showed a weakly significant difference between ex-cropland and ex-pine plantation soils, $U(8) = 2.000$, $Z = -2.193$, $p = 0.032$ (Table S5, Appendix). Herbs and grasses emerging spontaneously from the seed bank made up 55-65% of total biomass in grassland and ex-cropland soil, while seeded species made up more than half of biomass in ex-pine plantation soils.

Nonetheless, other than expected, soil type and species*soil type interactions had no significant effect on seedling emergence after 132 days in the field ($F(6,46) = 1.58$, $MSE = 110.23$, $p = 0.18$). This preliminary result of the GLM was supported by further, nonparametric tests insofar as seedling emergence did not differ significantly between pairs of soil types for any of the five species, with one exception: Native and non-native *Trifolium* both tended to have the lowest emergence in ex-pine plantation soils and the highest emergence on former cropland soils, and this difference was weakly significant (*Trifolium repens* $U(8) = 2.000$, $Z = -2.220$, $p = 0.032$; *Trifolium riograndense* $U(8) = 2.500$, $Z = -2.128$, $p = 0.032$).

Species effect on seedling emergence was highly significant ($F(3,6) = 119.34$, $MSE = 173.77$, $p = 0.00$; Table 2, Figure 1). *Vulpia bromoides* emergence was significantly highest of all species across soil types, and per soil types, in spite of seeds (like *Piptochaetium* seeds) being 15 months old at the start of the experiment. Across all soil types, *Vulpia* differed significantly a) from *Holcus lanatus*, U(26) = 0.000, Z = -4.496, p = 0.000, b) from *Trifolium repens*, U(26) = 0.000, Z = - 4.500, p = 0.000, c) from *Trifolium riograndense*, U(26) = 0.000, Z = -4.504, p = 0.000. These differences were only weakly significant ($p<0.05$) in native pasture soil, and in ex-pine plantation soil, and more pronounced in ex-cropland soil ($p<0.01$); detailed statistics results per soil type are given in Table S3, Appendix.

Holcus lanatus and *Trifolium riograndense* both achieved second highest emergence rates and did not differ significantly from each other, e.g. across all soil types U(28) = 77.000, Z = -1.476, p = 0.148.

Lowest overall emergence rates were found in the non-native clover cultivar. Across soil types, *Trifolium repens* differed significantly not only from *Vulpia* but from a) *Holcus lanatus*, U(28) = 0.000, Z = -4.674, p = 0.000 and b) from *Trifolium riograndense*, U(28) = 4.000, Z = -4.514, p = 0.000.

Discussion and conclusions

There was little indication that novel soils (ex-pine plantation, ex-cropland soil) favoured emergence of non-native species, as hypothesized. Emergence of *Vulpia bromoides*, originally a Eurasian species and now naturalised in Brazil, was higher than that of the other species and this difference was more pronounced in ex-cropland soil than in the other two soils with lower pH and nutrient status. This underlines its characterisation as a "ruderal" species by Brazilian field ecologists (G. Overbeck, pers. comm.). Non-native *Holcus lanatus* was not favoured by novel soil characteristics. *Trifolium repens*, also formerly a Eurasian species, did perform best in cropland soil but the same applied to its native congeneric, *Trifolium riograndense*. Both Fabaceae were probably favoured by the raised pH.

Non-native species do not yet play a major role on logged, re-grazed pine plantations in the study region, but they are an important component of secondary grassland communities on former cropland, accounting for 20% of vegetation cover on average (but making up less than 1% cover in remnant grassland; Koch et al. 2016). This is a globally reported phenomenon that even inspired development of the "novel ecosystem" concept (Cramer et al. 2008). Our study suggests that soil alteration alone does not account for non-native species success but that other factors such as disturbance and deliberate seeding must contribute to their establishment. This underlines the crucial role of restoration seeding to guide secondary grassland development to desired states.

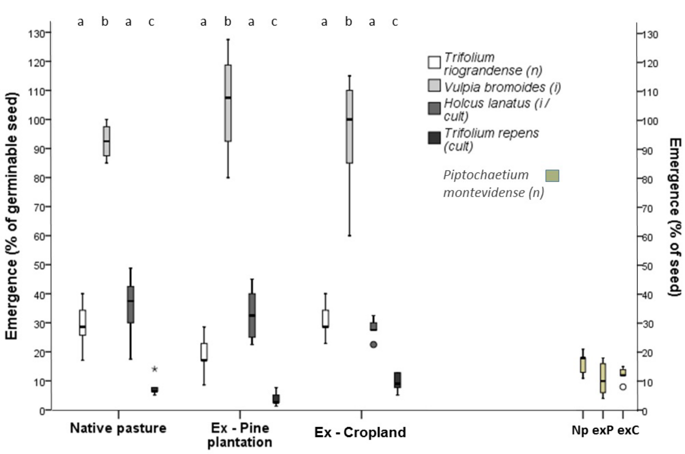

Figure 1. Emergence of five seeded cold-season species in three types of soil over 132 days in late summer – early winter 2015. n: native, i: introduced/naturalized, cult: cultivated. Soil type abbreviations correspond to types in left of graph.

Field emergence success of the native clover, *T. riograndense* is encouraging for future restoration projects, including use of restored grasslands for pasture. Increasing forage value of native grasslands has been a major incentive for introducing and overseeding legume cultivars; yet in this experiment the cultivar – by definition passed through selective enhancement - did not express best potential for emergence.

Piptochaetium montevidense has value as native winter and spring-growing forage grass that is also tolerant of elevated grazing pressure (Boldrini 2009; Maraschin 2009). In our experiment its emergence was lower than that of the other grasses. It is not clear whether this was due to higher age of the seeds, or whether processing and storage were not adequate. Laboratory germination protocols developed for other species from the same genus did not prove suitable.

Vulpia bromoides is not a cultivated forage species in its original range and was not deliberately introduced. It has naturalized spontaneously in Brazil and this experiment gave some indication for the reasons for this success. Seeds retained high germination potential for more than one year, and emergence was high irrespective of significant differences in soil chemistry. Its potential value as a forage species remains to be tested.

Non-native, naturalized *Holcus lanatus* demonstrated a good adaption to all site conditions, even to the remnant grasslands. Whether negative ecological and economic impacts are to be anticipated is not clear. A large body of literature exists for this species; key features that promote naturalization and invasiveness were summarized by Gucker 2008: *H. lanatus* produces large amounts of seeds, is dispersed by a variety of factors (water, animals, wind) and forms an abundant seed bank (notably, *Holcus* seed weight determined in this study was half as low or lower than that of the other species, Table 2). In its original range, adult plants with their velvety stem bases are not preferred cattle forage and it should therefore be grazed when young. Once established at undesired levels, the species cannot be easily removed as it tolerates both grazing and frequent fire (Gucker 2008). Summing up, it might be a potential forage species in novel soils and a potential invasive plant in native soils of our study region. Continuous monitoring of this species in the *Campos de Cima da Serra* is recommended.

Nonetheless, the results of this pilot study must be interpreted with caution and should principally serve to guide future, more extensive and better designed studies of non-native and native species emergence and establishment in remnant sites and under altered site conditions. 1) The range of tested species should be increased to include characteristic, summer-flowering tussock and prostrate grass species as well as representatives of further diverse plant families, e.g. Asteraceae and Cyperaceae. Both summer and winter vegetation period must be tested. 2) Improved replication of soil donor sites, or controlled manipulation of soil fertility, might reveal the range of soil conditions under which native species are at an advantage over non-natives, or at a disadvantage. 3) At present, germinability tests under laboratory conditions are tailored to commercial varieties (in which rapid germination is desirable) and must be adjusted to wild or semi-wild genotypes with prolonged germination periods. Current standard pre-treatments in the lab may also not be adequate to reveal emergence potential in the field.

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Table S1. Appendix: Information on studied species, seed acquisition and processing. The first three species are Poaceae, the Trifolium species are of the Fabaceae family.

Species name [1]	Species origin	Status in Brazil	Harvest site	Harvest and cleaning procedure
Piptochaetium montevidense (Spreng.) Parodi	Southern America	Native	Fertilized native pasture, 29°24'33.52"S, 50°21'17.10"W	Ca. 10 flower stalks harvested off 20 tussocks 15.12.2013, stored at room temperature ca. 3 and in fridge ca. 9 months; seed and chaff rubbed off inflorescences by hand, separated by repeated rubbing and sieving 30.12.2014 (yield ca. 10,000 seeds), fridge-stored until sowing 9.2.2015
Vulpia bromoides (L.) Gray	Eurasia	Naturalized [1]	Fertilized native pasture, 29°24'33.52"S, 50°21'17.10"W	1 handful (10-20) flower harvested off 10 tussocks 15.12.2013; stored at room temperature ca. 3 and in fridge ca. 9 months; spikelets rubbed off stems and between fingers to obtain single florets/seed 30.12.2014 (yield ca. 5,000 single seeds; do not separate easily), awns not removed, fridge-stored until sowing 9.2.2015
Holcus lanatus L.	Eurasia	Cultivated; naturalized [1]	Roadside grassland, 29°23'35.56"S, 50°18'31.82"W	Seed and chaff stripped off ca. 50 tussocks in field 15.12.2014, separated by sieving 10.-13.1.2015 (yield ca. 180,000 seeds), fridge-stored until sowing 9.2.2015
Trifolium riograndense Burkart	Southern America	Native	Fertilized native pasture, 29° 0'47.95"S, 50°25'21.76"W	Transect walks through area, >1000 entire flower heads collected 19.12.2014; stored at room temperature until dry; flower heads plucked apart, rubbed between hands and through metal sieve to break pods, then through plastic sieves to separate seed and chaff 10.-13.1.2015 (yield ca. 10,000 seeds); fridge-stored until sowing 9.2.2015.
Trifolium repens cultivar	Eurasia	Cultivated	Commercial source: Associação Rural, São Francisco de Paula-RS	Cultivar of unknown name produced in Uruguay, clean seed bought in January 2015, fridge-stored until sowing 9.2.2015

[1] Flora do Brasil 2020, under construction (see references)

Table S2 - Appendix: Lab germination testing procedures.

Species	Temperature (^{0}C)	Pre-treatment	First count	Last count
T. riograndense	20 – 30	Mechanical scarification; Pre-cooling, 8^{0}C, 5 days	7 days	14 days
V. bromoides	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	-	14 days
H. lanatus	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	6 days	14 days
T. repens	20	Mechanical scarification; Pre-cooling, 8^{0}C, 5 days	4 days	10 days
P. montevidense	20 – 30	KNO_3(0,2%); Pre-cooling, 8^{0}C, 5 days	7 days	14 days

Table S3 - Appendix: Statistics results for comparison of seedling emergence between soil types and species. Dependent variable: Percent of germinable sown seed emerged after four months in the field. Exception Piptochaetium datasets: Percent of sown seed emerged after four months in the field

GLM, fixed factor: soil type; random factor: species

	df (effect)	df (error)	F	MSE	p	
Intercept	1	3	4.618	20674.267	0.121	
Soiltype	2	6.028	0.022	173.601	0.978	
Species	3	6.007	119.340	173.771	0.000	***
Soiltype*species	6	46	1.577	110.234	0.175	

Kruskal-Wallis-Test, factor: species

Dataset	df	Chi-Square	p	
Soil types pooled	3	47.586	0.000	***
Native pasture	3	15.317	0.002	***
Ex-Pine plantation	3	16.089	0.001	***
Ex-Cropland	3	16.340	0.001	***

Mann-Whitney U-tests on differences between species (exact asymptotic significance)

Species 1	Species 2	df	U	Z	p	
Dataset: Soil types pooled						
Holcus lanatus	*Trifolium repens*	28	0.000	-4.674	0.000	***
Trifolium riograndense	*Holcus lanatus*	28	77.000	-1.476	0.148	
Trifolium riograndense	*Trifolium repens*	28	4.000	-4.514	0.000	***
Trifolium riograndense	*Vulpia bromoides*	26	0.000	-4.504	0.000	***
Vulpia bromoides	*Holcus lanatus*	26	0.000	-4.496	0.000	***
Vulpia bromoides	*Trifolium repens*	26	0.000	-4.500	0.000	***
Dataset: Native pasture						

Level of significance: * P<0.05, ** P<0.01, *** P<0.001

Table S3 - Appendix (cont.):

GLM, fixed factor: soil type; random factor: species						
	df (effect)	**df (error)**	**F**	**MSE**	**p**	
Holcus lanatus	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	7.000	-1.149	0.130	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Vulpia bromoides*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Holcus lanatus*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Trifolium repens*	7	0.000	-2.460	0.016	*
Dataset: Ex-Pine plantation						
Holcus lanatus	*Trifolium repens*	8	0.000	-2.619	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	3.000	-1.991	0.056	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Vulpia bromoides*	7	0.000	-2.460	0.016	*
Vulpia bromoides	*Holcus lanatus*	7	0.000	-2.449	0.016	*
Vulpia bromoides	*Trifolium repens*	7	0.000	-2.460	0.016	*
Dataset: Ex-Cropland						
Holcus lanatus	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Holcus lanatus*	8	8.000	-0.946	0.421	
Trifolium riograndense	*Trifolium repens*	8	0.000	-2.627	0.008	**
Trifolium riograndense	*Vulpia bromoides*	8	0.000	-2.619	0.008	**
Vulpia bromoides	*Holcus lanatus*	8	0.000	-2.619	0.008	**
Vulpia bromoides	*Trifolium repens*	8	0.000	-2.619	0.008	**

Kruskal-Wallis-Test, factor: soil type			
Dataset	**df**	**Chi-Square**	**p**
Species pooled	2	0.876	0.645
Holcus lanatus	2	1.516	0.469
Trifolium repens	2	5.937	0.051
Trifolium riograndense	2	5.141	0.076
Vulpia bromoides	2	1.304	0.521
Piptochaetium montevidense	2	3.047	0.218

Mann-Whitney U-tests on differences between soil types (exact asymptotic significance)						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: Species pooled						
Native pasture	Ex-Pine plantation	36	157.000	-0.686	0.506	
Native pasture	Ex-Cropland	37	187.500	-0.070	0.945	
Ex-Pine plantation	Ex-Cropland	37	157.500	-0.914	0.365	
Dataset: *Holcus lanatus*						
Native pasture	Ex-Pine plantation	8	11.000	-0.313	0.841	
Native pasture	Ex-Cropland	8	6.500	-1.261	0.222	
Ex-Pine plantation	Ex-Cropland	8	9.000	-0.738	0.548	
Dataset: *Trifolium repens*						
Native pasture	Ex-Pine plantation	8	4.000	-1.798	0.095	
Native pasture	Ex-Cropland	8	9.000	-0.740	0.548	
Ex-Pine plantation	Ex-Cropland	8	2.000	-2.220	0.032	*
Dataset: *Trifolium riograndense*						
Native pasture	Ex-Pine plantation	8	4.500	-1.697	0.095	
Native pasture	Ex-Cropland	8	11.000	-0.319	0.841	
Ex-Pine plantation	Ex-Cropland	8	2.500	-2.128	0.032	*
Dataset: *Vulpia bromoides*						
Native pasture	Ex-Pine plantation	6	4.000	-1.155	0.343	
Native pasture	Ex-Cropland	7	8.000	-0.494	0.730	
Ex-Pine plantation	Ex-Cropland	7	7.500	-0.615	0.556	
Dataset: *Piptochaetium montevidense*						
Native pasture	Ex-Pine plantation	8	6.000	-1.586	0.151	
Native pasture	Ex-Cropland	8	6.000	-1.366	0.222	
Ex-Pine plantation	Ex-Cropland	8	11.000	-0.314	0.841	

Level of significance: * P<0.05, ** P<0.01, *** P<0.001

Table S4 - Appendix: Comparison of selected soil chemistry traits between soil types

Kruskal-Wallis-Test, factor: soil type						
Dependent var.	**df**	**Chi-Square**	**p**			
pH [H2O]	2	10.272	0.006			
P [mg/dm³]	2	4.837	0.089			
Al [cmolc/dm³]	2	7.348	0.025			
Base saturation [%]	2	10.158	0.006			
Mann-Whitney-U-Test on differences between soil types (exact asymptotic significance)						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: pH [H2O]						
Native pasture	Ex-Pine plantation	8	2.500	-2.128	0.032	*
Native pasture	Ex-Cropland	8	2.500	-2.108	0.032	*
Ex-Pine plantation	Ex-Cropland	8	0.000	-2.627	0.008	**
Dataset: P [mg/dm³]						
Native pasture	Ex-Pine plantation	8	7.000	-1.156	0.310	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	6.000	-1.358	0.222	
Dataset: Al [cmolc/dm³]						
Native pasture	Ex-Pine plantation	8	7.500	-1.048	0.310	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	1.000	-2.402	0.016	*
Dataset: Base saturation [%]						
Native pasture	Ex-Pine plantation	8	3.000	-1.991	0.056	
Native pasture	Ex-Cropland	8	2.000	-2.193	0.032	*
Ex-Pine plantation	Ex-Cropland	8	0.000	-2.619	0.008	**

Level of significance: * P<0.05, ** P<0.01.

Table S5 - Appendix: Comparison of harvested dried biomass between soil types

Kruskal-Wallis-Test, factor: soil type						
Dependent var.	**df**	**Chi-Square**	**p**			
Total biomass [g]	2	0.430	0.112			
Herb biomass [g]	2	6.540	0.038	*		
Graminoid biomass [g]	2	3.860	0.145			
Mann-Whitney-U-Test on differences between soil types						
Soil type 1	**Soil type 2**	**df**	**U**	**Z**	**p**	
Dataset: Total biomass [g]						
Native pasture	Ex-Pine plantation	8	10.000	-0.522	0.690	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	5.000	-1.567	0.151	
Dataset: Herb biomass [g]						
Native pasture	Ex-Pine plantation	8	6.000	-1.358	0.222	
Native pasture	Ex-Cropland	8	4.000	-1.776	0.095	
Ex-Pine plantation	Ex-Cropland	8	2.000	-2.193	0.032	*
Dataset: Graminoid biomass [g]						
Native pasture	Ex-Pine plantation	8	10.000	-0.522	0.690	
Native pasture	Ex-Cropland	8	3.000	-1.984	0.056	
Ex-Pine plantation	Ex-Cropland	8	6.000	-1.358	0.222	

Level of significance: * P<0.05.

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Influence of landscape structure on Euglossini composition in open vegetation environments

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Abstract: The fauna of Euglossini bees is poorly known in savanna regions, making it difficult to understand how these bees use open vegetation environments. The aim of this study was to evaluate the influence of landscape structure on species abundance and composition of Euglossini bees in naturally heterogeneous savanna landscapes. Nine sites were sampled monthly using six traps with chemical baits. Three aromatic essences (eucalyptol, methyl salicylate and vanillin) were used to attract the Euglossini. Surrounding environmental conditions were measured using three independent variables, calculated in multiple scales: index of local vegetation and two landscape indices (Shannon Diversity and area-weighted shape). We compared the competing hypotheses through model selection based on Second-order Akaike Information Criterion (AICc). The four competing hypothesis were: (1) The local vegetation complexity favors Euglossini bees species richness and/or abundance (local vegetation hypothesis); (2) The proportion of the native vegetation types favors Euglossini bees species richness and/or abundance (habitat amount hypothesis); (3) Higher landscape diversity shall increase species richness of Euglossini bees (landscape heterogeneity hypothesis); (4) More complex landscape configuration shall favor the Euglossini bees richness and/or abundance (landscape heterogeneity hypothesis). We sampled 647 individuals belonging to six species of two distinct genera. Our results support the habitat amount hypothesis since bees' abundance was strongly related with the proportion of habitat in the surrounding landscape. This may be related to the availability of floral and nesting resources in some types of savanna vegetation.

Keywords: *bees, habitat amount hypothesis, landscape heterogeneity, landscape configuration*

Influência da estrutura da paisagem na composição de Euglossini em ambientes com vegetação aberta

Resumo: A fauna das abelhas da tribo Euglossini é pouco conhecida em regiões de savana, tornando difícil a compreensão de como essas abelhas usam ambientes com vegetação aberta. O objetivo desse estudo foi avaliar a influência da estrutura da paisagem na abundância e composição de espécies de abelhas Euglossini em paisagens naturalmente heterogêneas de savana. Nove locais foram amostrados mensalmente utilizando seis armadilhas com iscas químicas. As essências eucaliptol, salicilato de metila e vanilina foram utilizadas para atrair os machos de Euglossini. As condições ambientais foram medidas usando três variáveis, calculadas em múltiplas escalas: índice de vegetação local e dois índices de paisagem (diversidade de Shannon e o índice de forma ponderado pela área). Através da seleção de modelos baseada no critério de informação de Akaike de segunda ordem (AICc) comparamos as hipóteses alternativas: (1) Vegetação local mais complexa favorece as abelhas Euglossini (hipótese da vegetação local); (2) A proporção dos tipos de vegetação nativas favorece as abelhas Euglossini (hipótese da quantidade habitat); (3) A diversidade da paisagem favorece a riqueza de espécies de abelhas Euglossini (hipótese da heterogeneidade da paisagem); (4) Configuração mais complexa da paisagem favorece a riqueza e/ou abundância de abelhas Euglossini (hipótese da heterogeneidade paisagem). Nós amostramos 647 indivíduos pertencentes a seis espécies de dois gêneros distintos. Nossos resultados apoiam a hipótese de quantidade de habitat já que a abundância das abelhas foi fortemente relacionada com a proporção de habitat nas paisagens circundantes. Esses resultados podem estar relacionados com a disponibilidade de recursos florais e substratos para nidificação em alguns tipos de savana.

Palavras-chave: *abelhas, hipótese da quantidade de habitat, heterogeneidade da paisagem, configuração da paisagem*

Introduction

Given the importance of Euglossini bees for wild pollination as well as the practicity of its sampling, many studies adopted them as biological models in order to understand how bees in general use their environment and how they can be affected by habitat loss, fragmentation and homogenization in landscapes (Powell & Powell 1987, Tonhasca et al. 2002b, Viana et al. 2006, Brosi et al. 2007, Mendes et al. 2008, Brosi 2009, Knoll & Penatti 2012, Silveira et al. 2015, Aguiar et al. 2015). However, these studies present mixed results, with most of them poorly delineated and conducted in forested environments. Few studies were specifically designed to evaluate landscape effects on Euglossini bees, such as works by Brosi (2009) and Brosi et al. (2007) that showed evidences of positive relationships between Euglossini bees and the amount of border between forest and non-forest areas in landscapes, what suggests the association of these bees with transition environments. These results suggest that many Euglossini bees depend both on the forests and their surrounding open areas. Others also evidenced that Euglossini bees can cross large distances between forest patches or leave these forests during their resource foraging trips, what may help to explain why they appear to be little affected by fragmentation per se (Tonhasca et al. 2002b, Tonhasca et al. 2003, Milet-Pinheiro & Schlindwein 2005, Ramalho et al. 2009, Brosi et al. 2007, Rosa et al. 2016).

The Euglossini bees have Neotropical distribution and exist throughout South and Central America, especially in tropical forests, with some occurrences in the Southern United States (Michener 2007, Nemésio 2009). Males of these bees have close association with certain orchid species, based on supply and demand for aromatic compounds being therefore known as orchid bees. They are pollinators of many plant species representatives of dozens of botanical families (Dressler 1982, Cameron 2004) and are potentially long-range pollinators that can be important in highly heterogeneous environments such as the neotropical forests (Janzen 1971, Wikelski et al. 2010). Although commonly associated with forested environments, these bees are also present in open vegetation types such as the Brazilian savanna, called “Cerrado” (Knoll & Penatti 2012, Silveira et al. 2015). The Brazilian savanna is one of the world’s biodiversity hotspots and is under great pressure because of agricultural expansion and lack of conservation reserves (Myers et al. 2000, Klink & Machado 2005, Bellard et al. 2014). However, the Euglossini fauna in savanna environments is relatively unknown in contrast with forested environments which are well studied for this group, especially considering the savanna physiognomies with low stratification profiles. Such lack of information about the importance of open vegetation types such as savanna for these bees deeply limits our understanding of the effects of landscape structure on Euglossini communities.

Many factors can be determinant for the maintenance of Euglossini populations and community structures. The amount of floral resources, along with other local vegetation characteristics, can attract foraging bees, increasing their likelihood to pass through certain places (Chittka & Thomson 2001). Alternatively, residence of individuals in the landscape can be determined by the availability of limiting factors such as the amount of floral resources, nesting substrate or material for nest building in a broader area regulating the abundance or preventing the maintenance of bee populations (Potts et al. 2003, 2005). Therefore, the abundance and species richness can be directly proportional to the landscape coverage of the vegetation types that best meets the demands of the studied group, as expected according to the habitat amount hypothesis (Fahrig 2013). A third possibility is that the vegetation types with different floral compositions and physical features can have complementary roles for the bees (Dunning et al. 1992). For example, while some bee species can find nesting substrates in a given environment, the floral resources may be in another place, making necessary the presence of both landscape units for the presence of such species. Additionally, the differences in phenology may cause spatial and temporal variations in the floral resources availability among vegetation types. In this case, the most heterogeneous landscapes may favor the maintenance of a greater abundance and species richness because of the complementarity of vegetation types as expected according to the landscape heterogeneity hypothesis (Fahrig et al. 2011).

It is, thus, very difficult to evaluate the importance of each of these effects separately. However, the comparison of multiple concurrent hypothesis (e.g. local vegetation, habitat amount and landscape heterogeneity) through model selection approaches based on Akaike Information Criterion (AIC) can help us to find reasonable explanation of observed patterns (Burnham & Anderson 2002). The Savanna domains comprise different vegetation types, ranging from grasslands to forests and typically form wide vegetation mosaics (Klink & Machado 2005, Brasil 1992). This natural heterogeneity is the result of a long and dynamic evolutionary history, forming an ideal environment to establish relationships between composition of biological communities and structural landscape patterns (Fahrig et al. 2011, Fahrig 2013). In this context, the aim of this study was to evaluate the influence of landscape structure on the abundance and species composition of Euglossini community in naturally heterogeneous Savanna landscapes through the comparison among the four alternative hypothesis: (1) The local vegetation complexity favors Euglossini bees species richness and/or abundance (local vegetation hypothesis); (2) The proportion of the native vegetation types favors Euglossini bees species richness and/or abundance (habitat amount hypothesis); (3) Higher landscape diversity shall increase species richness of Euglossini bees (landscape heterogeneity hypothesis); (4) More complex landscape configuration shall favor the Euglossini bees richness and/or abundance (landscape heterogeneity hypothesis).

Methods

1. Study region, selection of sampling units and sampling bees

The study was conducted in Mucugê Municipal Park (12º59’S, 41º20’W), located in Chapada Diamantina, Bahia, Brazil. The study region has an altitude ranging between 900 and 1400 meters and its vegetation is composed of a mosaic of savanna formations in a continuous structural gradient ranging from grass-woody (characterized by graminoid hemicryptophytes, geophytes and spaced rachitic wooded plants) to wooded savanna (characterized by sparse nano-cryptophytes and hemicryptophytes), with predominance of rocky savannas (Juncá et al. 2005) (Figure 1). The climate type is tropical savanna (Aw) according to the classification of Köppen-Geiger, characterized by the average temperature of the coldest month of the year greater than 18 ° C, higher rainfall than the potential evapotranspiration and with two marked dry and wet seasons. Rainfall is concentrated in the summer (Peel et al. 2007). With an average annual rainfall of 1281 mm, this region has a rainy season from November to April, with the average cumulative rainfall for this period being 942 mm and 339 mm in the dry season. Maximum annual average temperature is 29 ° C and the minimum is 19.8 ° C (INMET 2015).

We randomly selected nine sample units at least 1.5 km apart from one another within an area of 38 km^2 on the official vegetation map for the state of Bahia (Diretoria de Desenvolvimento Florestal 1998), totaling three sample units for each of the three native vegetation types most abundant at the study area: grassy-woody savanna, rupestrian grassy-woody savanna and rupestrian wooded savanna (Figure 1). In order to sample Euglossini males traps were built with 2 l bottle polyethylene terephthalate (Neves & Viana 1997). Within each trap, a cotton wool wrapped in gauze was soaked with 2 ml of one of the chemical baits used, i.e. eucalyptol, methyl salicylate or vanillin. These aromatic essences are among the most attractive baits for this biological group as reported by the specialized literature (Oliveira & Campos 1996). In each sample unit six traps (two with each

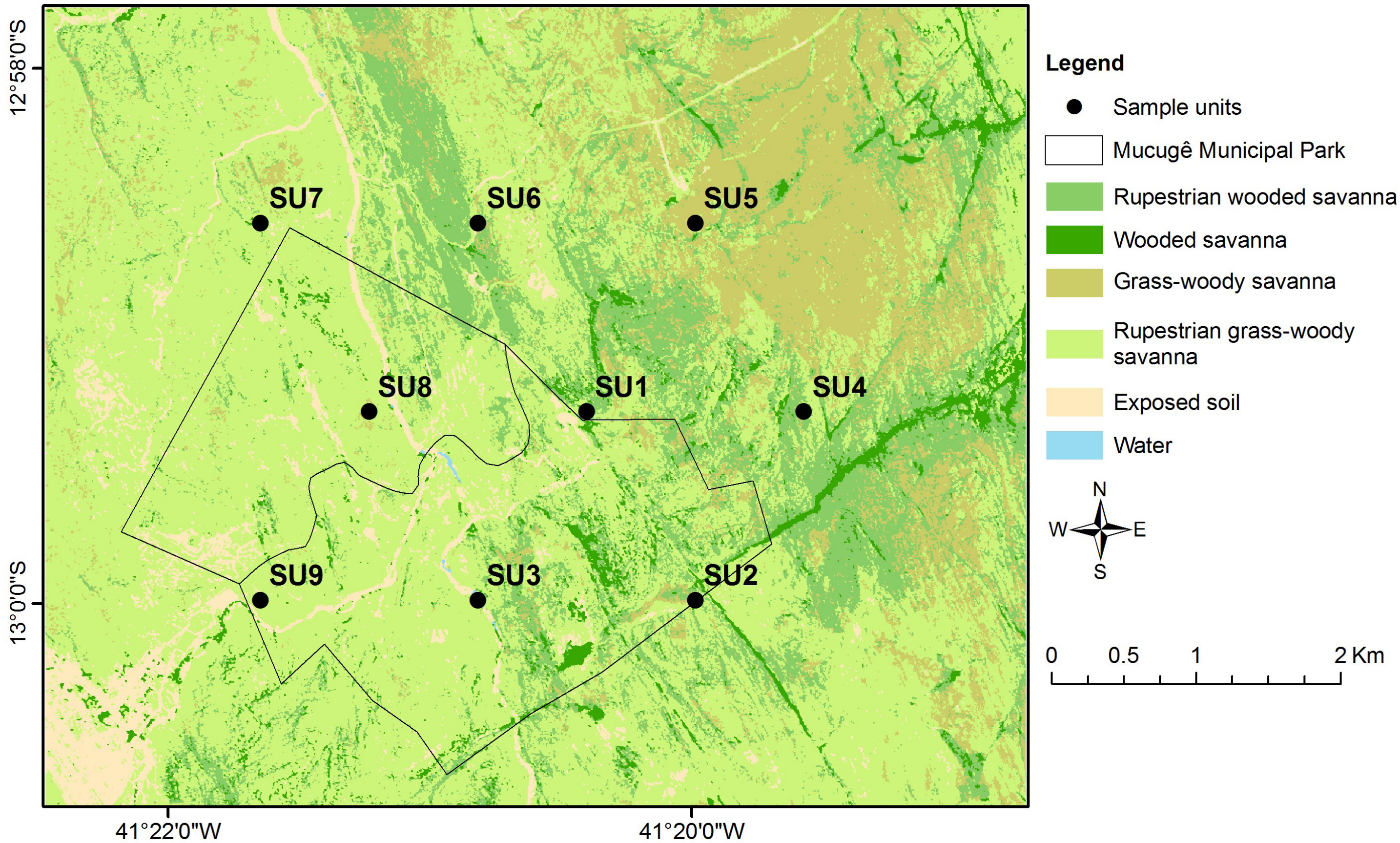

Figure 1 – Map showing the different class covers in the studded region; the black dots represent the location of the sample units, and the coordinates have the South America 1969 DATUM; the sampling units SU4, SU5 and SU6 correspond to the grass-woody savanna, SU1, SU2 and SU3 are wooded savanna units and SU7, SU8 and SU9 are rupestrian grass-woody savanna points.

chemical bait) were installed in wooden stakes 1.3 m above the ground and at least 25 m distant from each other, forming a square with 50 m side, totaling six traps. The traps were exposed for 10 hours, from 7:00 am to 17:00 h, totaling a sampling effort of 540 hours per sample unit for each of the 13 field campaigns. This campaigns occurred at least 20 days apart between January and December of 2008 and the traps positions were rotated clockwise each time. All collected specimens were identified at the species level by the first author with the aid of specialized bibliographic materials and confirmed by the experts Edinaldo Luz das Neves and André Nemésio (Nemésio 2009). We adopted the taxonomic nomenclature of the Catalogue of bees (Hymenoptera, Apoidea) in the Neotropical Region (Moure et al. 2007). All collected specimens were deposited in the Zoology Museum of UFBA - MZUFBA. More detail is available in the support information Appendix 1.

2. Measuring the surrounding environmental conditions and data analysis

In order to evaluate surrounding environmental conditions, we used a combination of remote sensing, geographic information systems (GIS) and field survey . The local vegetation structural characteristics was estimated through the 2-band enhanced vegetation index (Jiang et al. 2008), calculated based on data from KOMPSAT satellite images with four meter of spatial resolution, obtained in November 2008. This index is directly proportional to the density of photosynthetic active biomass. For each sampling unity we calculated the mean values of the pixels of the vegetation index layers within buffers with radii varying from 25 to 100 m, in increasing steps of 25 m. To calculate the proportions of each vegetation type and the landscape indices, a land cover map was created through a supervised classification of the same KOMPSAT satellite images using the maximum likelihood algorithm (Moreira et al. 2016). The map included four vegetation classes as defined by Veloso et al. (1991), with few adaptations: rupestrian wooded savanna, wooded savanna, grass-woody savanna and rupestrian grass-woody savanna, plus exposed soil and water (Figure 1). The landscape heterogeneity indices adopted were the landscape Shannon Diversity and the area-weighted shape index, which represent the compositional and configurational heterogeneity respectively (Fahrig et al. 2011, Turner & Gardner 2015). In addition, the proportion of the landscape covered by each vegetation type was also calculated. All landscape indices were based on buffers raging from 250 m to 1250 m with 250 m increases. The calculation of the EVI2 and land cover classifications were using the vegetation ArcGISTM 9.3 (ESRI® 2008) and 4.7 ENVITM© 2009 and ITT. Landscape pattern quantification was done using FRAGSTATS 3.3 (McGarigal et al. 2012). More detail is available in the support information Appendix 2.

To proceed with the analysis, it was necessary to select an adequate scale for the surrounding environmental conditions. The Euglossini bees are commonly referred as long range pollinators given their great flight capacity (Janzen 1971, Wikelski et al. 2010, Pokorny et al. 2014). However, despite their capabilities, most individuals only travel short distances (up to 600 m) when foraging and the frequency of observations is inversely related to the traveled distance (Milet-PinheiroI & Schlindwein 2005, Pokorny et al. 2014). Wikelski et al. (2010) reported mean traveling distances of 1516 m for *Exaerete frontalis* (Guérin-Méneville, 1845), which is a large bee for

Euglossini standards. The fact is that there is very little information on the actual home range of these bees. Even if the maximum home range of a species is well known the correspondence with the scale of measurement is not direct because multiple levels of influence are present (Moreira et al. 2015). Therefore, no particular scale was assumed in this work. Instead, the scale with the highest coefficient of determination (R^2) was selected from a range of scales, from 25 to 100 m for the local vegetation and from 250 to 1250 m for the landscape structure, both are compatible with what was found for other native bees (Steffan-dewenter et al. 2002, Moreira et al. 2015). These coefficients were calculated for each combination of the dependent variables (species richness, total abundance and abundance of each species) and independent (local vegetation structure, landscape diversity, configuration and proportion of each vegetation type). Finally, to assist the interpretation of the results an exploratory principal component analysis (PCA) was performed with the proportions of each vegetation cover. These statistical analyses were performed using the R 2.15.0 program, with the packages stats and vegan version 2.2-1 (R Development Core Team 2009).

To compare the alternative hypotheses for the relationship between the surrounding environmental conditions with Euglossini bees community characteristics, a model selection approach based on Akaike Information Criterion (AIC) was used. The four a priori theoretical hypotheses were confronted for each dependent variable: (1) The local vegetation complexity favors Euglossini bees species richness and/or abundance (local vegetation hypothesis); (2) The proportion of the native vegetation types favors Euglossini bees species richness and/or abundance (habitat amount hypothesis); (3) Higher landscape diversity shall increase species richness of Euglossini bees (landscape heterogeneity hypothesis); (4) More complex landscape configuration shall favor the Euglossini bees richness and/or abundance (landscape heterogeneity hypothesis). A set of mathematical models describing each of these hypotheses were included in the model selection procedure along with a null model represented by a constant. The hypothesis 1, 3 and 4 had one model with the local vegetation index, landscape Shannon Diversity and the Configuration, and only positive relationships were admitted in agreement with the theoretical expectations. Since a vegetation class could not be defined as the most likely habitat a priory, the hypothesis 2 was represented by four models with the proportion of the vegetation classes, what corresponds to eight statistical hypothesis since both positive and negative relationships were considered as equally likely a priory. Only models with one variable were included in the model selection given the restrictive number of degrees of freedom (Burnham & Anderson 2002). The models were compared using the values of the second-order Akaike information criterion (AICc), which is suitable for small samples ($n<40$) (Burnham & Anderson 2002). The delta AICc (Δi) value for each model, namely, the difference between the AICc value for that model with the lowest AICc in the set, was used to evaluate the plausibility of the candidate models. Models with values of delta AICc (Δi) ≤ 2 were considered equally plausible. We also considered the Akaike weights (Wi) of the models to evaluate the relative amount of evidence for the best model (Burnham & Anderson 2002). The statistical analyses were performed using the R 2.15.0 program, with the packages stats and 'bbmle' version 1.0.16 (R Development Core Team 2009). All data used in this analysis are available in the online supplementary information Appendix 3.

Results

The 647 Euglossini individuals collected were classified into two genera and six species (Table 1). The Euglossini community proved to be dominated by three more abundant species, *Eulaema nigrita* Lepeletier (1841), *Euglossa leucotricha* Rebêlo & Moure (1996) and *Euglossa melanotricha* Moure (1967), which occurred in all sample units and represent approximately 96.9% of the total abundance. *Euglossa fimbriata* Rebêlo & Moure (1968) accounts for 2.6% and was collected in eight of the nine sampling units. The other species, *Euglossa cordata* Linnaeus (1758) and *Euglossa securigera* Dressler (1982) were much less frequent.

In general, our results support that the amount of surrounding habitat in the landscape has a positive and strong influence on species richness and abundance (Table 2). The total abundance is best explained by a positive relationship with the proportion of grass-woody savanna in the surrounding landscape. This model is well supported and has a good fit with the data (Table 2; Figure 2 A). The model selection for species richness is inconclusive since the null model presented the lowest AICc value (Table 2; Figure 2 B).

Analyzed separately, the four most abundant species show two different trends. The first is for *Euglossa fimbriata* and *Eulaema nigrita* that were best explained by a positive relationship with the proportion of grass-woody savanna in the landscape (Table 2; Figure 2 C and D). For *Eulaema nigrita* the best model was well supported with a good fit to the data. *Euglossa fimbriata* also presented a positive tendency with the same factor. However, the model selection for this specie showed inconclusive results considering that the best model has poor support ($Wi<0.6$) and that the null model can be considered equally plausible ($\Delta i \leq 2$). Even so, there is a clear tendency of positive relation between *Euglossa fimbriata* and the grass-woody savanna proportion in the landscape (Figure 2 C). The second trend was presented by *Euglossa leucotricha* and *Euglossa melanotricha* as negative relationship with the rupestrian grass-woody savanna (Table 2; Figure 2 E and F). The best model for *Euglossa leucotricha* is well supported and presents a good fit with the data (Figure 2 E). Although the best model for *Euglossa melanotricha* do not have a high weight of evidence ($Wi<0.6$), it is substantially more supported ($\Delta i \geq 2$) than the second model in the rank (Table 2; Figure 2 F).

Discussion

All results supported the habitat amount hypothesis proposed by Fahrig (2013), which predicts that species abundance must be primarily driven by the proportion of habitat in the surrounding landscape. However, the observed species did not respond similarly among each other to the amount

Table 1: Abundance and richness distribution of Euglossini bees in all sample units.

Species	Abundance									Total	%
	SU1	SU2	SU3	SU4	SU5	SU6	SU7	SU8	SU9		
Eulaema nigrita	26	65	15	16	102	5	16	15	32	292	45.13
Euglossa cordata	0	0	0	0	1	0	0	1	0	2	0.31
Euglossa fimbriata	1	4	2	1	5	1	0	2	1	17	2.63
Euglossa leucotricha	26	23	21	24	41	28	16	11	14	204	31.51
Euglossa melanotricha	22	9	11	14	20	26	15	12	2	131	20.25
Euglossa securigera	0	0	0	1	0	0	0	0	0	1	0.15
Total	75	101	49	56	169	60	47	41	49	647	100
Richness	4	4	4	5	5	4	3	5	4	-	-

SUi – sample units.

Table 2 – Model selection results for the six dependent variables.

Variable	Model	AICc	AICcΔi	df	AICcWi
Richness	$y = \beta_0$	33.2	0	1	0.364
	$y = -\beta_1$ RGWS (750) + β_0	36.3	3.1	2	0.076
Abundance	$y = +\beta_1$ GWS (250) + β_0	90	0	2	1
	$y = -\beta_1$ RGWS (250) + β_0	121.8	31.8	2	<0.001
Euglossa leucortricha	$y = -\beta_1$ RGWS (250) + β_0	54.2	0	2	0.8564
	$y = +\beta_1$ LDI (1250) + β_0	58.7	4.5	2	0.0897
Euglossa melanotricha	$y = -\beta_1$ RGWS (250) + β_0	66.1	0	2	0.4769
	$y = +\beta_1$ LDI (250) + β_0	68.1	2	2	0.1735
Euglossa fimbriata	$y = +\beta_1$ GWS (250) + β_0	32.2	0	2	0.285
	$y = +\beta_1$ LCC (500) + β_0	32.7	0.6	2	0.214
	$y = \beta_0$	33.7	1.5	1	0.135
	$y = -\beta_1$ RGWS (250) + β_0	34.5	2.4	2	0.087
Eulaema nigrita	$y = +\beta_1$ GWS (250) + β_0	128.3	0	2	1
	$y = +\beta_1$ LCC (1000) + β_0	162.7	34.4	2	<0.001

AICc – Akaike's Information Criterion corrected for small samples; AICcΔi - AICc differences; df – degree of freedom; AICcWi – Akaike weight; GWS - grass-woody savanna; RGWS - rupestrian grass-woody savanna; LCC - landscape configuration complexity; LDI - Landscape diversity index, between parentheses are the scales of measurement.

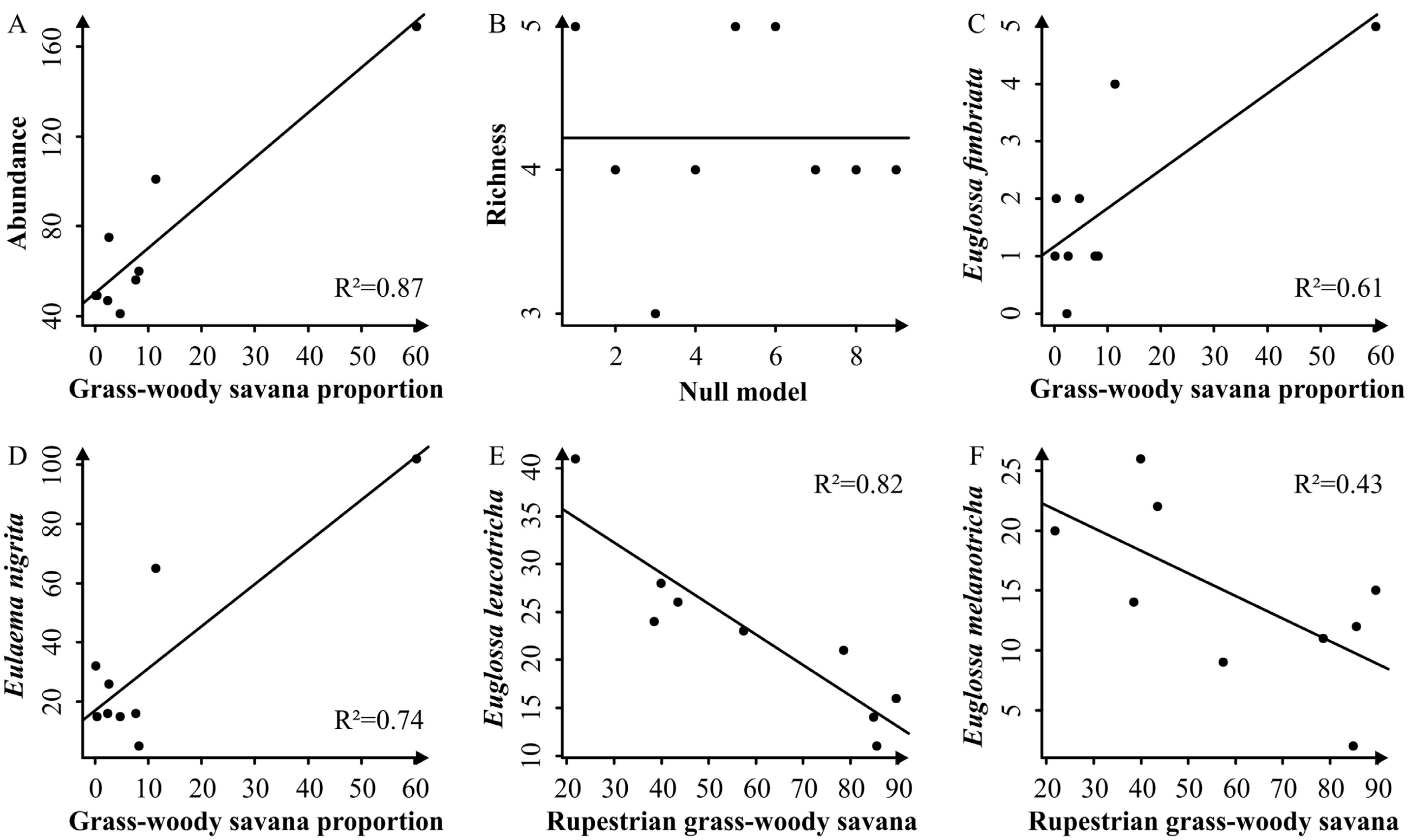

Figure 2 – Relationships between the dependent variables with the selected explanatory factors. The solid lines represent fitted models and the dots represent the observed results for each sample unity.

of habitat. A possible explanation for this result is that this specific variation in responses may be associated with the floral and nesting resources availability in the landscapes. To accept this statement, it is necessary to assume that the abundance of the male bees attracted to the chemical baits is a function of the reproductive success of their mothers and inversely related with the distance between the baits and their original nests. This is a reasonably logic assumption considering that the reproductive success is determmined by the population recruitment, once the original nests are the center of dispersion of the males and that the bait efficiency is reduced with the distance (Milet-Pinheiro & Schlindwein 2005, Aguiar et al. 2015, Rosa et al. 2015). Therefore, the amount of vegetation types with more trophic and nesting resources around the sample unit will be positively related with species abundance at the sample unit (Potts et al. 2003, 2005). There is some evidence in the literature to support such claims. For example, the females of all Euglossini species collected can build their nests on preexisting cavities in the ground, including the savanna soil (Zucchi et al. 1969, Augusto & Garófalo 2007, 2009). These bees can also occupy empty termites and ant nests (Zucchi et al. 1969). Combining the fact that such conditions are common in grass-woody savanna and that preferences for more open areas were previously reported, one can propose that this vegetation type is a potential adequate environment to the nesting behavior of the most abundant Euglossini bees in the studied region, although such proposition calls for additional empirical verification (Rebêlo & Garófalo 1997, Silveira et al. 2015, Juncá et al. 2005).

The scarcity or total absence of floral and nesting resources in the landscape can also explain the negative effect of the proportion of rupestrian grass-woody savanna. In this vegetation type, *Euglossa leucotricha* and *Euglossa melanotricha,* both with preference for more dense/vegetated or forested areas, the soft ground is practically absent and the scattered plants grow on bare rock and are rachitic because of the dry oligotrophic conditions (Juncá et al. 2005, Silveira et al. 2015). Such environment with low humidity and high temperature variations can be challenging for Euglossini bees to build their nests (Dressler 1982, Roubik 1992, Cameron 2004). This leads us to the question of why *Euglossa leucotricha* and *Euglossa melanotricha* did not respond directly to the proportion of grass-woody savanna like the other two species? The missing piece here is the wooded savanna. Like the grass-woody savanna, the wooded savanna also has appropriate conditions for Euglossini bees to build their nests (Juncá et al. 2005). In addition, both vegetation types have richer and more abundant floral resources than the rupestrian grass-woody savanna (Moreira et al. 2015, Moreira et al. 2016). Therefore, the amount of resources present on the landscape are inversely proportional to the rupestrian grass-woody savanna cover (Figure 3, Moreira et al. 2016). If the proposed effects are true, one can conclude that rupestrian grass-woody savanna is unfavorable for Euglossini bees. Furthermore, in the studied region *Euglossa leuchotricha* and *Euglossa melanotricha* can use both grass-wood savanna and wooded savanna, when *Euglossa fimbriata* and *Eulaema nigrita* may use preferentially the first one.

The differences among species in their responses to vegetation types, as discussed above, also illustrate the problem associated with confusions regarding the use of the terms 'vegetation types' and 'habitat', commonly used as synonyms (Mitchell 2005). This is especially important considering that the species studied here are also present in forested environments (Tonhasca et al. 2002a, Tonhasca et al. 2003, Milet-Pinheiro & Schlindwein 2005, Ramalho et al. 2009, Brosi et al. 2007, Aguiar et al. 2015, Rosa et al. 2015). Studies that aim to evaluate the effects of habitat loss on Euglossini bees in forested regions generally assume binary landscapes including only the categories 'habitat' (forest) and 'inhospitable matrix' (non-forest) (Tonhasca 2002a, Tonhasca et al. 2003, Milet-Pinheiro & Schlindwein 2005, Ramalho et al. 2009, Brosi et al. 2007). However many species do not necessarily perceive the environment in that way. Our results show that this approach may be inadequate for community level analyses, and is likely a reason for some of the mixed results reported in the literature. There are two alternatives to deal with this problem. The first is to analyze only the species that are closely associated with forests and therefore are dependent on these environments (Pardini et al. 2010). The second alternative is to explicitly consider the landscape's heterogeneity instead of the single habitat notion for the community level (Fahrig et al. 2013, Moreira et al. 2015). If the last one is done, we can greatly increase our understanding of the relationship between Euglossini and the spatial structure of the surrounding environments.

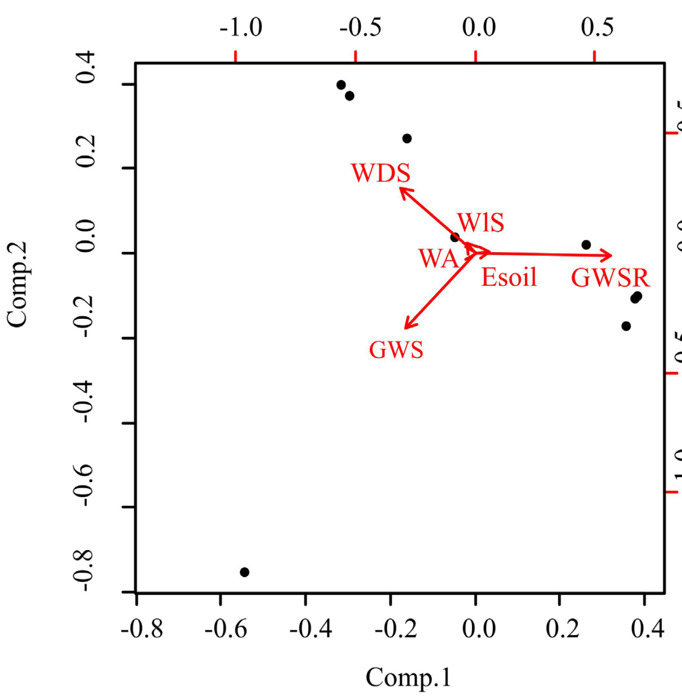

Figure 3 - Principal components of the proportion of the six cover types in the landscape; dots represent the sample units; Red vectors represent the factors directions; GWS - grass-woody savanna; RGWS - rupestrian grass-woody savanna; WA - water; WDS - wooded savanna; WIS - wooded savanna; Esoil - exposed ground.

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Supplementary material

The following online material is available for this article:

Appendix 1: Detailed description of the selection of the sampling unities

Appendix 2: Detailed local vegetation and landscape structure measurement scheme

Appendix 3: Dataset

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inventory

Fishes from the Jaru Biological Reserve, Machado River drainage, Madeira River basin, Rondônia State, northern Brazil

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Abstract: This work assessed freshwater fishes collected at 12 stations located along the Tarumã River, middle Machado River, Madeira River basin. The fieldwork took place in May and September 2015 during the high and low water seasons, respectively. We gathered 1,482 specimens representing seven orders, 30 families, 54 genera and 74 species using seine, gill, and hand nets. The family Characidae was the most representative, exhibiting the highest number of species captured. The species with the greatest abundance were *Hemigrammus vorderwinkleri, Hyphessobrycon bentosi, Hemigrammus* cf. *bellottii, Bryconella pallidifrons,* and *Apistogramma resticulosa.* Two species that remained unidentified are probably a new species. This study represents the third fish survey totally conducted at a Conservation Unit in the Rondônia State, and will certainly provide valuable information for future investigations on biodiversity conservation in the Machado River.

Keywords: *Amazon, freshwater, conservation, ichthyofauna, inventory.*

Peixes da Reserva Biológica do Jaru, drenagem do rio Machado, bacia do rio Madeira, Estado de Rondônia, norte do Brasil

Resumo: Este trabalho avaliou os peixes de água doce coletados em 12 estações localizadas ao longo do rio Tarumã, médio rio Machado, bacia do rio Madeira. O trabalho de campo ocorreu em maio e em setembro de 2015 durante os períodos de cheia e seca, respectivamente. Um total de 1.482 exemplares, sete ordens, 30 famílias, 54 gêneros e 74 espécies foram amostrados com malhadeiras, picaré e puça. Characidae foi a família com maior número de espécies capturadas. As espécies com maior abundância foram *Hemigrammus vorderwinkleri*, *Hyphessobrycon bentosi, Hemigrammus* cf. *bellottii*, *Bryconella pallidifrons* e *Apistogramma resticulosa*. Duas espécies não foram identificadas e provavelmente podem ser novas espécies. Este estudo representa a terceira pesquisa de peixes totalmente realizada em uma Unidade de Conservação no Estado Rondônia, e certamente fornecerá informações valiosas para futuros estudos sobre a conservação da biodiversidade do rio Machado.

Palavras-chave: *Amazônia, água doce, conservação, ictiofauna, inventário.*

Introduction

Among the 33,984 fish species described around the world (Eschmeyer & Fong 2016), at least 13,000 varieties are unique to freshwater environments (Nelson et al. 2016). The Neotropical realm (South and Central America) encompasses more than 4,000 valid fish species (Reis 2013), with the Amazon River basin harboring the world's greatest diversity of freshwater fish (Freitas et al. 2010, Winemiller et al. 2016, Reis et al. 2016). To date, most studies of the Amazonian ichthyofaunal diversity have concentrated in the floodplains adjacent to large rivers (cf. Lowe-McConnell 1999) and next to urban areas (Mendonça et al. 2005, Souza et al. 2016). Notwithstanding, there are few reports on the ichthyofauna inhabiting environmental areas of high conservation value (Camargo & Giarrizzo 2007, Oliveira et al. 2009, Pedroza et al. 2012, Vieira et al. 2016).

Despite concerns about the effectiveness of conservation units in the tropics, there is growing evidence that they have been surprisingly useful tools for curbing deforestation (*e.g.* Jaru Biological Reserve) (Bruner et al. 2001) or conservation of fish species (Frederico et al. 2016). Historically, the majority of the current protected areas in Brazil were created with disregard for the aquatic environment (Agostinho et al. 2005, Abell et al. 2007). Nevertheless, it is uncontestable that they can protect many water bodies and, thus, play a highly important role in the conservation of freshwater organisms (Agostinho et al. 2005).

This study presents a survey of the ichthyofauna of the Tarumã River, a right-bank tributary of the Machado River, located within the Jaru Biological Reserve. This area displays virtually unchanged habitats in respect to its external environment. As the Tarumã River flows within a legally protected area, the objective of this study is to provide a first ichthyofaunal inventory, which may eventually support future studies on fish biology and conservation.

Material and Methods

1. Study area

The Jaru Biological Reserve (Rebio Jaru) was established on July 11, 1979, under Federal Decree-law number 83,716, and is managed by the Instituto Chico Mendes de Conservação da Biodiversidade/Ministério do Meio Ambiente (ICMBio/MMA). Marked by the high degree of conservation, the rain forest of the Rebio Jaru is practically intact. The reserve has a humid tropical climate with temperatures varying between 23°C and 26°C, and the average annual rainfall ranges from 1,700 to 2,400 mm. The dry season occurs between May and October (Justina 2009).

The Rebio Jaru hydrographic network is part of the Machado River Basin located in eastern Rondônia State, northern Brazil. The Tarumã River, the main sub-basin of the Rebio Jaru, runs almost entirely (99%) within the Rebio Jaru. The average depth of the Tarumã River during the dry season was 2.8 ± 0.9 m; the average width, 32.8 ± 7.8 m; and the average water speed, 0.4 ms^{-1}. On the other hand, the average depth, width, and water flow values are 5.6 ± 1.2 m; 41.9 ± 4.0 m; and 0.3 ± 0.1 ms^{-1} during the wet season, respectively. The Tarumã River has many rapids flowing across the granitic formation of the Serra da Providência and Jamari complex (Justina 2009). The high transparency of the water (average transparency $_{Dry}$ = 1.2 ± 2.1 m; and average transparency $_{Wet}$ = 1.1 ± 0.4 m) due to the low amount of sediment, characterizes the Tarumã as a clear water river.

2. Data collection

We performed collections of freshwater fish in the Tarumã River in May and September 2015. Each expedition lasted four days. Our samplings comprised 12 sites in two different aquatic environments: five in the main channel and seven in the small stream channels (igarapés) of the Tarumã River (Table 1, Figure 1).

The physical conditions of some sampling stations in the Tarumã River basin are shown in Figure 2.

Stream 4 (S4) – igarapé, 1.81 m wide and 0.26 m deep, preserved riparian vegetation, swift current, sand, pebbles, and dead leaves at the bottom (Figure 2a).

Stream 6 (S6) – igarapé, 1.39 m wide and 0.21m deep, preserved riparian vegetation, slow current, sand, pebbles, large branches and trunks, and dead leaves at the bottom (Figure 2b).

River 1 (R1) - stretch, 112 m wide and 6.2 m deep located near the mouth of the Tarumã River, preserved riparian vegetation and slow current (Figure 2c).

Stream 5 (S5) – igarapé, 0.70 m wide and 0.25 m deep, preserved riparian vegetation, slow current, sand, pebbles, large branches and trunks at the bottom (Figure 2d).

Stream 3 (S3) – igarapé, 1.00 m wide and 0.21 m deep, preserved riparian vegetation, swift current, sand, bare ravine, large branches and trunks at the bottom (Figure 2e).

River 4 (R4) - stretch, 30 m wide and 4.5 m deep located near the head of the Tarumã River, preserved riparian vegetation, slow current (Figure 2f).

We accomplished the collections in the river channels using a total of eight gill nets with standard size meshes of 2 x 20 m, and fishing nets with mesh sizes varying from 30 to 100 mm (between opposite knots). The fishing nets were set at each sampling site during the morning, from 8:00 am to 12:00 pm, and at night, from 8:00 pm to 5:00 am. For the same period, we used a trotline with four 5/0 hooks with ends tied either to the bank vegetation or to mooring spikes. We used some pieces of piranha, *Serrasalmus rhombeus* Linnaeus 1766, as baits attached to the trotline hooks.

In small streams, the fish collection in a stretch of 80 m lasted one hour during the daytime. Three collectors used hand gathering techniques with a seine net (1.5 × 2 m, 2 mm mesh) and a hand net (0.5 × 0.8 m, 2 mm mesh) along the entire stretch, selecting the best technique for each environment. Before the collections, the ends of the sampling sections were blocked with fine-mesh nets (5 mm between opposite knots) to prevent fish escapes, regardless of the capture method used. Abiotic data, such as depth, width, and soil were observed *in situ*. We sacrificed the specimens in a solution of clove oil (Eugenol, 2 drops per liter; cf. American Veterinary Medical Association 2001). After that, the fish were fixed in 10% formalin solution and then preserved in 70% ethanol. For species identifications, we consulted the most currently accepted taxonomic literature and identification keys (Queiroz et al. 2013b). The classification followed Nelson et al. (2016). The specimens were deposited in the Coleção de Peixes, Universidade Federal do Mato Grosso, Cuiabá, MT, Brazil (CPUFMT); Laboratório de Ictiologia de Ribeirão Preto da Universidade de São Paulo, Ribeirão Preto, SP, Brazil (LIRP) and Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brazil (MZUSP) (Appendix 1). The fish sampling was authorized by the *Instituto Chico Mendes de Conservação da Biodiversidade* (ICMBio, License: 48723–2/2015).

Results

Our sampling comprised 1,482 specimens representing seven orders, 33 families, 54 genera, and 74 species. A total of 1,263 specimens representing six orders, 20 families, and 50 species were collected in the streams and 219 specimens; five orders, 13 families, and 24 species were collected in the river (Table 2). Characiformes, Siluriformes, and Cichliformes represented 59% (47 species), 21% (17 species), and 10%

Table 1. Sampling sites in the Tarumã River basin with environmental classification, geographic coordinates, and altitude.

Site	Environments	Geographic coordinates	Altitude (m)
R1	River	9°27’19” S, 61°40’43” W	75
R2	River	9°32’15” S, 61°40’13” W	90
R3	River	9°42’46” S, 61°39’42” W	111
R4	River	9°46’23” S, 61°38’45” W	119
R5	River	9°47’04” S, 61°40’19” W	141
S1	Stream	9°27’28” S, 61°40’34” W	80
S2	Stream	9°27’51” S, 61°40’ 8” W	82
S3	Stream	9°31’44” S, 61°40’13” W	90
S4	Stream	9°32’31” S, 61°38’54” W	181
S5	Stream	9°41’47” S, 61°39’47” W	97
S6	Stream	9°44’05” S, 61°39’31” W	126
S7	Stream	9°46’58” S, 61°40’11” W	130

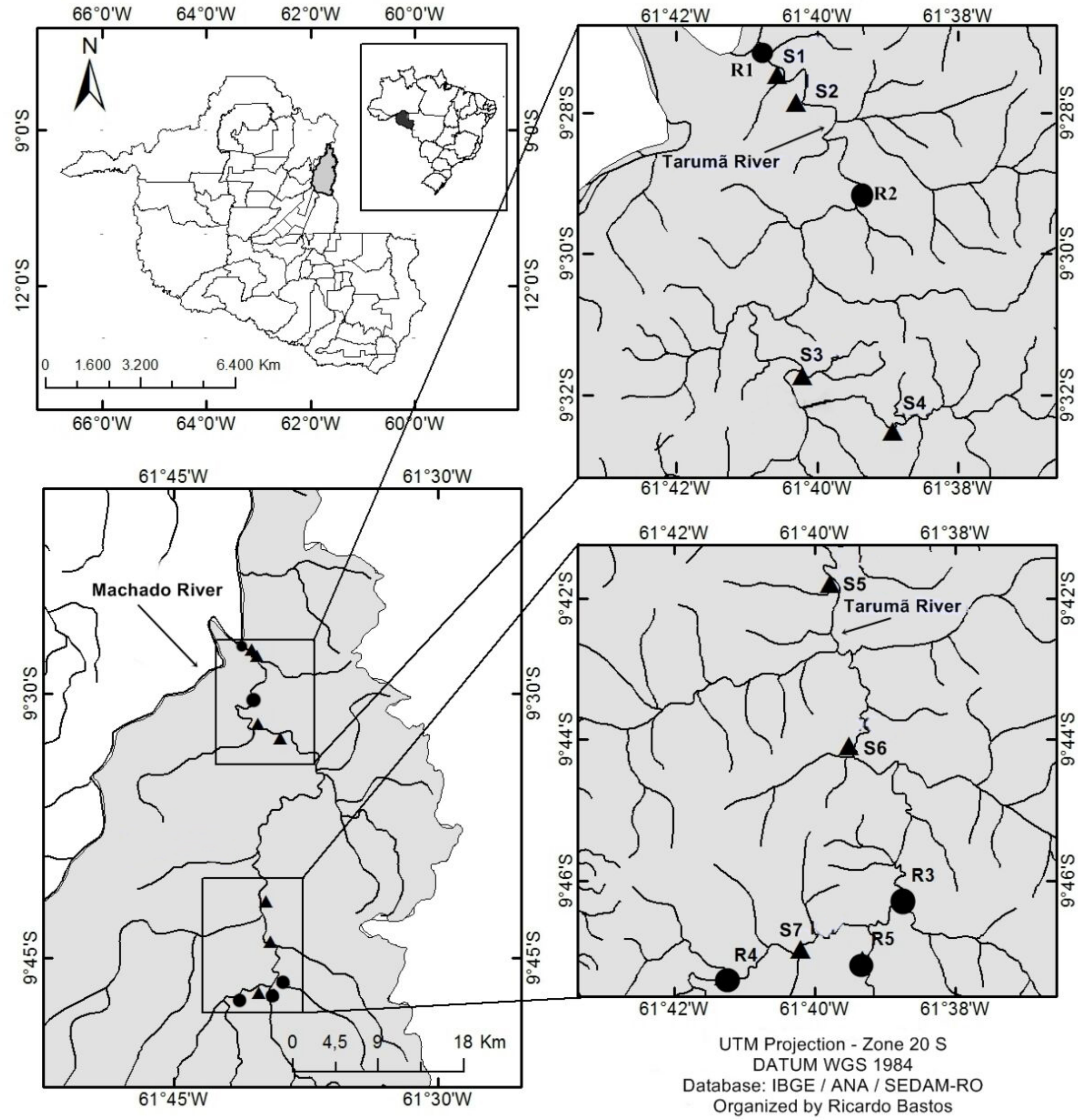

Figure 1. Map of the study area showing the collection stations in the drainage systems in the Jaru Biological Reserve (shaded area), Rondônia, Brazil. Triangles represent streams and circles represent collection points in the river channels of the Tarumã River.

(eight species) of all species, respectively. Nevertheless, Cichliformes were the second most dominant regarding the abundance of capture (6%, n = 101). The Myliobatiformes, Beloniformes, and Synbranchiformes orders showed richness and abundance lower than 4% (n = 6) and 2% (n = 6), respectively (Figure 3). In the streams the Characiformes, Siluriformes, and Cichliformes represented 52% (26 species), 28% (14 species), and 10% (five species) of all species, respectively. Nevertheless, Cichliformes were the second most dominant regarding the abundance of capture (7%, n = 97). In the river the results were similar to streams, where the Characiformes, Siluriformes, and Cichliformes represented 75% (18 species), 8% (two species), and 8% (two species) of all species, respectively. The other orders showed richness and abundance lower than 6% (Table 2). The families with the highest richness and abundance were Characidae (25 species, 31%; n = 874, 58%), Serrasalmidae (six species, 8%; n = 129, 9%), and Cichlidae (seven species, 9%; n = 100, 7%) (Figure 4). In the stream environment the families with the highest richness and abundance were Characidae (18 species, 36%; n = 1085, 78%), Cichlidae (five species, 10%; n = 97, 7%) and Crenuchidae (four species, 8%; n = 92, 6%), for the river environment the families Characidae (20 species, 5%; n = 208, 50%), Serrasalmidae (21 species, 5%; n = 127, 31%), and Cynodontidae (two species, 8%; n = 23, 5%) were the most representative in richness and abundance. The other families showed richness and abundance lower than 8% (Table 2). *Hemigrammus vorderwinkleri* Géry 1963 (n = 202), *Hyphessobrycon bentosi* Durbin 1908 (n = 196), *Hemigrammus* cf. *bellottii*

Figure 2. Habitats of some collection stations in the drainage systems in the Jaru Biological Reserve, Tarumã River Basin, Rondônia, Brazil. (a) stream 4; (b) stream 6; (c) stretch 1 of the river channels; (d) stream 5; (e) stream 3; (f) stretch 4 of the river channels.

Steindachner 1882 (n = 185), *Bryconella pallidifrons* Fowler 1946 (n = 147) (n = 100), and *Apistogramma resticulosa* Kullander 1980 (n = 86) were the most abundant species in the total of specimens collected and these species also were predominant in the stream environment. The species *Serrasalmus rhombeus* (n = 45), *Serrasalmus compressus* (n = 30) and *Myloplus lobatus* (n = 24) were the most abundant in the river environment.

Discussion

Most of the several studies conducted in different portions of the Madeira River Basin in the Brazilian territory have focused either on a relatively small area or on specific tributaries. These investigations identified 122 species from the Jamari River (Santos 1996), 133 from the Marmelos River (Camargo & Giarrizzo 2007), 447 from the Aripuanã and middle Madeira rivers (Py-Daniel et al. 2007), 74 from the Belmont Stream (Araújo et al. 2009), 160 from the Guariba and Roosevelt Rivers (Pedroza et al. 2012), 189 from the Cuniã Lake (Queiroz et al. 2013a), and 174 from the middle Madeira River (Torrente-Vilara et al. 2011). A more comprehensive inventory identified 820 species along the Madeira River Basin (Queiroz et al. 2013b). More specifically, in the Machado River Basin, 48 species were recorded near the urban area of the Cacoal town (Perin et al. 2007), and 140 species in different tributaries (Casatti et al. 2013). However, contrary to the previously mentioned works, only the present study and the assays of Queiroz et al. (2013a) and Vieira et al. (2016) were conducted entirely in a Conservation Unit in the Rondônia State, resulting in the identification of 74, 189, and 141 species, respectively. The current work contributed 24 species to the general inventory of the Machado River and 26 species to the inventory conducted in Conservation Units in the Rondônia State. Additionally, the material deposited in the Brazilian collections enabled the accomplishment of recent taxonomic studies, which contributed to enhancing the knowledge of the ichthyofauna of both the Madeira River (Rocha et al. 2008a, Rocha et al. 2008b, Zanata & Ohara 2009, Ribeiro et al. 2011, Ohara 2012, Marinho & Ohara 2013, Zanata & Ohara 2015, Ohara & Lima 2015a, Tencatt & Ohara 2016a, Ohara & Neuhaus 2016, Tencatt & Ohara 2016b, Ohara et al. 2016a, Pastana & Ohara 2016), and the Machado River (Ohara & Lima 2015b, Ohara & Marinho 2016, Ohara et al. 2016b).

Among the sampled species from the Tarumã River, almost 44% (35 species) appear on the list of ornamental fish (Brasil 2012). Some of those species, such as *Apistogramma resticulosa*, *Hemigrammus ocellifer*, *Hemmigramus vorderwinkleri*, *Hemmigramus* cf. *bellottii*, *Hyphessobrycon bentosi*, *H. copelandi*, *Elachocharax pulcher*, and *Serrasalmus rhombeus* (Table 2) were well represented in our survey, suggesting a relatively high local abundance. However, according to the Environmental Crimes Law number 9605, of February 12, 1998, the capture of ornamental fish in the Rebio Jaru is prohibited (ICMBio 2010).

Table 2. Fish captured in the Tarumã River, Rondônia, in May and September 2015. Method of collection: T – Trotline, G – Gillnets and S/H – Seine nets (*picarés*) and hand net (puçá). * Potential for the ornamental fish trade (Brasil 2012), [1] species additional to species list to studied watershed. Systematic positions were based on Nelson et al. (2016).

Class/Order/Family/Species	Streams	River	Method
CLASS CHONDRICHTHYES			
MYLIOBATIFORMES			
Potamotrygonidae			
Potamotrygon falkneri Castex & Maciel 1963[1]	-	1	T
CLASS OSTEICHTHYES			
CHARACIFORMES			
Acestrorhynchidae			
Acestrorhynchus falcatus Bloch 1794*	2	-	S/H
Acestrorhynchus falcirostris Cuvier 1819*	-	2	G
Alestidae			
Chalceus guaporensis Zanata & Toledo-Piza 2004	-	1	G
Characidae			
Axelrodia stigmatias Fowler 1913	7	-	S/H
Bario steindachneri Eigenmann 1893	1	-	S/H
Bryconella pallidifrons Fowler 1946	147	-	S/H
Brycon amazonicus Spix & Agassiz 1829	-	2	G/T
Brycon cf. *pesu* Müller & Troschel 1845[1]	-	3	G
Brycon falcatus Müller & Troschel 1844[1]	-	2	G
Creagrutus anary Fowler 1913[1]	6	-	S/H
Hemigrammus analis Durbin 1909	25	-	S/H
Hemigrammus cf. *bellottii* Steindachner 1882*	185	-	S/H
Hemigrammus ocellifer Steindachner 1882*	45	-	S/H
Hemigrammus melanochrous Fowler 1913	51	-	S/H
Hemigrammus vorderwinkleri Géry 1963*	202	-	S/H
Hyphessobrycon agulha Fowler 1913*	26	-	S/H
Hyphessobrycon copelandi Durbin 1908*	42	-	S/H
Hyphessobrycon bentosi Durbin 1908*	196	-	S/H
Hyphessobrycon sweglesi Géry 1961	2	-	S/H
Jupiaba zonata Eigenmann 1908*[1]	2	-	S/H
Moenkhausia collettii Steindachner 1882*	5	-	S/H
Moenkhausia oligolepis Günther 1864*	10	-	S/H
Moenkhausia mikia Marinho & Langeani 2010	1	-	S/H
Phenacogaster cf. *retropinnus* Lucena & Malabarba 2010[1]	3	-	S/H
Roeboides affinis Günther 1868[1]	-	4	G
Triportheus albus Cope 1872*	-	4	G
Gasteropelecidae			
Carnegiella strigata Günther 1864*	25	-	S/H
Crenuchidae			
Characidium cf. *pellucidum* Eigenmann 1909	20	-	S/H
Characidium aff. *zebra* Eigenmann 1909[1]	2	-	S/H
Elachocharax pulcher Myers 1927*	68	-	S/H
Microcharacidium sp. [1]	2	-	S/H
Erythrinidae			
Hoplias malabaricus Bloch 1794*	9	-	S/H
Lebiasinidae			
Pyrrhulina cf. *brevis* Steindachner, 1876	3	-	S/H
Ctenolucidae			
Boulengerella cuvieri Spix & Agassiz 1829	-	16	G
Hemiodontidae			
Hemiodus unimaculatus Bloch 1794 *	-	1	G
Anostomidae			
Leporinus friderici Bloch 1794	-	5	G
Cynodontidae			
Hydrolycus armatus Jardine 1841[1]	-	17	G/T
Hydrolycus tatauaia Toledo-Piza, Menezes & Santos, 1999*[1]	-	6	G
Serrasalmidae			

Table 2. Continued...

Class/Order/Family/Species	Streams	River	Method
Myloplus lobatus Valenciennes 1850[1]	-	24	G
Myloplus rubripinnis Müller & Troschel 1844*	-	24	G
Pygocentrus nattereri Kner 1858*	-	4	G
Serrasalmus compressus Jégu, Leão & Santos, 1991[1]	-	30	G
Serrasalmus rhombeus Linnaeus 1766*	-	45	G
Prochilodontidae			
Prochilodus nigricans Agassiz 1829	-	21	G
SILURIFORMES			
Aspredinidae			
Bunocephalus coracoideus Cope 1874*[1]	9	-	S/H
Trichomycteridae			
Ituglanis amazonicus Steindachner 1882	1	-	S/H
Callichthyidae			
Corydoras cf. *trilineatus* Cope 1872[1]	1	-	S/H
Corydoras cf. *armatus* Günther 1868[1]	5	-	S/H
Cetopsidae			
Helogenes marmoratus Günther 1863*	5	-	S/H
Heptapteridae			
Mastiglanis asopos Bockmann 1994	1	-	S/H
Nemuroglanis furcatus Ribeiro, Pedroza & Rapp Py-Daniel, 2011[1]	16	-	S/H
Pimelodella howesi Fowler 1940	4	-	S/H
Pimelodella cf. *steindachneri* Eigenmann 1917[1]	1	-	S/H
Pseudopimelodidae			
Microglanis poecilus Eigenmann 1912*[1]	1	-	S/H
Doradidae			
Acanthodoras spinosissimus Eigenmann & Eigenmann 1888*[1]	1	-	S/H
Physopyxis lyra Cope 1871	1	-	S/H
Loricariidae			
Ancistrus sp.	1	-	S/H
Rineloricaria lanceolata Günther 1868*	2	-	S/H
Pimelodidae			
Pimelodus ornatus Kner 1858*[1]	-	2	T
Platynematichthys notatus Jardine 1841[1]	-	1	T
GYMNOTIFORMES			
Hypopomidae			
Hypopygus lepturus Hoedeman 1962*	15	-	S/H
Rhamphichthyidae			
Gymnorhamphichthys rondoni Miranda-Ribeiro 1920*	2	-	S/H
Gymnotidae			
Gymnotus coropinae Hoedeman 1962	8	-	S/H
BELONIFORMES			
Belonidae			
Potamorrhaphis guianensis Jardine 1843*	3	-	S/H
SYNBRANCHIFORMES			
Synbranchidae			
Synbranchus madeirae Rosen & Rumney 1972	2	-	S/H
CICHLIFORMES			
Cichlidae			
Aequidens cf. *tetramerus* Heckel 1840*	4	-	S/H
Apistogramma resticulosa Kullander 1980*	86	-	S/H
Crenicara punctulatum Günther 1863*	3	-	S/H
Crenicichla regani Ploeg 1989*	2	-	S/H
Crenicichla santosi Ploeg 1991[1]	2	-	S/H
Cichla pleiozona Kullander & Ferreira 2006	-	2	G
Satanoperca jurupari Heckel 1840*	-	1	G
ACANTHURIFORMES			
Sciaenidae			
Petilipinnis grunniens Jardine & Schomburgk 1843[1]	-	1	G

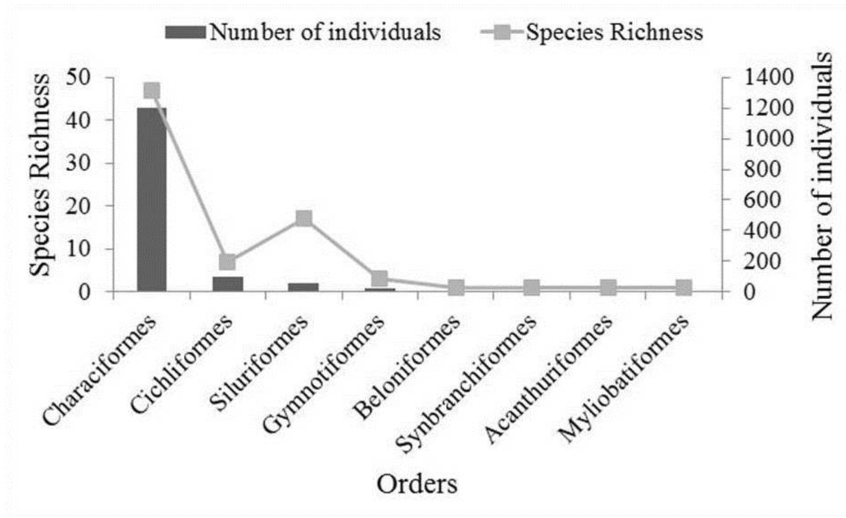

Figure 3. Number of individuals and richness of species of the fish orders collected in the Tarumã River Basin, Rondônia, Brazil.

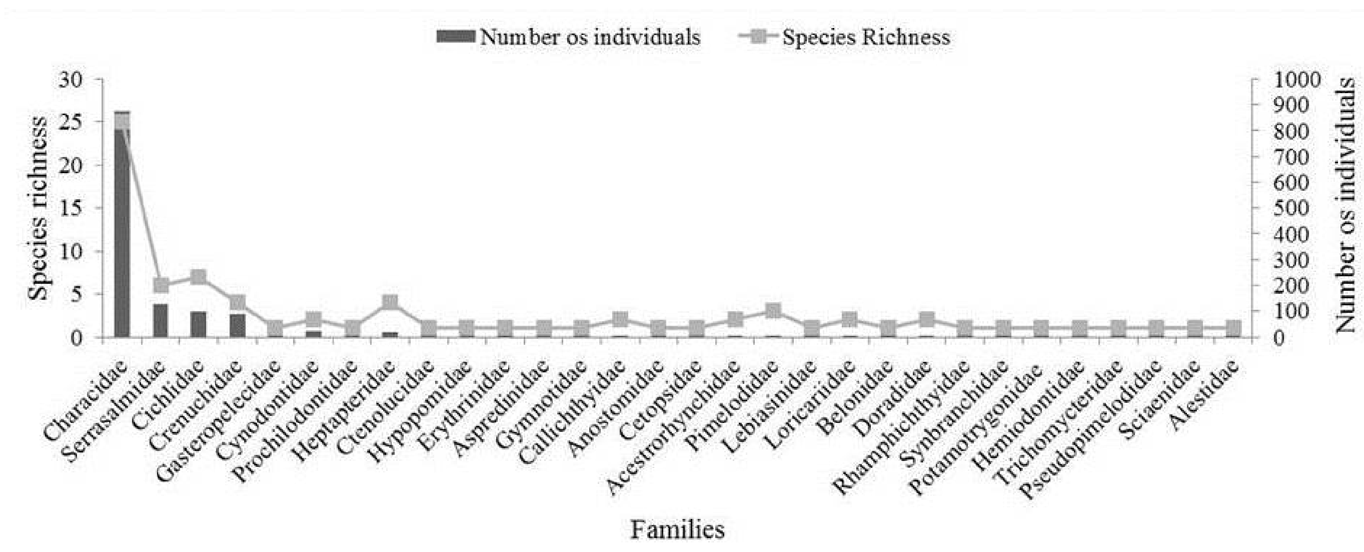

Figure 4. Number of individuals and richness of species of the fish families collected in the Tarumã River Basin, Rondônia, Brazil.

The presence of migratory species in the Tarumã River indicates that this area can be used for reproduction and/or feeding. (*Brycon amazonicus* Spix & Agassiz 1829, *B. falcatus* Müller & Troschel 1844, *Triportheus albus* Cope 1872, *Prochilodus nigricans* Agassiz 1829, *Myloplus lobatus* Valenciennes 1850, *Myloplus rubripinnis* Müller & Troschel 1844, and *Platynematichthys notatus* Jardine 1841).

Some rare species, considered as such due to their absence in most major ichthyological collections, were recorded in the Tarumã River. These included the Characiformes *Axelrodia stigmatias* Fowler 1913, *Bario steindachneri* Eigenmann 1893, *Chalceus guaporensis* Zanata & Toledo-Piza 2004, *Creagrutus anary* Fowler 1913, and *Hemigrammus melanochrous* Fowler 1913, the Siluriformes, *Nemuroglanis furcatus* Ribeiro, Pedroza & Rapp Py-Daniel, 2011, and the Cichliformes, *Crenicichla santosi* Ploeg 1991. Two taxa were provisionally identified, due to their uncertain taxonomic status. They may be records of new species, such as *Microcharacidium* sp. (Crenuchidae) and *Ancistrus* sp. (Loricariidae). We emphasize that none of the sampled species are on the IUCN Red List.

Several species were discriminated with the use of "cf" or "aff", indicating that the number of new species may be higher. For example, the species identified herein as *Brycon* cf. *pesu* Müller & Troschel 1845 belongs to a complex of species, where new species are undergoing a description process (Zanata & Lima pers. comm.). Several species, namely, *Hemigrammus* cf. *bellottii*, *Phenacogaster* cf. *retropinnus* Lucena & Malabarba 2010, *Characidium* cf. *pellucidum* Eigenmann 1909, *Characidium* aff. *zebra* Eigenmann 1909, *Corydoras* cf. *armatus* Günther 1868, and *Corydoras* cf. *trilineatus* Cope 1872, belong either to poorly known taxonomic groups or represent still undescribed species, meaning that further taxonomic studies will be necessary.

Our work highlights the importance of conducting studies within protected areas and strengthens the role of territorial spaces with relevant environmental characteristics in a context where only three inventories within 14 Conservation Units in Rondônia have taken place. The presence of protected areas may help mitigate environmental impacts and maintain the biological integrity of a region surrounded by a long history of anthropogenic disturbances (deforestation, gold mining, the construction of the BR 364 road and, more recently, the building of large hydroelectric dams).

Appendix 1

Voucher specimens.

MYLIOBATIFORMES: *Potamotrygon falkneri**; **CHARACIFORMES:** *Acestrorhynchus falcatus* (MZUSP 118769, MZUSP 118812), *Acestrorhynchus falcirostris* (UFRO-I 5523, UFRO-I 18313), *Axelrodia stigmatias* (MZUSP 118772, MZUSP 118786), *Bario steindachneri* (MZUSP 118734), *Bryconella pallidifrons* (MZUSP 118744, MZUSP 118791, MZUSP 118724, MZUSP 118759), *Brycon amazonicus* (MZUSP 14017), *Brycon* cf. *pesu* (LIRP 11773, UFRO-I 14213, UFRO-I 15506), *Brycon falcatus* (LIRP 13045, 10269), *Chalceus guaporensis* (UFRO-I 4321, UFRO-I 17473), *Creagrutus anary* (MZUSP 118745, MZUSP 118779), *Hemigrammus ocellifer* (MZUSP 118738, MZUSP 118767, MZUSP 118798, MZUSP 118725), *Hemigrammus* cf. *bellottii* (MZUSP 118765, MZUSP 118741, MZUSP 118790, MZUSP 118733), *Hemigrammus melanochrous* (MZUSP 118743, MZUSP 118802, MZUSP 118726), *Hemigrammus analis* (MZUSP 118758, MZUSP 118788), *Hemigrammus vorderwinkleri* (MZUSP 118766, MZUSP 118794), *Hyphessobrycon agulha* (MZUSP 118785, MZUSP 118742, MZUSP 118797), *Hyphessobrycon copelandi* (MZUSP 118763), *Hyphessobrycon bentosi* (MZUSP 118764, MZUSP 118792), *Hyphessobrycon sweglesi* (MZUSP 118732), *Jupiaba zonata* (MZUSP 118762), *Moenkhausia oligolepis* (MZUSP 118753), *Moenkhausia mikia* (MZUSP 118755), *Moenkhausia collettii* (MZUSP 118770), *Phenacogaster* cf. *retropinnus* (MZUSP 118754, MZUSP 118789), *Roeboides affinis* (CPUFMT 3393), *Triportheus albus* (CPUFMT 3398), *Carnegiella strigata* (MZUSP 118760), *Characidium* cf. *pellucidum* (MZUSP 118757, MZUSP 118801, MZUSP 118727), *Characidium* aff. *zebra* (MZUSP 118774, MZUSP 118793), *Elachocharax pulcher* (MZUSP 118756, MZUSP 118796, MZUSP 118719), *Microcharacidium* sp. 2 (MZUSP 118781, MZUSP 118778), *Hoplias malabaricus* (MZUSP 118751, MZUSP 118768, MZUSP 118776, MZUSP 118813), *Pyrrhulina* cf. *brevis* (MZUSP 118740, MZUSP 118730), *Boulengerella cuvieri* (CPUFMT 3392), *Hemiodus unimaculatus* (UFRO-I 12750, UFRO-I 14109), *Leporinus friderici* (CPUFMT 3400), *Hydrolycus armatus* (CPUFMT 3390), *Hydrolycus tatauaia* (LIRP 10293, 10298), *Myloplus lobatus* (CPUFMT 3394), *Myloplus rubripinnis* (CPUFMT 3397), *Pygocentrus nattereri* (CPUFMT 3401), *Serrasalmus compressus* (CPUFMT 3399), *Serrasalmus rhombeus* (CPUFMT 3391), *Prochilodus nigricans* (CPUFMT 3396); **SILURIFORMES:** *Bunocephalus coracoideus* (MZUSP 118784, MZUSP 118747, MZUSP 118787, MZUSP 118714), *Ituglanis amazonicus* (MZUSP 118808), *Corydoras* cf. *trilineatus* (MZUSP 118748), *Corydoras* cf. *armatus* (MZUSP 118752), *Helogenes marmoratus* (MZUSP 118800, MZUSP 118715), *Mastiglanis asopos* (MZUSP 118737), *Nemuroglanis furcatus* (MZUSP 118739, MZUSP 118721, MZUSP 118805), *Pimelodella howesi* (MZUSP 118782, MZUSP 118736), *Pimelodella* cf. *steindachneri* (MZUSP 118729), *Microglanis poecilus* (MZUSP 118717), *Acanthodoras spinosissimus* (MZUSP 118810), *Physopyxis lyra* (MZUSP 118771), *Ancistrus* sp. 1 (MZUSP 118728), *Rineloricaria lanceolata* (MZUSP 118735, MZUSP 118773), *Pimelodus ornatus* (LIRP 11969, 12177), *Platynematichthys notatus* (UFRO-I 3835); **GYMNOTIFORMES:** *Hypopygus lepturus* (MZUSP 118803, MZUSP 118723), *Gymnorhamphichthys rondoni* (MZUSP 118775, MZUSP 118799), *Gymnotus coropinae* (MZUSP 118783, MZUSP 118809, MZUSP 118722); **BELONIFORMES:** *Potamorrhaphis guianensis* (MZUSP 118811); **SYNBRANCHIFORMES**: *Synbranchus madeirae* (MZUSP 118795, MZUSP 118720); **CICHLIFORMES**: *Aequidens* cf. *tetramerus* (MZUSP 118780, MZUSP 118777, MZUSP 118718, MZUSP 118814), *Apistogramma resticulosa* (MZUSP 118761, MZUSP 118806, MZUSP 118731), *Crenicara punctulatum* (MZUSP 118746, MZUSP 118804), *Crenicichla regani* (MZUSP 118749), *Crenicichla santosi* (MZUSP 118750, MZUSP 118807), *Cichla pleiozona* (CPUFMT 3395), *Satanoperca jurupari* (UFRO-I 16652, UFRO-I 17429); **ACANTHURIFORMES**: *Petilipinnis grunniens* (UFRO-I 4883, LIRP 10405).* In process of deposit in Museu de Zoologia da Universidade de São Paulo, São Paulo, SP, Brazil (MZUSP).

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Author Contributions

Each author’s contributions were as follows: Igor Costa; contribution to data acquisition, analysis and interpretation of data, drafting of the manuscript and critical revision as for important intellectual content. Willian Ohara: contribution to the analysis and interpretation of data, drafting of the manuscript, and critical revision for adding substantive intellectual content. Missilene Almeida: contribution to data acquisition and drafting of the manuscript.

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Record of morphological deformities in *Corydoras* aff. *longipinnis* in two reservoirs under urban influence in Upper Iguaçu and Southern Coastal basins in Paraná State, Brazil

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Abstract: In this work we report on the presence of morphological deformities in *Corydoras* aff. *longipinnis* from two reservoirs in Paraná State, Brazil. The frequency of deformities in the fish populations was 11% in Iraí and 10.5% in Capivari reservoirs. Considering that the frequency may be associated with impacts caused by anthropic activities, this information can be used as a metric in management systems and environmental monitoring.

Keywords: *Anthropization, Corydoradinae, Malformations, Neotropical region, Urban reservoirs.*

Registro de anomalias morfológicas em *Corydoras* aff. *longipinnis* em dois reservatórios sob influência urbana no Paraná, Brasil

Resumo: Neste trabalho relatamos a presença de deformidades morfológicas em *Corydoras* aff. *longipinnis* de dois reservatórios no estado do Paraná, Brasil. A frequência das deformidades observadas nas populações analisadas foi de 11% no reservatório de Iraí e 10,5% no reservatório de Capivari. Considerando que a frequência pode ser associada a impactos causados por atividades antrópicas, essa informação pode ser usada como uma métrica em sistemas de gestão e monitoramento ambiental.

Palavras-chave: *Antropização, Corydoradinae, Malformações, região Neotropical, Reservatórios Urbanos.*

Introduction

Deformities in aquatic organisms are widely used as an environmental indicator, and their presence are related to several anthropic impacts (Sanders et al. 1999, Sun et al. 2009, Flores-Lopes & Reuss-Strenzel 2011). These deformities can originate from a direct interference in the organisms development caused by chemical compounds (such as heavy metals), or can be result of environmental changes due to anthropic activities that decreases population size favoring endogamy (Reash & Berra 1989; Sánchez et al. 2011, Messaoudi et al. 2009). In addition, environmental changes can make populations susceptible to parasites, cause parasite populations to increase, or cause intermediate host populations to increase. The resulting increase in parasitic infestation can cause diseases which in turn result in morphological deformities (Cunningham et al. 2005, Johnson et al. 2007).

Morphological deformities registered in Neotropical fish comprise dysplasia (atrophy, hypertrophy, torsion, deformation) on opercular bones, maxilla-mandibular apparatus, branchiostegal bones, fins and eyes. Also, tumors (neoplasias) on bones and skin and deformations on vertebral column (kyphosis, lordosis and scoliosis) can be observed (Flores-Lopes & Reuss-Strenzel, 2011). The occurrence of these morphological conditions coupled with other metrics, such as proportion of taxonomic groups, quantity of tolerant species, trophic position of species, fluctuating asymmetry, and the fish health, are present in many environmental assessments (Jaramillo-Villa & Caramaschi, 2008; Flores-Lopes et al. 2010).

The present work sought to register, identify, illustrate and quantify the morphological abnormalities in *Corydoras* aff. *longipinnis* from two reservoirs under urban influence. Since the occurrence of deformities in this species have been registered in polluted waters (Malabarba et al. 2004; Flores-Lopes & Reus-Strenzel 2011), the importance of this kind of evaluation in environmental assessments can be highlighted considering that poor environmental conditions can be related to occurrence of deformities.

Material and Methods

The two studied reservoirs are Iraí and Capivari, respectively located in the upper Iguaçu and Southern Coastal river basins, Paraná State, Brazil. The Iraí reservoir is located in the metropolitan area of Curitiba (25º24’33”S 49º6’16”) and is used for water supply (Rodrigues et al., 2005). The Capivari is located 50 km from Curitiba (25º10’46”S 48º52’51”W) and its water is used by a hydroelectric plant (Parigot de Souza power plant) (Rodrigues et al., 2005). Both reservoirs are located in the Atlantic forest biome, presenting fragments of semideciduous ombrophilous forest, and araucaria forest (Maack, 2012).

The fish were collected in 2002, using cast nets and trawl nets, fixed in formaldehyde 10% and conserved in alcohol 70º GL. Species identification followed Tencatt et al. (2016). Each individual was examined using a visual method to identify morphological abnormalities, following Flores-Lopes & Reuss-Strenzel (2011). The frequency of deformities was calculated based on the number of abnormality occurrences relative to the total number of individuals. The use of the number of occurrences instead of the number of individuals with abnormalities was adopted because some individuals presented more than one abnormality.

Examined specimens of *Corydoras* aff. *longipinnis* are hosted in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUP) - Universidade Estadual de Maringá. Collection codes of the examined material are: 17742 (55), from Capivari reservoir; and 17738 (48); 17739 (6); 17740 (2) 17741 (42); 17743 (29); 17744 (7); 17745 (3); 17746 (153); 17747 (35), from Iraí reservoir (numbers in parentheses indicate the quantity of analyzed specimens).

Results and Discussion

A total of 380 individuals were analyzed, 325 from Iraí and 55 from Capivari reservoirs. When considering both reservoirs, an 11% frequency of deformities was detected. The deformities detected were dysplasia in the fins (7.10% frequency), dysplasia in the maxilla and jaws (0.26%), dysplasia in the branchiostegal bones (0.53%), dysplasia in the bone plates (0.53%), deformations in the vertebral column (0.26%), neoplasias (0.53%) and other dysplasias (lumps and hollows, 2.10%) (Figure 1 and Figure 2). These results are summarized in Table 1.

As previously discussed, most morphological deformities are related to negative anthropogenic influences on the environment (Sanders et al. 1999). An evaluation of water quality in the studied reservoirs, assessed from 2005 to 2008 classified Iraí as critically degraded (or polluted) and Capivari as moderately degraded (Instituto Ambiental do Paraná, 2009). In the same document, sewage discharges and industrial activities were also recorded from tributaries, resulting in an increase of nutrient concentrations in Capivari reservoir and consequently a decline in water quality.

More accurate analysis could elucidate which factors are responsible for the morphological deformities reported. Considering the industrial activities and sewage discharges, the presence of heavy metals and other kinds of pollutants, such as pesticides can be designated as potential causes of the deformities that were found.

Comparisons between populations found in conserved locations and those collected from polluted locals, can contribute to verify the natural frequency of occurrence of deformities in fish populations. Messaoudi et al. (2009) verified that polluted locals presented a frequency of deformities three times greater in relation to unpolluted areas. However, this comparison was made in marine environment, using other species. In Neotropical region, *C.* aff. *longipinnis* from the Guaíba lake, which is under urban influence, Flores-Lopes & Reuss-Strenzel (2011) registered 43 and 81 occurrences of deformities in 3270 and 2601 individuals, respectively; clearly contrasting to the 49 occurrences in 705 individuals observed herein. Despite the lower number of examined specimens, the frequency observed herein

Figure 1. Fins and spines deformities in *Corydoras* aff. *longipinnis*: (1) dorsal spine dysplasia; (2) adipose fin dysplasia; (3) pectoral spine dysplasia; (4) pectoral spine neoplasia; (5) anal-fin rays dysplasia.

Figure 2. Bones dysplasia in *Corydoras* aff. *longipinnis*: (1) vertebral column; (2) head with hollow; (3) branchiostegal bones.

Table 1. Number of occurrences and frequency of deformities in *Corydoras* aff. *longipinnis* sampled in the Iraí and Capivari reservoirs.

	Iraí reservoir		Capivari reservoir		Total	
	Occurrence	Frequency	Occurrence	Frequency	Occurrence	Frequency
Dysplasia in fins and spines	24	7.38%	3	5.45%	27	7.10%
Dysplasia in maxilla and jaws	1	0.31%	0	0%	1	0.26%
Dysplasia on branchiostegal bones	1	0.31%	1	1.81%	2	0.53%
Dysplasia on bone plates	2	0.61%	0	0%	2	0.53%
Neoplasias	2	0.61%	0	0%	2	0.53%
Column deformities	1	0.31%	0	0%	1	0.26%
Othes dysplasias	6	1.85%	2	3.63%	8	2.10%
Total	37	11.21%	6	10.53%	43	11.31%

(≈ 6.9%) is at least two times proportionally higher than in the Guaíba Lake (≈ 1.3-3.1%).

The use of Iraí and Capivari reservoirs for human activities, such as water supply, energy generation, fishing, and recreation, emphasizes the importance of conducting proper environmental monitoring in these water bodies, since deformities in fish could indicate low water quality and presence of harmful substances. These statements highlight the use of fish as a potential biological indicator, but there is a need for more detailed analysis concerning the specific cause of deformities. Encouraging further survey efforts is essential to improve the monitoring and assessment of aquatic environments.

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Ichthyofauna of Mundaú river basin, Ceará State, Northeastern Brazil

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Abstract: Mundaú river basin is located at Center-North Ceará State and occupies a total area of 2,227 km^2, including Estuário do Rio Mundaú Environmental Protection Area. This study aimed to catalog the fishes of this basin. Collections were performed with active and passive gear in 35 sampling sites, between 2012 and 2014, in several habitats (main channels, streams, floodplains, permanent and temporary pools, ponds, and dams). A total of 2,545 specimens were collected, belonging to 55 species distributed in 10 orders, 31 families, and 50 genera; 30 of these are strictly freshwater species, and 25 estuarine-marine species. Three species (*Hemigrammus guyanensis* Gery, 1995, *H. rodwayi* Durbin, 1909 and *Poecilia sarrafae* Bragança & Costa, 2011) represent new records for the Mid-Northeastern Caatinga ecoregion. Besides, two cynolebiid species, *Hypsolebias* sp. and *Anablepsoides cearensis* (Costa & Vono, 2009), were found and the latter, currently classified as critically endangered, had its occurrence area widened.

Keywords: *intermittent river, semiarid, Mid-Northeastern ecoregion, freshwater neotropical fishes.*

Ictiofauna da bacia do rio Mundaú, Estado do Ceará, Nordeste do Brasil

Resumo: A bacia do Rio Mundaú está situada no centro-norte do Estado do Ceará, e drena uma área de 2.227 km^2 de Caatinga, incluindo a Área de Proteção Ambiental do Estuário do Rio Mundaú. Este estudo teve como objetivo realizar um inventário dos peixes desta bacia. As coletas foram realizadas com petrechos ativos e passivos, em 35 pontos amostrais entre 2012 e 2014 em diversos habitats (canal principal, córregos, alagados, poças permanentes e temporárias, lagoas e açudes) ao longo de toda a bacia. Um total de 2.545 indivíduos foram coletados, pertencentes a 55 espécies distribuídas em 10 ordens, 31 famílias e 50 gêneros; destas, 30 são estritamente de água doce e 25 estuarino-marinhas. Três espécies (*Hemigrammus guyanensis* Gery,1995, *H. rodwayi* Durbin, 1909 e *Poecilia sarrafae* Bragança & Costa, 2011) constituem novas ocorrências para a ecorregião do Nordeste Médio-Oriental. Além disso, foram registradas duas espécies de cinolebídeos, *Hypsolebias* sp. e *Anablepsoides cearensis* (Costa & Vono, 2009), a última classificada como criticamente ameaçada de extinção, teve sua área de ocorrência ampliada.

Palavras-chave: *rios intermitentes, semiárido, ecorregião Nordeste Médio-Oriental, peixes de água doce neotropical.*

Introduction

Freshwater ichthyofaunal studies in Brazil are mainly focused on South and Southeastern regions (Langeani et al. 2009). While these regions are well documented, the Northeastern region is in need of studies, especially the Mid-Northeastern Caatinga Ecoregion (MNCE) (Rosa et al. 2003; Ramos et al. 2005). This ecoregion includes coastal basins located between São Francisco and Parnaíba river basins, draining Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará, and a small portion of Piauí States (Rosa et al. 2003). Albert et al. (2011) listed 88 freshwater fish species from MNCE; however, new species are being found and described (*e. g. Parotocinclus seridoensis* Ramos, Barro-Neto, Britski & Lima, 2013; *Serrapinnus potiguar* Jerep & Malabarba, 2014; and *Hypsolebias martinsi* Britzke, Nielsen & Oliveira, 2016). The estimative of freshwater fish species number at Brazilian Northeast may be premature, given the lack of taxonomic revisions and few representative regional collections (Rosa et al. 2003; Langeani et al. 2009, Ramos et al. 2014).

Ichthyofaunistic inventories at MNCE basins are scarce (Langeani et al. 2009), and the existing ones usually deal with small portions of bigger basins (*e.g.* Silva et al. 2014), or reservoirs (*e.g.* Gurgel-Lourenço et al. 2013, Sánchez-Botero et al. 2014). Few coastal basins in this hydrographical region were fully cataloged, for example Gramame river basin, in

Paraíba State, in which (Gomes-Filho & Rosa 2001) there were recorded 23 species; Curimataú river basin in Paraíba and Rio Grande do Norte States, 22 species (Ramos et al. 2005), and Pratagi river microbasin, also in Rio Grande do Norte State, 22 species (Paiva et al. 2014).

MNCE is largely inserted in the Brazilian semiarid, an area of Caatinga's phytophysionomy where rivers are mostly intermittent resulting in a simple hydrographical network (Rosa et al. 2003), except for headwaters and coastal areas in Alagoas, Pernambuco, Paraíba, and Rio Grande do Norte States, which are greatly nested within the Atlantic Forest (Rosa & Groth 2004, Paiva et al. 2014). Therefore, these semiarid river systems are constantly being modified through their damming as an attempt to assure the inland population a hydric demand during long drought periods.

Except Jaguaribe river basin and a small portion of Parnaíba river basin, Ceará hydrographic network is composed by small-sized coastal basins. Some of these basins may probably have an important role to the conservation of Caatinga's fishes, although they have been considered insufficiently known (MMA 2007). Among these, there is Mundaú river basin, located in Center-North Ceará State, whose freshwater ichthyofauna is virtually unknown. Soares-Filho et al. (2010) cataloged the estuarine porcion of this basin and reported 53 estuarine and marine fish species. The Estuário do Rio Mundaú Environmental Protection Area (EPA) is located at the lower extent of the basin, covering 1,596.37 hectares of Ceará West coast, and it is considered a priority area for fish conservation (SEMACE 2014). Because of the basin's importance for the Caatinga fish fauna knowledge, this study aimed to survey the fishes of Mundaú river basin.

Material and methods

1. Study area

Mundaú river basin (Figure 1) covers, approximately, an area of 2,227 km^2 (COGERH 2014), draining regional residual massifs, backland depressions, and seaside plains (IPECE 2012). The two main tributaries of Mundaú river basin, Cruxati in the west and Mundaú in the east, emerge from Uruburetama Massif at elevations up to 970 m, and are intermittent. Along their courses, these rivers are fed by various first order waterbodies, like Sororô, Tabocas, Laginhas, and Torrados streams. Mundaú river main course runs 97.6 km north to south, and disembogues in the Atlantic Ocean, while Cruxati river runs 77.5 km from headwaters to Mundaú river's confluence (COGERH 2014).

The local climate is semiarid with mean annual precipitation of 1,110.6 mm, and 1,914.7 mm evaporation; mean annual temperatures between 26ºC and 28ºC, and highest precipitations occurring from January to June (FUNCEME 2015).

1.1. Sampling design

Acquisition of specimens was performed in 35 locations along the entire Mundaú river basin. For better coverage, collections were made at Cruxati and Mundaú rivers' main channels, as well as in streams, ponds, dams, and bogs (Table 1). Sampling took place during dry and wet seasons, between 2012 and 2014, covering localities from Mundaú river headwaters, 900 m

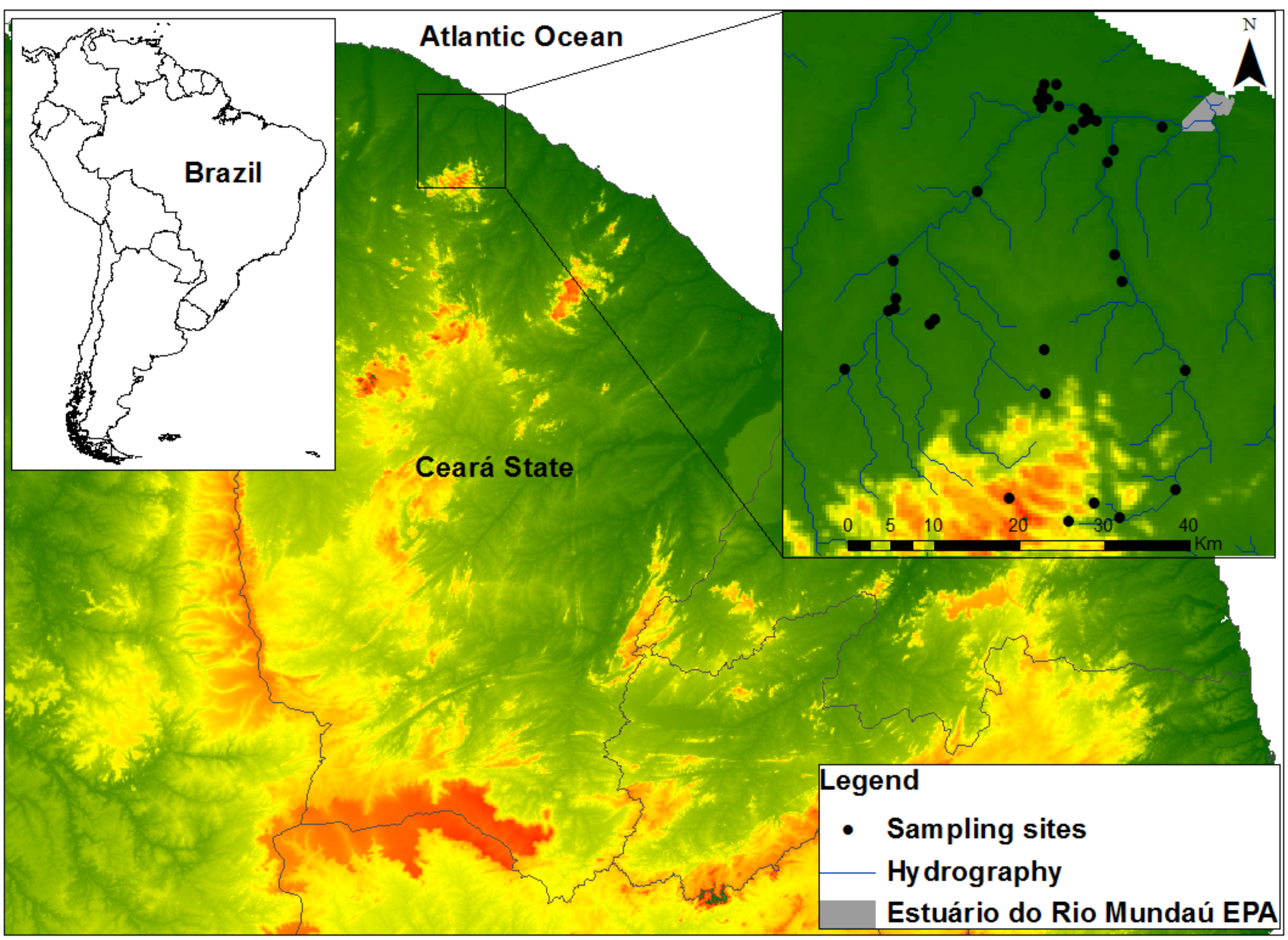

Figure 1. Mundaú river basin location in Ceará State, northeastern Brazil, evidencing the sampling sites. Hot colors represent higher altitudes.

Table 1. Geographic coordinates, elevation, tributary and main habitat of the sampling sites along the Mundáu river basin.

Geographic Coordinates	Elevation (m)	Tributary	Habitat
3.62527° S 39.49782° W	108	Mundaú	Main channel
3.21225° S 39.45283° W	11	Mundaú	Estuary
3.46911° S 39.42811° W	51	Mundaú	Main channel
3.59491° S 39.43911° W	89	Mundaú	Main channel
3.60494° S 39.61399° W	913	Mundaú	Headwater
3.62936° S 39.55111° W	273	Mundaú	Main channel
3.46814° S 39.78777° W	56	Cruxati	Stream
3.44799° S 39.57688° W	80	Cruxati	Pond
3.42094° S 39.69766° W	62	Cruxati	Stream
3.41619° S 39.69288° W	70	Cruxati	Rock pool
3.40675° S 39.74111° W	44	Cruxati	Main channel
3.40438° S 39.73577° W	43	Cruxati	Stream
3.39431° S 39.73433° W	41	Cruxati	Main channel
3.37569° S 39.49455° W	34	Mundaú	Dam
3.61006° S 39.52488° W	145	Mundaú	Headwater stream
3.35383° S 39.73588° W	37	Cruxati	Pool on the main channel
3.34763° S 39.50277° W	23	Mundaú	Pool on the main channel
3.28102° S 39.64833° W	17	Cruxati	Pool on the main channel
3.24950° S 39.51011° W	6	Mundaú	Estuary
3.23722° S 39.50433° W	8	Mundaú	Estuary
3.21582° S 39.54688° W	10	Mundaú	Floodplain
3.20777° S 39.53649° W	9	Mundaú	Floodplain
3.20650° S 39.52169° W	8	Mundaú	Estuary
3.20456° S 39.53277° W	9	Cruxati	Dam
3.19706° S 39.53098° W	9	Mundaú	Estuary
3.19319° S 39.53528° W	8	Cruxati	Floodplain
3.19197° S 39.57933° W	13	Cruxati	Estuary
3.19114° S 39.56165° W	7	Mundaú	Pool on the main channel
3.18395° S 39.58353° W	21	Cruxati	Stream
3.18297° S 39.57402° W	14	Cruxati	Pond
3.17528° S 39.57939° W	33	Cruxati	Stream
3.16827° S 39.56391° W	29	Cruxati	Floodplains
3.16748° S 39.57683° W	26	Cruxati	Floodplains
3.49422° S 39.57544° W	102	Cruxati	Stream
3.20361° S 39.52817° W	7	Mundaú	Estuary

higher than the estuary. Due to river intermittence, dry season samples were taken only in remaining pools at the main watercourse, while wet season sampling covered locations in the main flow and marginal environments.

Fish were captured using seines (seine nets 20 x 2.5m, mesh size 10 mm and 4 x 2 m, mesh size 5 mm), castnets (2 m height, mesh size 15 mm), gillnets of various mesh sizes (15, 25, 35, 45 and 55 mm), and dip nets (mesh size 5 and 10 mm) and bottle traps. Fishes were anesthetized with 30 mL of a 10% clove oil solution (clove oil 10 mL; ethyl alcohol 90 mL) in 970 mL of water (Lucena et al. 2013), fixed in a 4% formaldehyde solution during a minimal eight-day period, and conserved in 70% ethanol solution. Fish surveys were conducted according to governmental laws (Permit n° 17632-2/ICMBio). Fishes were screened, identified, and deposited at the ichthyological collections of Universidade Federal do Rio Grande do Norte (UFRN) and Universidade Federal da Paraíba (UFPB). The fishes were identified to the lowest taxonomic level according to specialized sources, that is, group specific identification keys, systematic reviews, original descriptions (e.g. Araújo et al. 2004, Britski et al. 1984, Costa 2007, Marceniuk 2005, Kullander 1988, Ploeg 1991, Ramos 2012), and specialist support. Species classified as new occurrences were evaluated according to Reis et al. (2003), Rosa et al. (2003), and Buckup et al. (2007). The nomenclature followed the recommendation by Eschmeyer (2015), and species habitat definition followed Fishbase (2014).

Results

A total of 2,545 fish specimens were collected, belonging to 55 species, 50 genera, 31 families, and 10 orders (Table 2). Of those species, 30 (54.5%) are strictly freshwater, seven (12.7%) estuarine, and 14 (25.4%) marine (Table 2). *Astyanax* aff. *bimaculatus* (Linnaeus, 1758) was widely distributed, being present in 19 of 35 sampling sites. *Poecilia vivipara* Bloch & Schneider, 1801, *Awaous tajasica* (Lichtenstein, 1822), and *Dormitator maculatus* (Bloch, 1792) were the only species present in both fresh Bragança & Costa, 2011 and brackish waters. *Oreochromis niloticus* (Linnaeus, 1758) and *Poecilia reticulata* Peters, 1859 are exotic introduced species. *Poecilia sarrafae*, *Hemigrammus guyanensis* Géry, 1959, and *H. rodwayi* Durbin, 1909 are new occurrences for MNCE, and *Nannostomus beckfordi* Günther, 1872 and *Callichthys callichthys* (Linnaeus, 1758) are new records for Ceará State.

Among freshwater fishes, Characiformes was the most representative order in number of species (53.3%), while among marine and estuarine fishes it was Perciformes (80%). The orders Siluriformes and Cyprinodontiformes represent 10.9% and 9.1% of total species, with six and five species,

Table 2. List of fish species and information on physiology, habitat, abundance and voucher of the Mundaú river basin. Abbreviations: (E)Estuarine, (F) freshwater; (M) marine; (NR) new record for the MNCE; (CR) Critically endangered; (I) introduced; (*) Photographed voucher.

ORDER/Family/*Species*	FISIOLOGY	MAIN CHANNEL	STREAM	FLOOD PLAIN	ROCK POOL	POND	DAM	TOTAL ABUNDANCE	VOUCHER
ELOPIFORMES (1)									
Elopidae (1)									
Elops saurus Linnaeus, 1766	M	6	0	0	0	0	0	6	UFRN 2612
CHARACIFORMES (16)									
Curimatidae (1)									
Steindachnerina notonota (Miranda Ribeiro, 1937)	F	122	22	20	0	8	25	197	UFRN 2642
Prochilodontidae (1)									
Prochilodus brevis Steindachner, 1875	F	42	8	0	5	15	29	99	UFRN 3530
Anostomidae (1)									
Leporinus piau Fowler, 1941	F	7	15	0	0	0	0	22	UFRN 1421
Erythrinidae (1)									
Hoplias malabaricus (Bloch, 1794)	F	14	4	2	0	3	4	27	UFRN 2582
Lebiasinidae (1)									
Nannostomus beckfordi Günther, 1872(NR)	F	24	19	15	0	3	44	105	UFRN 2591
Characidae (10)									
Astyanax aff. *bimaculatus* (Linnaeus 1758)	F	204	36	4	23	8	24	299	UFRN 1420
Astyanax aff. *fasciatus* (Cuvier 1819)	F	114	15	0	0	20	0	149	UFRN 1432
Cheirodon jaguaribensis Fowler,1941	F	5	0	0	0	0	0	5	UFRN 2523
Compsura heterura (Eigenmann, 1915)	F	101	12	11	0	8	0	132	UFRN 1430
Hemigrammus guyanensis Géry, 1959(NR)	F	5	6	1	0	0	0	12	UFRN 2599
Hemigrammus rodwayi Durbin, 1909(NR)	F	41	30	4	0	3	0	78	UFRN 2562
Hyphessobrycon sp.	F	0	32	0	0	0	4	36	UFRN 2602
Phenacogaster calverti (Fowler, 1941)	F	114	8	12	0	8	11	153	UFRN 2557
Serrapinnus heterodon (Eigenmann 1915)	F	187	39	11	12	17	22	288	UFRN 1431
Serrapinnus piaba (Lütken, 1875)	F	132	18	11	7	12	11	191	UFRN 1429
Crenuchidae (1)									
Characidium bimaculatum Fowler, 1941	F	10	5	0	0	0	0	15	UFRN 1439
SILURIFORMES (6)									
Ariidae (2)									
Sciades herzbergii (Bloch, 1794)	M	11	0	0	0	0	0	11	UFRN 3550
Auchenipteridae (1)									
Trachelyopterus galeatus (Linnaeus, 1766)	F	4	0	0	0	0	0	4	UFRN 3531
Callichthyidae (1)									
Callichthys callichthys (Linnaeus, 1758)	F	1	1	0	0	0	0	2	UFRN 2607
Loricariidae (2)									
Hypostomus cf. *pusarum* (Starks, 1913)	F	9	0	0	0	0	4	13	UFRN 2584
Parotocinclus cearensis Garavello, 1977	F	4	0	0	0	0	0	4	UFRN 1422

Table 2. Continued...

ORDER/Family/*Species*	FISIOLOGY	MAIN CHANNEL	STREAM	FLOOD PLAIN	ROCK POOL	POND	DAM	TOTAL ABUNDANCE	VOUCHER
BATRACHOIDIFORMES (1)									
Batrachoididae (1)									
Batrachoides surinamensis (Bloch & Schneider, 1801)	M	1	0	0	0	0	0	1	UFRN 3545
MUGILIFORMES (1)									
Mugilidae (1)									
Mugil curema Velenciennes, 1836	M	24	0	0	0	0	0	24	UFRN 2611
CYPRINODONTIFORMES (5)									
Cynolebiidae (2)									
Anablepsoides cearensis (Costa &Vono, 2009) (CR)	F	0	9	20	0	0	0	29	UFRN 3046*
Hypsolebias sp.	F	8	12	8	0	0	0	28	UFRN 1446
Poeciliidae (3)									
Poecilia reticulata Peters,1859(I)	F	36	31	0	0	0	0	67	UFRN 2581
Poecilia sarrafae Bragança & Costa, 2011(NR)	F	0	20	26	0	0	0	46	UFRN 2574
Poecilia vivipara Bloch & Schneider, 1801	F	113	28	10	29	12	6	198	UFRN 1419
SYNBRANCHIFORMES (1)									
Synbranchidae (1)									
Synbranchus marmoratus Bloch, 1795	F	2	0	0	0	0	0	2	UFRN 3534
PERCIFORMES (20)									
Centropomidae (1)									
Centropomus undecimalis (Bloch, 1792)	M	5	0	0	0	0	0	5	UFRN 3546
Carangidae (2)									
Hemicaranx sp.	M	4	0	0	0	0	0	4	UFRN 3541
Oligoplites saurus (Bloch & Schneider, 1801)	M	4	0	0	0	0	0	4	UFRN 3548
Lutjanidae (1)									
Lutjanus sp.	M	3	0	0	0	0	0	3	UFRN 3538
Gerreidae (3)									
Eugerres brasilianus (Cuvier, 1830)	M	4	0	0	0	0	0	4	UFRN 3549
Diapterus auratus Ranzani,1842	M	23	0	0	0	0	0	23	UFRN 3655
Eucinostomus argenteus Baird & Girard, 1855	M	8	0	0	0	0	0	8	UFRN 3654
Haemulidae (2)									
Genyatremus luteus (Bloch, 1970)	M	5	0	0	0	0	0	5	UFRN 3535
Haemulopsis corvinaeformis (Steindachner 1868)	M	8	0	0	0	0	0	8	UFRN 3542
Sparidae (1)									
Archosargus probatocephalus (Walbaum, 1792)	E	10	0	0	0	0	0	10	UFRN 4281
Sciaenidae (2)									
Bairdiella ronchus (Cuvier, 1830)	M	5	0	0	0	0	0	5	UFRN 3537
Larimus breviceps Cuvier, 1830	M	11	0	0	0	0	0	11	UFRN 3540
Ephippidae (1)									
Chaetodipterus faber (Broussonet, 1782)	M	15	0	0	0	0	0	15	UFRN 3547
Cichlidae (3)									
Cichlasoma orientale Kullander,1983	F	41	8	5	0	6	8	68	UFRN 1426
Crenicichla menezesi Ploeg, 1991	F	11	12	0	0	0	6	29	UFRN 1442

Table 2. Continued...

ORDER/Family/*Species*	FISIOLOGY	MAIN CHANNEL	STREAM	FLOOD PLAIN	ROCK POOL	POND	DAM	TOTAL ABUNDANCE	VOUCHER
Oreochromis niloticus (Linnaeus, 1758) [(I)]	F	23	3	0	0	8	12	46	UFRN 2588
Eleotridae (2)									
Dormitator maculatus (Bloch,1792)	E	6	0	5	0	0	0	11	UFRN 2613
Eleotris pisonis (Gmelin,1789)	E	13	0	0	0	0	0	13	UFRN 2577
Gobiidae (2)									
Awaous tajasica (Lichtenstein, 1822)	F	2	0	0	0	0	0	2	UFPB 10028
Gobionellus oceanicus (Pallas, 1770)	M	3	0	0	0	0	0	3	UFRN 3415
PLEURONECTIFORMES (3)									
Paralichthyidae (1)									
Citharichthys sp.	E	4	0	0	0	0	0	4	UFRN 3553
Achiridae (2)									
Achirus achirus (Linnaeus, 1758)	E	3	0	0	0	0	0	3	UFRN 3552
Trinectes paulistanus (Miranda Ribeiro, 1915)	E	4	0	0	0	0	0	4	UFPB 10030
TETRAODONTIFORMES (1)									
Tetraodontidae (2)									
Lagocephalus laevigatus (Linaeus, 1766)	M	4	0	0	0	0	0	4	UFRN 3544
Sphoeroides testudineus (Linnaeus, 1758)	E	10	0	0	0	0	0	10	UFRN 3543
Total of species (n = 55) **New record (n = 4)**		**1,570**	**393**	**165**	**76**	**131**	**210**	**2,545**	

respectively. The most diverse families were Characidae, with 10 species, representing 18.2% of total, followed by Cichlidae and Poeciliidae with three species each (5.5%) (Table 2). Of the five Cyprinodontiformes species listed, two belong to the family Cynolebiidae (*Anablepsoides cearensis* and *Hypsolebias* sp.). The non-annual killifish *A. cearensis* (Figure 2a and 2b) was the only threatened species in Mundaú river basin, listed as Critically Endangered (CR) in the Brazilian threatened fauna Red List (ICMBio 2014, Brasil 2014). This species was collected in two oxbow lakes (Figure 2d and 2e) and in a shallow perennial stream, located in an Arbustive Caatinga forest fragment (Figure 2 c), with similar features to the described type locality of the species (Costa & Vono 2009).

Discussion

The predominance of the orders Characiformes and Siluriformes in the freshwater ichthyofauna of Mundaú river basin follows the pattern found among freshwater fishes in Brazilian Northeast (Ramos et al. 2005, Nascimento et al. 2014, Ramos et al. 2014, Silva et al. 2014), in Brazil (Buckup et al. 2007), and in Neotropical region (Reis et al. 2003, Lévêque et al. 2008). However, when estuarine and marine species are also considered, the order Perciformes is the most abundant, corroborating with other MNCE species listings (Soares-Filho et al. 2010, Paiva et al. 2014).

Astynax aff. *bimaculatus* was recorded in 54% of sampling sites, being the most abundant species. It belongs to the *Astyanax bimaculatus* group composed by 22 valid species distributed along almost all drainages in South America, representing one of the most abundant morphotypes (Lucena & Soares 2016). Together with *Poecilia vivipara*, *Serrapinus heterodon*, *S. piaba*, and *Steindachnerina notonota*, they represented the most common species and could exhibit small sized body, generalist ecophysiological habits, and partitioned or all year reproduction. These features, likewise others related to anthropic disturbances, such hypoxia and eutrophic habitats, are usually present in the most frequent and abundant species in freshwater community studies in MNCE (Sánchez-Botero et al. 2014, Silva et al. 2014). However, most of the species were restricted to a few habitats, reflecting narrow ecological conditions for their occurrence. The estuarine-marine fishes were mainly juveniles of species previously recorded in Mundaú Estuary, including some important ones for artisanal fisheries (Soares-Filho et al. 2010). Of the 25 non-freshwater species registered in the basin in our study, six are not mentioned in Soares-Filho et al. (2010): *Citharichthys* sp. *Hemicaranx* sp., *Genyatremus luteus, Haemulopsis corvinaeformis, Larimus breviceps*, and *Sciades herzbergii*. The same authors also observed the goliath grouper *Epinephelus itajara* (Lichtenstein, 1822) at Mundaú Estuary, a critically endangered marine species (Brasil 2014).

The occurrence of cynolebiids at temporary pools in the main channel and floodplains in the medium and lower ranges of Mundaú river basin may reinforce the biological importance of this drainage as a priority area for Caatinga conservation, as suggested by Brasil (2007) and in the recent reevaluation (Brasil 2016). *Anablepsoides cearensis* was only known from the type locality, a shallow stream in São Gonçalo do Amarante, Ceará (Costa & Vono 2009). Thus, its distribution record was increased about 77 km west, to the lower portion of Mundaú river, in Itapipoca. Morphological differences between *Hypsolebias* sp. and its Caatinga congeners (Costa 2007) suggests that it may be a new species. The only species of the genus described for MNCE are: *H. antenori* (Tulipano, 1973), which occurs at the coastal basins east of Mundaú drainage, between Messejana municipality, in Ceará State, and Areia Branca municipality, in Rio Grande do Norte State (Costa 2007); *H. longignatus* Costa 2008 from Pacoti river basin, in Aquiraz municipality (Costa 2008), and *H. martinsi* from Icaraizinho river, in Amontada municipality (Britzke et al. 2016),

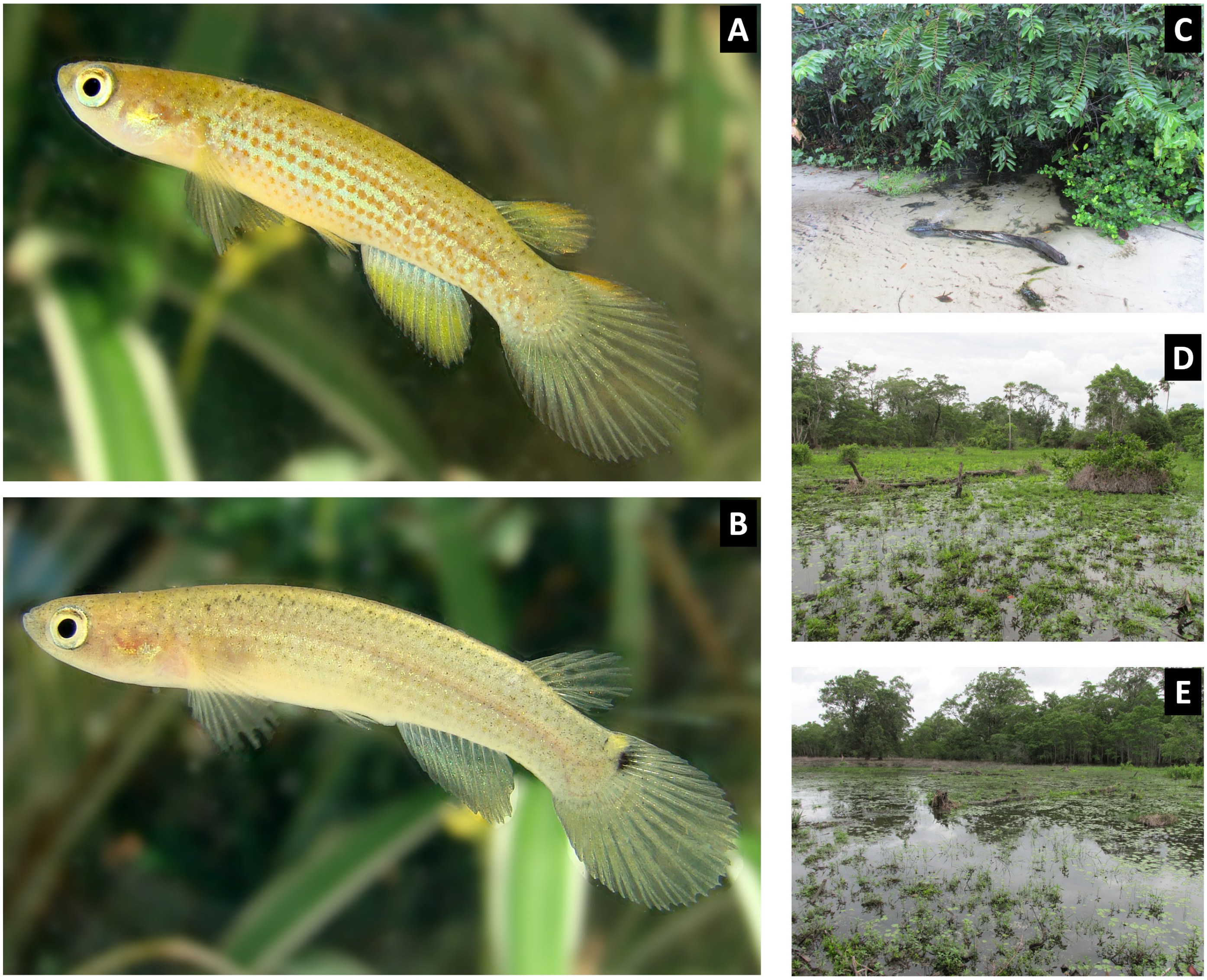

Figure 2. *Anablepsoides cearensis*, a critically endangered freshwater fish species collected in permanent pools in the lower portion of the Mundaú river basin. A = male, B = female, C, D, E = sampling sites.

both from small basins in Ceará State. Geographically, our record of *Hypsolebias* sp. is between the type locality of *H. longignatus* and *H. martinsi*, being the former eastern from Mundaú river basin and distant 140 km, while the latter is on the northwest and closer, about 25 km in the adjacent Aracatiaçu river basin. However, *Hypsolebias* sp. seems to belong to the *H. antenori* group, and thus very distinct from *H. longignatus*, which belongs to the *H. flammeus* group. The species collected in Mundaú river basin differs from *H. martinsi* by the presence of vertical bars in body, and from *H. antenori* by the virtual absence of round blotches on caudal peduncle. Additional morphological and molecular studies should be done in order to determine its specific status. This record emphasizes the classification of MNCE as a freshwater ecoregion with a high percentage of endemic species (Albert et al. 2011).

Among the species labeled as new occurrences for MNCE, *P. sarrafae* was considered as endemic to Parnaíba river basin (Bragança & Costa 2011, Ramos et al. 2014), and this is the first record outside Maranhão-Piauí ecoregion. *Hemigrammus guyanensis* was putatively endemic to Guiana Shield, while *H. rodwayi* and *Nannostomus beckfordi* are considered as native from Amazon-Orinoco-Guianas Core (Albert et al. 2011) occurring in the Guiana, Suriname, French Guiana, and Northern Brazil (Reis et al. 2003, Buckup et al. 2007). *H. rodwayi* is also found in several MNCE coastal rivers: Mamanguape (UFPB 5685) and Abiaí (UFPB 9384) river basins, in Paraíba State, and Doce (UFRN 2483) and Jundiaí drainages (UFRN 2595), in Rio Grande do Norte State (unpublished data). The occurrence of those Amazonian species in Brazilian Northeast might be an indicative of a past connection between the Atlantic and Amazon forests in more humid periods (Wang et al. 2004).

Menezes et al. (2007) reported that *Nannostomus beckfordi*, recorded from Bahia and Alagoas states, was introduced in the Brazilian Northeast due to aquarism. However, the presence of this species in Mundaú river basin in Ceará State is the northern and westernmost record in MNCE, and it could be a relictual distribution and another evidence of the preterit connection between the Neotropical forested biomes. Currently, *N. beckfordi* is considered a poorly defined species complex, which needs a taxonomic review (Benzaquem et al. 2015). The native or introduced status of this taxon needs to be addressed once endemic undescribed species could be unprotected.

The introduction of *Poecilia reticulata* in Brazilian Northeast rivers can also be a result of aquarism. However, this species has been introduced in several countries for mosquito larvae control, such as *Aedes aegypti* (Lindholm et al. 2005). Meanwhile, larger species, such as tilapia *Oreochromis niloticus*, were introduced to fish farming in the reservoirs of the northeastern Brazil by the Departamento Nacional de Obras Contra Seca (DNOCS) in the 1970 (Paiva & Mesquita 2013). In addition to competing for resources with native species, tilapias are quite resistant, easily surviving in disturbed environments (Leão et al. 2011).

A total of 55 species were found in Mundaú river basin, of which only two are introduced. The high species richness, together with new records for MNCE, which includes two cynolebiids, one of them a critically endangered species (*Anablepsoides cearensis*), highlights the importance of preserving this basin, mainly the lowland stretches. However, some anthropic impacts were observed along Mundaú river basin waterbodies, mainly associated to riparian forest removal for agriculture and cattle raising in many stretches of the rivers and streams sampled; river sand extraction; damming; irregular occupation of river margins by human habitation; and domestic sewage discharge. The ichthyological survey of Mundaú river basin contributes to the knowledge of Caatinga fish fauna, and corroborates its importance as a priority area for the conservation of aquatic biota of this semiarid region.

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Ichthyofauna of the "Cachoeira de São Roberto" and fishes of lower Preto River, upper Paraná River basin, Brazil

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Abstract: Rheophilic environments typically houses fish species with specific ecological requirements. Thus, the suppression of these environments can lead to damaging impacts to local and regional fauna. In this work the ichthyofauna of the "Cachoeira de São Roberto" was inventoried, with a historical review of fish collected in the lower Preto River basin. The sampling sites included two reaches (named R1 and R2): R1 in the "Cachoeira de São Roberto" and R2 refers to two km upstream. The fishes were sampled bimonthly during one year (April 2013 to February 2014) using small and dip nets. Voucher specimens were catalogued in the Fish Collection of "Departamento de Zoologia e Botânica do Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista 'Júlio de Mesquita Filho', câmpus de São José do Rio Preto, SP" (DZSJRP). Historical records from lower Preto River basin were composed by fish species sampled downstream of spillway of the dam in the municipality of São José do Rio Preto, SP, using the database of fish collection of DZSJRP. The ichthyofauna of the sampled reaches was composed by 53 species, distributed in 16 families and seven orders. The greatest richness was found in the upstream site (R2) with the presence of *Aphyocheirodon hemigrammus* and *Myleus tiete*, two Brazilian threatened fish species. Chao index suggested the occurrence of 64 species for the inventoried reaches. Considering all portion of lower Preto River basin, including historical records, 69 species were found, four of these species have not described yet. The present study highlights the importance of inventories in rheophilic environments, which usually includes sensitive, threatened, and species with restricted distribution. Besides that, inventories can provide technical data to support decisions about potential environmental impacts helping with the management and conservation of fish fauna.

Keywords: *diversity, rheophilic environment, Small Hydroelectric Plants, threatened species.*

Ictiofauna da Cachoeira de São Roberto e peixes do baixo rio Preto, bacia do alto rio Paraná, Brasil

Resumo: Ambientes reofílicos normalmente abrigam espécies de peixes com exigências ecológicas. Assim, a supressão desses ambientes pode levar a impactos danosos à fauna local e regional. Neste trabalho inventariou-se a ictiofauna da Cachoeira de São Roberto, com uma revisão histórica dos peixes coletados na bacia do baixo rio Preto. Os locais amostrados incluíram dois trechos (nomeados R1 e R2): R1 na área da Cachoeira de São Roberto e R2, dois km a montante de R1. As coletas ocorreram bimestralmente durante um ano (abril de 2013 a fevereiro de 2014), utilizando puçá e arrasto. Os peixes coligidos foram depositados na Coleção de Peixes do Departamento de Zoologia e Botânica do Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista "Júlio de Mesquita Filho", câmpus de São José do Rio Preto, SP (DZSJRP). Registros históricos da bacia do baixo rio Preto foram representados pelas espécies de peixes coletadas a jusante do vertedouro da represa municipal de São José do Rio Preto, SP depositados na coleção DZSJRP. A ictiofauna nos trechos inventariados (R1 e R2) foi representada por 53 espécies, distribuídas em 16 famílias e sete ordens. A maior riqueza foi encontrada no R2, com registro de *Aphyocheirodon hemigrammus* e *Myleus tiete*, duas espécies da ictiofauna brasileira ameaçadas de extinção. Estimativas de diversidade (Chao index) sugeriram a ocorrência de 64 espécies nos trechos inventariados. Considerando toda porção da bacia do baixo rio Preto, incluindo os registros históricos, registrou-se 69 espécies, quatro delas ainda não descritas formalmente. O presente estudo ressalta a importância de inventários em áreas reofílicas, que normalmente inclui espécies sensíveis, ameaçadas e com distribuição restrita. Além disso, inventários podem fornecer dados técnicos para subsidiar decisões sobre impactos ambientais auxiliando a gestão e conservação da fauna de peixes.

Palavras-chave: *diversidade, ambiente reofílico, Pequenas Centrais Hidrelétricas, espécies ameaçadas.*

Introduction

The Northwest of São Paulo State, Brazil, shows few riffle or waterfall areas, most of them not completely inventoried. Some of these areas have been target and submerged by hydroelectric plants (Agostinho et al. 2007, Júlio Junior et al. 2009). "Salto do Ferrador" (in municipality of Icém, SP) and "Cachoeira dos Índios" (in municipality of Ouroeste, SP) are two examples of these waterfalls situated in the Grande River and replaced by "Marimbondo" and "Água Vermelha" hydroelectric plants, respectively. In addition to the hydroelectric plants in the principal rivers of the region (*e.g.* Tietê and Grande rivers), Small Hydroelectric Plants (SHPs) have been employed in tributaries of these drainages (Agostinho et al. 2007). Recently, two riffle areas were target by this kind of project: "Cachoeira do Talhadão", in the Turvo River, Duplo Céu District, municipality of Palestina, SP, and in the confluence of Preto and Turvo Rivers near to "Cachoeira de São Roberto", lower Preto River basin, municipality of Pontes Gestal, SP (HABTEC 2010).

Riffle areas with rocky substrates and high water velocity are important to the maintenance of a specific biota (Garavello & Garavello 2004). The suppression and/or simplification of these areas by dam-buildings is undeniably associated to biological homogenization of aquatic ecosystems (Rahel 2002, Agostinho et al. 2008). Environmental homogenization can lead to the increase of generalist species and decrease of specialist species (Hoeinghaus et al. 2009). Moreover, conversion of lotic to lentic environment can favor species with preference to lentic waters, specially non-native Cichlidae [e.g. *Coptodon rendalli* (Boulenger), *Oreochromis niloticus* (Linnaeus), *Cichla* spp.] found in the upper Paraná River basin (Langeani et al. 2007).The "Cachoeira de São Roberto", a recreation riffle area, was target by a Small Hydroelectric Plant project without a previous or appropriate ichthyofaunistic inventory. The dam-building adjacent to this area could suppress this riffle environment and jeopardize fish fauna in the lower Preto River. Due to the lack of knowledge about the ichthyofauna and the possibility of SHP's building in this area, the aims of this study were to inventory the fish fauna of "Cachoeira de São Roberto" and to regain the fish historical records of the lower Preto River, Northwest of São Paulo State.

Material and Methods

The Preto River is one of the most important rivers of Northwest of São Paulo State; its source is in the municipality of Cedral, SP and it runs across nearly 25 km until the urban area of São José do Rio Preto, SP, where it becomes highly modified by stream flow channelization, impoundments, and lack of riparian forest (ComiteTG 2009). There are two dams and in the end of the second, the river has a spillway with *circa* four meters. After that, it runs for approximately 150 km until its confluence with the Turvo River, in municipality of Pontes Gestal, SP, Grande River drainage in the upper Paraná River basin.

The Northwest of São Paulo State has only 4% of its native vegetation, which has been replaced by agricultural crops since the beginning of the XIX century (Nalon et al. 2008). One of the remaining native riparian forest along Preto River is on the "Cachoeira de São Roberto", a tourist and recreational area with a series of small riffles situated approximately five km from its mouth, in the municipality of Pontes Gestal, SP. The area shows a tropical semi-humid climate, with temperature around 24ºC and pluviosity around 1,418 mm annual (PMPONTESGESTAL 2015).

Two sites in the lower Preto River basin were sampled (Figure 1): Reach 1 (R1), "Cachoeira de São Roberto" located approximately five km upstream from its mouth into the Turvo River (20°11'S 49°41'W), which is characterized by high water velocity with some declivity, substrate composed primarily by slab and basaltic boulders, besides the presence of sparse riparian forest composed by few trees (width of the riparian strip is approximately 10 m from each bank); Reach 2 (R2), located almost two km upstream from R1 (river distance), near to the mouth of the Botelho Stream (20°11'S 49º41'W). It is characterized by lower water velocity than R1, sandy substrate with some pebbles, and marginal vegetation composed mainly by grasses (Poaceae) (Figure 2). These two different reaches (R1 and R2) were selected aiming to know the ichthyofauna that could be affected by the Small Hydroelectric Plant.

Sampling was conducted bimonthly during one year (April 2013 to February 2014) in both reaches. A total of six sample collections were made, three in the dry season (April to August) (the period of low rainfall, 307 mm precipitation) and three in the wet season (October to February) (a period of high rainfall, 605 mm total). Pluviometric data was obtained by "Coordenadoria de Assistência Técnica Integral (CATI)" from the municipality of Votuporanga, SP.

Fishes were sampled using a small net (2.0 x 0.95 m, 2 mm mesh size) and a dip net (0.8 x 0.4 m, 2 mm mesh size). Standardized sampling effort (involving three people) was performed for two hours over approximately 150 m in each reach. Fishes were fixed in 10% formalin for 72 hours and after transferred to 70% alcohol to final preservation. Specimens were identified and vouchered at the fish collection of the "Departamento de Zoologia e Botânica do Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista 'Júlio de Mesquita Filho', câmpus de São José do Rio Preto, SP" (DZSJRP) (Figures 3, 4).

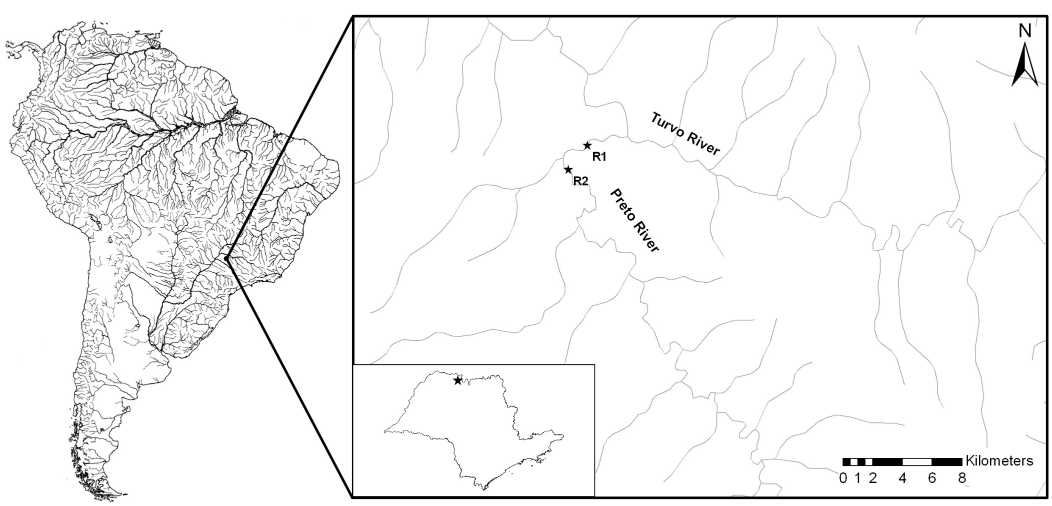

Figure 1. Location of the two reaches (R1 and R2) in the lower Preto River in the Northwest of São Paulo State, Brazil. Modified from Zeni et al. (2015).

Figure 2. Reaches sampled in the lower Preto River: a) Riffles area of the "Cachoeira de São Roberto" (R1); b) Run area, near Botelho Stream (R2). Modified from Zeni et al. (2015).

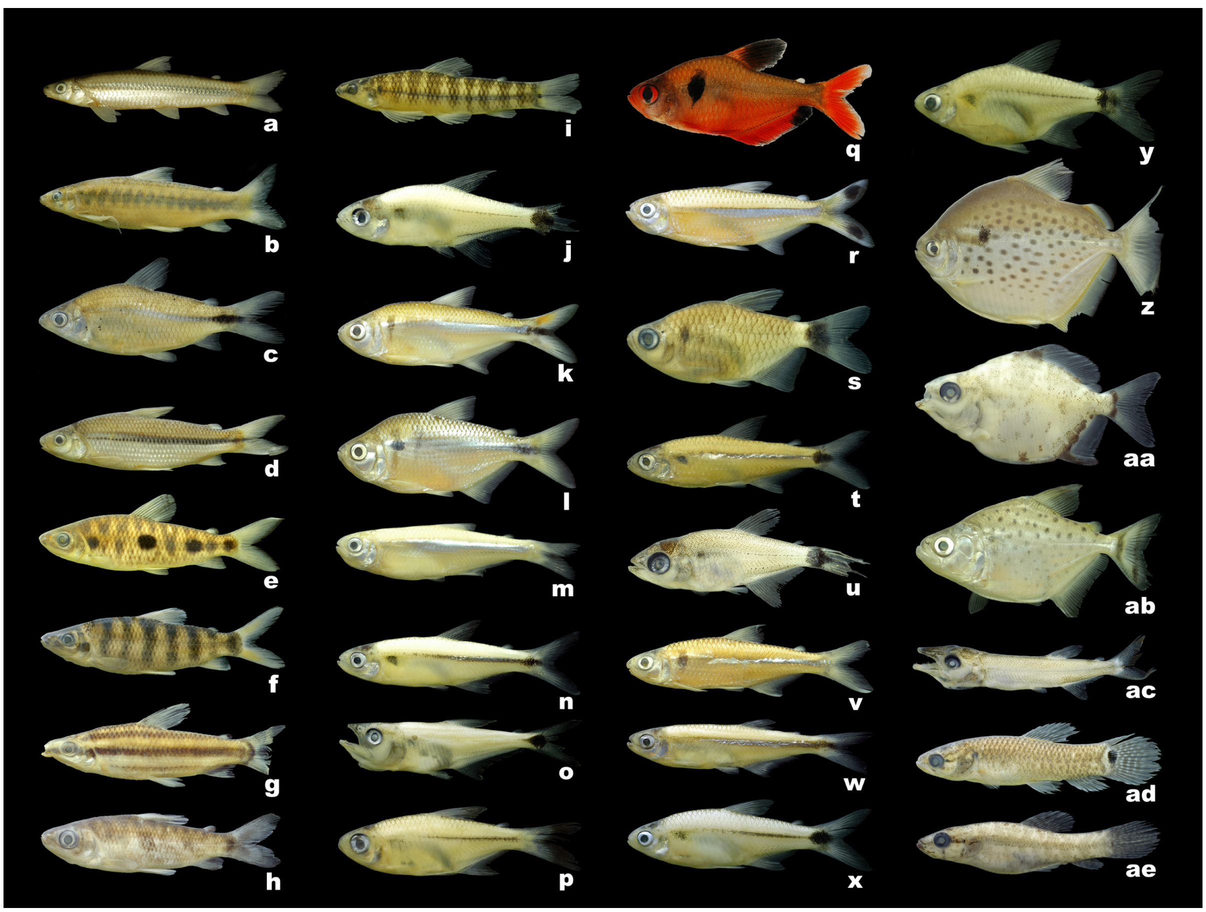

Figure 3. Fishes from the sampled areas of the lower Preto River. Characiformes. a) *Apareiodon affinis*, DZSJRP 19519, 62.6 mm SL; b) *Parodon nasus*, DZSJRP 19516, 74.5 mm SL; c) *Cyphocharax modestus*, DZSJRP 19541, 56.8 mm SL; d) *Steindachnerina insculpta*, DZSJRP 19501, 84.7 mm SL; e) *Leporinus friderici*, DZSJRP 19496, 81.9 mm SL; f) *Leporinus octofasciatus*, DZSJRP 19499, 72.2 mm SL; g) *Leporinus striatus*, DZSJRP 19543, 41.1 mm SL; h) *Megaleporinus macrocephalus*, DZSJRP 19500, 61.2 mm SL; i) *Characidium* cf. *zebra*, DZSJRP 19507, 46.4 mm SL; j) *Aphyocheirodon hemigrammus*, DZSJRP 19542, 22.0 mm SL; k) *Astyanax fasciatus*, DZSJRP 19511, 68.0 mm SL; l) *Astyanax lacustris*, DZSJRP 19517, 60.8 mm SL; m) *Bryconamericus stramineus*, 19518, 41.0 mm SL; n) '*Cheirodon*' *stenodon*, DZSJRP, 19512, 26.3 mm SL; o) *Galeocharax gulo*, DZSJRP 19530, 29.8 mm SL; p) *Hemigrammus marginatus*, DZSJRP 19525, 27.6 mm SL; q) *Hyphessobrycon eques*, DZSJRP 17694, 28.3 mm SL; r) *Moenkhausia intermedia*, DZSJRP 19508, 60.4 mm SL; s) *Moenkhausia* cf. *sanctaefilomenae*, DZSJRP 19539, 29.2 mm SL; t) *Odontostilbe* sp., DZSJRP 19495, 29.2 mm SL; u) *Oligosarcus pintoi*, DZSJRP 19533, 15.5 mm SL; v) *Piabina argentea*, DZSJRP 19494, 43.2 mm SL; w) *Planaltina britskii*, DZSJRP 19498, 31.5 mm SL; x) *Serrapinnus heterodon*, DZSJRP 19523, 30.7 mm SL; y) *Serrapinnus notomelas*, DZSJRP 19527, 28.1 mm SL; z) *Metynnis lippincottianus*, DZSJRP 19509, 99.1 mm SL; aa) *Myleus tiete*, DZSJRP 19526, 23.7 mm SL; ab) *Serrasalmus maculatus*, DZSJRP 19515, 42.5 mm SL; ac) *Acestrorhynchus lacustris*, DZSJRP 19532, 23.9 mm SL; ad) *Erythrinus erythrinus*, DZSJRP 19535, 31.0 mm SL; ae) *Hoplias* cf. *malabaricus*, DZSJRP 19522, 28.4 mm SL.

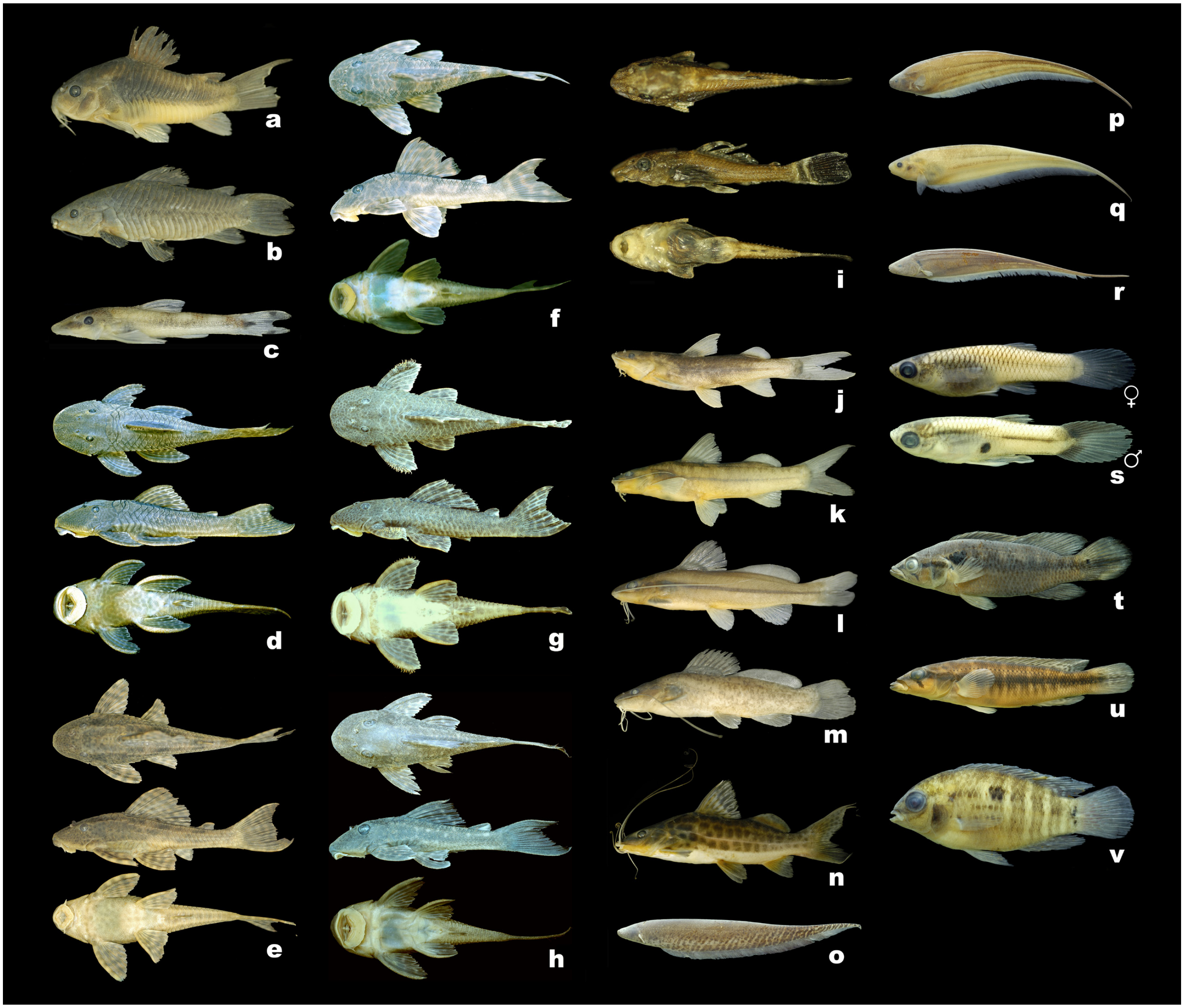

Figure 4. Fishes from the sampled areas of the lower Preto River. Siluriformes: a) *Corydoras aeneus*, DZSJRP 19524, 38.3 mm SL; b) *Hoplosternum littorale*, DZSJRP 19537, 96.4 mm SL; c) *Curculionichthys insperatus*, DZSJRP 19513, 25.7 mm SL; d) *Hypostomus albopunctatus*, DZSJRP 19502, 123.6 mm SL, dorsal, lateral, and ventral views; e) *Hypostomus ancistroides*, DZSJRP 19536, 93.5 mm SL, dorsal, lateral, and ventral views; f) *Hypostomus* cf. *iheringii*, DZSJRP 19510, 82.5 mm SL, dorsal, lateral, and ventral views; g) *Hypostomus nigromaculatus*, DZSJRP 19521, 83.6 mm SL, dorsal, lateral, and ventral views; h) *Hypostomus strigaticeps*, DZSJRP 19520, 64.2 mm SL, dorsal, lateral, and ventral views; i) *Pterygoplichthys ambrosettii*, DZSJRP 19534, 29.3 mm SL, dorsal, lateral, and ventral views; j) *Cetopsorhamdia iheringi*, DZSJRP 19514, 52.5 mm SL; k) *Imparfinis schubarti*, DZSJRP 19504, 73.0 mm SL; l) *Pimelodella avanhandavae*, DZSJRP 19505, 73.1 mm SL; m) *Rhamdia* cf. *quelen*, DZSJRP 19506, 70.9 mm SL; n) *Pimelodus maculatus*, DZSJRP 19503, 145.0 mm SL. Gymnotiformes: o) *Gymnotus sylvius*, DZSJRP 19529, 149.7 mm SL; p) *Eigenmannia guairaca*, DZSJRP 19546, 175.0 mm SL; q) *Eigenmannia virescens*, DZSJRP 19540, 115.0 mm SL; r) *Sternopygus macrurus*, DZSJRP 19544, 157.0 mm SL. Cyprinodontiformes: s) *Poecilia reticulata*, DZSJRP 19538, female 16.4 mm SL and male 13.2 mm SL. Perciformes: t) *Crenicichla britskii*, DZSJRP 19545, 88.1 mm SL; u) *Crenicichla jaguarensis*, DZSJRP 19497, 75.0 mm SL; v) *Laetacara araguaiae*, DZSJRP 19528, 32.9 mm SL.

Species abundance in R1 and R2 reaches was grouped and randomized as a function of the sampling effort, i.e., the number of sampling events (12 events, six from R1 and six from R2), to generate an accumulation curve. The Chao 1 index (with 100 permutations), which is a function of the ratio of the number of species with only one specimen in a sample (*singletons*) by the number of species with two specimens (*doubletons*) was used to estimate fish richness in the area (R1 plus R2).

Historical records from lower Preto River basin were composed by fish species sampled downstream of spillway of the dam in the municipality of São José do Rio Preto, SP, using the database of fish collection of DZSJRP. These data were compiled to the fish checklist of lower Preto River, excluding its abundances (Table 1).

Results

A total of 3,662 individuals were recorded in the sampling for both reaches, belonged to 53 species, 16 families, and seven orders. Characiformes represented the richest order, with 58.5% of the sampled species, followed

Table 1. Fishes of the lower Preto River basin: abundance (N) of the fish species (Orders and Families) in each reach (R1 and R2). Fish classification to families follows Reis et al. (2003), except to Serrasalmidae, according to Calcagnotto et al. (2005). * indicates threatened species; hyphen (-) indicates absence of quantitative data because are occurrence of species according to historical records.

Species	Reach 1	Reach 2
	N	N
CHARACIFORMES		
Parodontidae		
Apareiodon affinis (Steindachner, 1879)	14	0
Parodon nasus Kner, 1859	2	0
Curimatidae		
Cyphocharax modestus (Fernández-Yépez,1948)	0	5
Cyphocharax vanderi (Britski, 1980)	-	-
Steindachnerina insculpta (Fernández-Yépez, 1948)	2	6
Anostomidae		
Leporellus vittatus (Valenciennes, 1850)	-	-
Leporinus friderici (Bloch, 1794)	7	1
Leporinus lacustris Campos, 1945	-	-
Leporinus paranensis Garavello & Britski, 1987	-	-
Leporinus octofasciatus Steindachner, 1915	2	0
Leporinus striatus Kner, 1858	0	1
Megaleporinus macrocephalus (Garavello & Britski, 1988)	1	0
Schizodon altoparanae Garavello & Britski, 1990	-	-
Crenuchidae		
Characidium cf. *zebra* Eigenmann, 1909	25	16
Characidium sp.	-	-
Characidae		
Aphyocharax dentatus Eigenmann & Kennedy, 1903	-	-
Aphyocheirodon hemigrammus Eigenmann, 1915*	0	1
Astyanax fasciatus (Cuvier, 1819)	280	12
Astyanax lacustris (Lütken, 1875)	26	13
Bryconamericus stramineus Eigenmann, 1908	860	3
'*Cheirodon*' *stenodon* Eigenmann, 1915	10	58
Galeocharax gulo (Cope, 1870)	0	1
Hemigrammus marginatus Ellis, 1911	0	6
Hyphessobrycon eques (Steindachner, 1882)	0	1561
Moenkhausia intermedia Eigenmann, 1908	18	14
Moenkhausia cf. *sanctaefilomenae* (Steindachner, 1907)	0	3
Odontostilbe sp.	4	20
Oligosarcus pintoi Campos, 1945	0	2
Piabina argentea Reinhardt, 1867	30	26
Planaltina britskii Menezes, Weitzman & Burns, 2003	85	5
Serrapinnus heterodon (Eigenmann, 1915)	8	37
Serrapinnus notomelas (Eigenmann, 1915)	1	87
Serrapinnus sp.	-	-
Triportheus nematurus (Kner, 1858)	-	-
Serrasalmidae		
Metynnis lippincottianus (Cope, 1870)	1	0
Myleus tiete (Eigenmann & Norris, 1900)*	0	1
Serrasalmus maculatus Kner, 1858	1	5
Acestrorhynchidae		
Acestrorhynchus lacustris (Lütken, 1875)	0	4
Erythrinidae		
Erythrinus erythrinus (Bloch & Schneider, 1801)	0	1
Hoplias cf. *malabaricus* (Bloch, 1794)	0	3
SILURIFORMES		
Callichthyidae		
Corydoras aeneus (Gill, 1858)	0	1
Hoplosternum littorale (Hancock, 1828)	0	3

Table 1. Continued...

Species	Reach 1	Reach 2
	N	N
Loricariidae		
Curculionichthys insperatus (Britski & Garavello, 2003)	1	0
Hypostomus albopunctatus (Regan, 1908)	25	0
Hypostomus ancistroides (Ihering, 1911)	58	49
Hypostomus cf. *iheringii* (Regan, 1908)	58	3
Hypostomus margaritifer (Regan, 1908)	-	-
Hypostomus nigromaculatus (Schubart, 1964)	47	0
Hypostomus paulinus (Ihering, 1905)	-	-
Hypostomus strigaticeps (Regan, 1908)	1	0
Pterygoplichthys ambrosettii (Holmberg, 1893)	0	10
Heptapteridae		
Cetopsorhamdia iheringi Schubart & Gomes, 1959	5	0
Imparfinis schubarti (Gomes, 1956)	5	0
Pimelodella avanhandavae Eigenmann, 1917	4	5
Rhamdia cf. *quelen* (Quoy & Gaimard, 1824)	4	2
Pimelodidae		
Pimelodus maculatus Lacepède, 1803	1	0
GYMNOTIFORMES		
Gymnotidae		
Gymnotus sylvius Albert & Fernandes-Matioli, 1999	23	41
Sternopygidae		
Eigenmannia guairaca Peixoto, Dutra & Wosiacki, 2015	0	15
Eigenmannia virescens (Valenciennes, 1836)	3	3
Sternopygus macrurus (Bloch & Schneider, 1801)	0	4
CYPRINODONTIFORMES		
Poeciliidae		
Poecilia reticulata Peters, 1859	1	11
SYNBRANCHIFORMES		
Synbranchidae		
Synbranchus cf. *marmoratus* Bloch, 1795	-	-
PERCIFORMES		
Cichlidae		
Cichlasoma paranaense Kullander, 1983	-	-
Coptodon rendalli (Boulenger, 1897)	-	-
Crenicichla britskii Kullander, 1982	1	5
Crenicichla jaguarensis Haseman, 1911	1	2
Laetacara araguaiae Ottoni & Costa, 2009	0	1
Oreochromis niloticus (Linnaeus, 1758)	-	-
Satanoperca sp.	-	-

by Siluriformes (26.4%), Gymnotiformes (7.5%), Perciformes (5.7%), and Cyprinodontiformes (1.9%). The most representative families were Characidae, with 16 species, followed by Loricariidae, with seven species. Anostomidae and Heptapteridae were both represented by four species each, whereas Cichlidae, Serrasalmidae, and Sternopygidae presented three species; Callichthyidae, Curimatidae, Erythrinidae, and Parodontidae, two species and the other five families (Acestrorhynchidae, Crenuchidae, Gymnotidae, Pimelodidae, and Poeciliidae) were represented only by one species each (Table 1).

Fish abundance was higher in R2 (2,047 individuals) than in R1 (1,615 individuals). *Astyanax fasciatus* (Cuvier), *Bryconamericus stramineus* Eigenmann (*sensu* Eigenmann 1908), and *Planaltina britskii* Menezes, Weitzman & Burns were the most abundant species in the R1 ("Cachoeira de São Roberto"), while *Hyphessobrycon eques* (Steindachner), *Serrapinnus notomelas* (Eigenmann), and '*Cheirodon*' *stenodon* Eigenmann were the most abundant ones in the R2.

Twenty-two species were common to both reaches (Table 1). However, each reach showed unique species: R1 presented 13 species found only in this reach and among these species, *Hypostomus* cf. *iheringii* (Regan) and *H. nigromaculatus* (Schubart) were the most abundant ones, with 58 and 47 individuals, respectively. In R2, 18 species were exclusive, where *Hyphessobrycon eques* was the most abundant (1,561 individuals), followed by *Eigenmannia guairaca* Peixoto, Dutra & Wosiacki, with 15 individuals. Twelve species were represented by only one individual (Table 1). *Aphyocheirodon hemigrammus* Eigenmann and *Myleus tiete* (Eigenmann & Norris), both recorded in R2, are categorized as threatened species for Brazil and São Paulo State (Agostinho et al. 2003, Oyakawa et al. 2009, ICMBio 2015).

The estimated richness of the sampled reaches was 65 species (±8). This result was supported by the species accumulation curve, which was increasing without reaching an asymptote (Figure 5).

In addition to the current sampled ichthyofauna (53 species), 16 additional species were added to this inventory by the historical records in the lower Preto River basin (downstream of spillway in municipality of the São José do Rio Preto, SP) (Figure 6). The origin of ichthyofauna in the lower Preto River was predominantly by native species (88.4%) with eight non-native

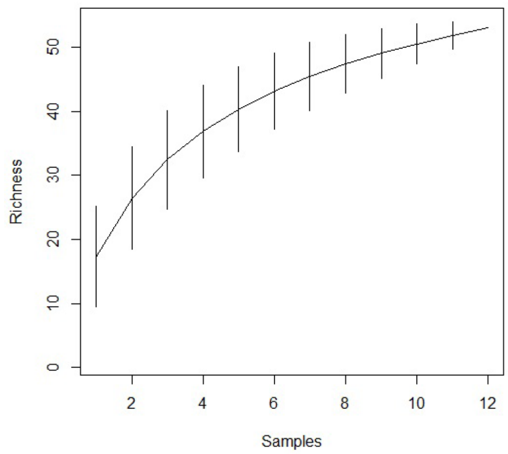

Figure 5. Species accumulation curve based on sampling efforts in the two reaches.

species [*Coptodon rendalli*, *Erythrinus erythrinus* (Bloch & Schneider), *Laetacara araguaiae* Ottoni & Costa, *Megaleporinus macrocephalus (Garavello & Britski)*, *Metynnis lippincottianus* (Cope), *Oreochromis niloticus, Poecilia reticulata* Peters and *Triportheus nematurus* (Kner)]. According to Ota (2013), it is not possible to affirm that *Satanoperca* sp. is a native or introduced species in the upper Paraná River basin. Only four species could not be identified at the species level because they probably are species not described: *Characidium* sp. (cf. Silveira, 2008), *Odontostilbe* sp. (cf. Bührnheim, 2006), *Satanoperca* sp. (cf. Ota, 2013), and *Serrapinnus* sp. (F. Jerep, *pers. comm.*).

Discussion

Higher abundance of Characiformes and Siluriformes in the lower Preto River basin, upper Paraná River basin was already expected, since they are commonly found in the Neotropical region (Britski 1992, Lowe-McConnell 1999, Reis et al. 2003, 2016, Eschmeyer et al. 2016).

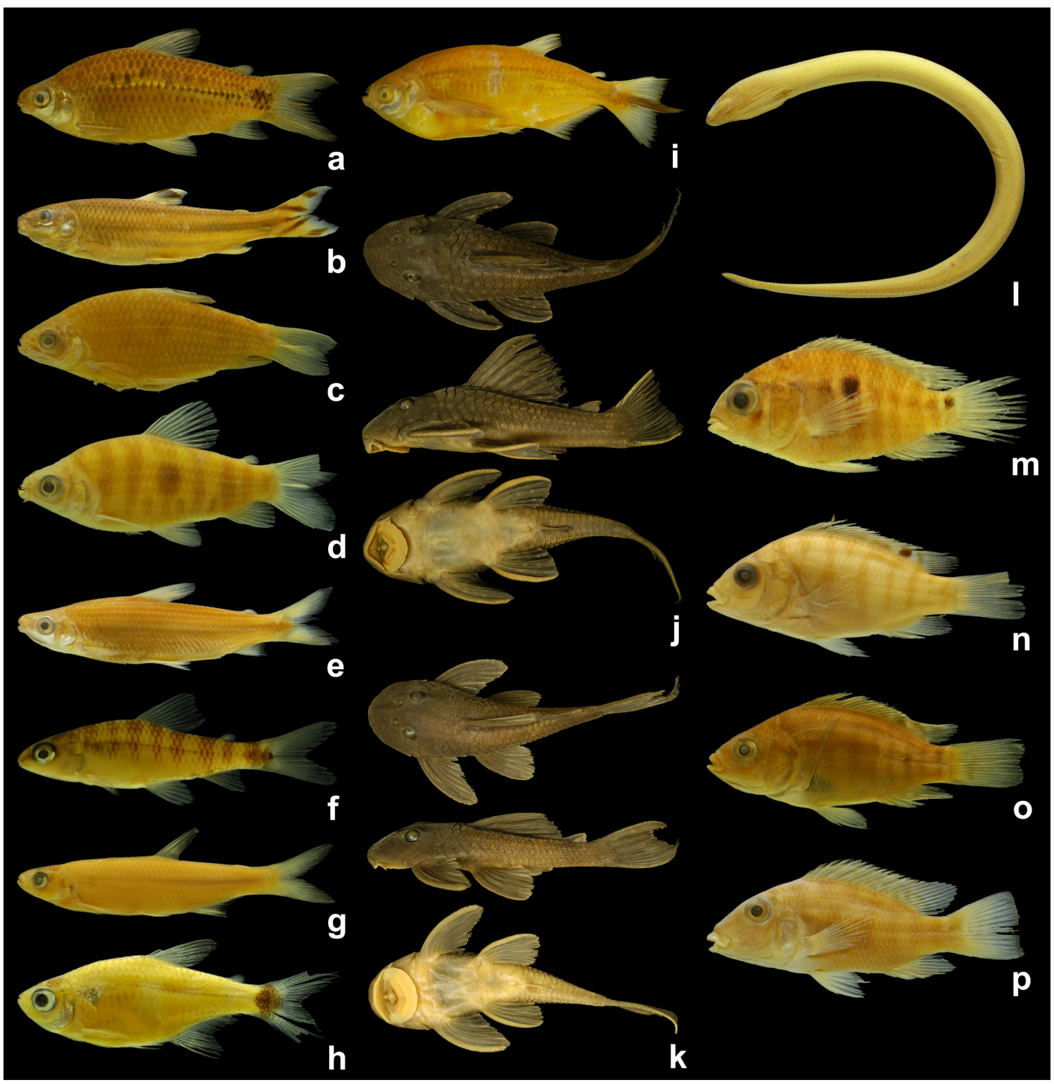

Figure 6. Additional fishes from the lower Preto River, catalogued at the fishes collection (DZSJRP). Characiformes: a) *Cyphocharax vanderi*, DZSJRP 4820, 73.4 mm SL; b) *Leporellus vittatus*, DZSJRP 3678, 159.0 mm SL; c) *Leporinus lacustris*, DZSJRP 8, 75.4 mm SL; d) *Leporinus paranensis*, DZSJRP 2197, 47.2 mm SL; e) *Schizodon altoparanae*, DZSJRP 2853, 93.7 mm SL; f) *Characidium* sp., DZSJRP 18356, 17.9 mm SL; g) *Aphyocharax dentatus*, DZSJRP 3751, 47.8 mm SL; h) *Serrapinnus* sp., DZSJRP 19244, 21.8 mm SL; i) *Triportheus nematurus*, DZSJRP 621, 117.9 mm SL. Siluriformes: j) *Hypostomus margaritifer*, DZSJRP 17721, 131.0 mm SL; k) *Hypostomus paulinus*, DZSJRP 17719, 59.1 mm SL. Synbranchiformes: l) *Synbranchus* cf. *marmoratus*, DZSJRP 1705, 129.0 mm SL. Perciformes: m) *Cichlasoma paranaense*, DZSJRP 1747, 27.5 mm SL; n) *Coptodon rendalli*, DZSJRP 2011, 25.3 mm SL; o) *Oreochromis niloticus*, DZSJRP 18370, 28.9 mm SL; p) *Satanoperca* sp., DZSJRP 18384, 38.0 mm SL.

The richness recorded in the sampled area (53 species) was similar to others studies performed in the Northwest of São Paulo State (Garutti 1988, Casatti et al. 2009, Ferreira & Casatti 2012), although these studies had been performed in streams using different methodologies. In addition to the 53 sampled species, 16 species were regained from historical records, comprising a total of 69 species to the lower Preto River. These data are, probably, underestimated, due to the fact they are the first ones for the area.

The higher abundance of *Astyanax fasciatus*, *Bryconamericus stramineus*, and *Planaltina britskii* in R1 than in R2 is, probably, because these species are drift feeders (Casatti & Castro 1998) that can obtain the maximum food intake at sites with an uninterrupted and lotic water flow. Nonetheless, Parodontidae and *Hypostomus* spp. were also more abundant in this reach. These species have low body depth, ventral mouth, and pectoral fins used to deflect high water flow and cling to the rocky substrate (Garavello & Garavello 2004), the predominant substrate type in riffle areas like R1. In R2, where grasses were present in the river bank, the highest abundance was assigned to *H. eques* and *S. notomelas*. The presence of grass in the river bank can provide an increase of food resources availability, such as aquatic insects, often associated with marginal grasses (Ceneviva-Bastos & Casatti 2014, Zeni & Casatti 2014). Moreover, this condition can be important to the establishment and growth of *Aphyocheirodon hemigrammus* and *Myleus tiete*, threatened species in Brazil, since grasses leaves and roots can be used as refuges from predators, acting as a nursery for large-bodied species (Araújo & Garutti 2003). In fact, the juvenile specimen of *Myleus tiete* (23.8 mm SL) recorded in this study was sampled in R2. According to Zeni et al. (2015), juvenile specimens from others species [*Acestrorhynchus lacustris* (Lütken), *Erythrinus erythrinus*, *Galeocharax gulo* (Cope), *Hoplias* cf. *malabaricus* (Bloch), and *Pterygoplichthys ambrosettii* (Holmberg)] were found associated to this area during reproductive period.

Myleus tiete is a migratory fish and, currently, considered as an endangered species (EN) (ICMBio 2015), since few and small populations are geographically fragmented and isolated, specially due to the numerous dam-buildings in the drainages of upper Paraná River basin. Modification of lotic to lentic system can jeopardize this migratory species by preventing the life cycle to be completed, since some stages can occur only in lotic environments. Furthermore, the suppression of riparian vegetation mainly caused by agriculture practices can directly affect *M. tiete* feeding habits as well as *A. hemigrammus*. *Aphyocheirodon hemigrammus* occurs, preferentially, in lentic and floodplain environments and it has been considered vulnerable to extinction (VU) (ICMBio 2015), mainly due meso- and microhabitat homogenization associated to siltation, which has been decreasing this species' population. Although both species can be found in different habitats (lotic and lentic environment), in the lower Preto River they were found only in the lentic reach (R2). As a conclusion, this type of environment can be important to the occurrence of lentic-adapted species, as *A. hemigrammus*, and juvenile of rheophilic species, as *M. tiete*.

Large rheophilic fishes previously reported from the "Cachoeira de São Roberto", such as *Megaleporinus obtusidens* (Valenciennes) ('piapara'), *Brycon* sp. ('piracanjuba'), and *Salminus brasiliensis* (Cuvier) ('dourado') (Borges 2007) were not found during more than one year of sampling. The absence of these species, even juvenile specimens, may be due to the failure in the sampled methods used in this study or result of the human impacts on the Preto River. Land use changes, removal of riparian vegetation, physical habitat homogenization due to the invasion of grass from pastures, organic pollution, and more recently, dam-building in the urban sections of the river, and the presence of non-native species can affect the occurrence of large rheophilic species (Agostinho et al. 2005). Besides these species, local fishermen have recognized *Prochilodus lineatus* (Valenciennes) ('corimba') and *Salminus hilarii* Valenciennes ('tabarana') in the area, but not recorded in this study.

Among the species found in the lower Preto River basin, most of them were common for the "Unidade de Gerenciamento de Recursos Hídricos (UGRH) da bacia do Turvo-Grande" (cf. Garutti 1988, Lemes & Garutti 2002, Andrade 2003, Casatti et al. 2009, Rocha et al. 2009), with exception of *Aphyocheirodon hemigrammus* and *Myleus tiete*. Besides the threatened species, four undescribed species were recorded (*Characidium* sp., *Odontostilbe* sp., *Satanoperca* sp. and *Serrapinnus* sp.). This condition enhances the high diversity of the upper Paraná River basin (Langeani et al. 2007; Carvalho & Langeani 2013) and reinforces the importance of the Preto River basin to shelter new and endangered species.

The occurrence of fishes in the lower Preto River comprised 11.6% of non-native species introduced in the upper Paraná River from different vias (Langeani et al. 2007, Azevedo-Santos et al. 2015). Pisciculture and aquarism are probable the principal causes of fish introduction observed in the lower Preto River basin and in others Brazilian river basins (Alves et al. 2007, Vitule 2009; Azevedo-Santos et al. 2015). *Megaleporinus macrocephalus*, *Coptodon rendalli,* and *Oreochromis niloticus* were both introduced by pisciculture (Langeani et al. 2007), whereas *Laetacara araguaiae* was probably introduced via upper rio Araguaia basin (*pers. obs*.), and *Poecilia reticulata* could had been introduced by aquarism or mosquito control (Deacon et al. 2011). *Erythrinus erythrinus* was probable introduced by sport fishing, whereas *Triportheus nematurus* was probably distributed in the upper Paraná after the Itaipu dam construction (Langeani et al. 2007). *Metynnis lippincottianus* had probably been accidentally introduced in the upper Paraná River basin (Ota 2015).

In the historical records in the lower Preto River basin there are three non-native species (*Triportheus nematurus*, *Oreochromis niloticus*, and *Coptodon rendalli*), whereas two of them are Perciformes. Cichlidae was one of the most diverse families in these records and maybe this was caused by their association with lentic environment, since the sampling site of these records was marginal lagoons of the Preto River, near the District of Macaúbas, municipality of Mirassolândia, SP. Perciformes has showed high occurrence in the upper Paraná River basin through natural (Langeani et al. 2007) and artificial environments, as the dam in the upper portion of the Preto River, in São José do Rio Preto, SP (Andrade 2003).

Dam-building could have affected lower Preto River if the SHPs projects in Turvo River had been approved. Physical homogenization caused by dam-building and the introduction of non-native species are synergistic processes responsible for biotic homogenization of communities (Rahel 2002). Fortunately, these projects were denied by the "Companhia Ambiental do Estado de São Paulo" (CETESB), as long as the alterations caused by them are environmentally impracticable by jeopardizing hydrological flow and leading to the suppression of more than 60% of the remaining forest fragments. The final decision of CETESB (2012) concluded that "Cachoeira de São Roberto" is situated in the future reservoir area, being affected by changes in water dynamic of the Preto River. In fact, dam-building change lotic into lentic environment and this can affect species with specific adaptations to survive in high flow environments and not only decreasing native species richness (Agostinho et al. 2007, Agostinho et al. 2008, Winemiller et al. 2016), but also increasing the abundance of non-native species (Hoeinghaus et al. 2009). Thus, the suppression of "Cachoeira de São Roberto" would affect the occurrence and abundance of several native species, such as *Hypostomus* spp., Parodontidae, and rheophilic species, as *Myleus tiete*, that could not resist to habitat alterations, besides increase the occurrence of non-native species, such *O. niloticus* and *C. rendalli*. In this context, ichthyofaunistic inventory is important not only to know fish richness, which can include new and threatened species, but also to guide and/or support management and conservation of aquatic systems, mainly in those areas target by dam-building.

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Diet of two syntopic species of Crenuchidae (Ostariophysi: Characiformes) in an Amazonian rocky stream

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Abstract: This study assessed the diet of two poorly known syntopic fish species of the family Crenuchidae, *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus*, in a Presidente Figueiredo´ rocky stream, Amazonas, Brazil. The stomach contents were analyzed and their Frequency of Occurrence (FO %) and Relative Volume (Vol %) were combined in a Feeding Index (IAi). We examined 20 individuals of *C.* aff. *declivirostre* and 23 of *L. omospilus*. The Morisita-Horn Index was used to estimate the overlap between the diets of these species. Immature insects were the most valuable items consumed by both fish species. The diet of *C.* aff. *declivirostre* was mainly composed of larvae and pupae of Chironomidae, while *L. omospilus* predominantly consumed larvae of Hydroptilidae, Hydropyschidae and Pyralidae. Thus, both species were classified as autochthonous insectivorous. *Characidium* aff. *declivirostre* was considered a more specialized species, probably reflecting lower feeding plasticity or the use of more restricted microhabitats compared to *L. omospilus*. When the food items were analyzed at the family taxonomic level, the diet overlap between these species was considered moderate (Morisita-Horn Index = 0.4). However, a more thorough analysis, at the genus level, indicates a very low diet overlap. Therefore, we conclude that the feeding segregation between *C.* aff. *declivirostre* and *L. omospilus* may favor their co-existence, despite their high phylogenetic closeness.

Keywords: *stream fish, co-existence, feeding overlap.*

Dieta de duas espécies sintópicas de Crenuchidae (Ostariophysi: Characiformes) em um riacho rochoso amazônico

Resumo: O presente estudo investigou a dieta de duas espécies de peixes pouco conhecidas da família Crenuchidae, *Characidium* aff. *declivirostre e Leptocharacidium omospilus*, sintópicas de um riacho de corredeira do município de Presidente Figueiredo, Estado do Amazonas, Brasil. O conteúdo estomacal de cada exemplar foi analisado pelos métodos de Frequência de Ocorrência (FO%) e Volume Relativo (Vol%) e, posteriormente, combinados para o cálculo do Índice Alimentar (IAi). Foram analisados 20 indivíduos de *C.* aff. *declivirostre* e 23 de *L. omospilus*. A sobreposição entre a dieta das duas espécies foi estimada pelo Índice Simplificado de Morisita-Horn. Insetos imaturos foram os principais itens consumidos por ambas as espécies. A dieta de *C.* aff. *declivirostre* mostrou preferência acentuada por larvas e pupas de Chironomidae, enquanto que a dieta de *L. omospilus* concentrou-se em larvas de Hydroptilidae, de Hydropyschidae e de Pyralidae. Portanto, as espécies foram classificadas troficamente como insetívoras consumidoras de itens autóctones. A dieta de *C.* aff. *declivirostre* foi mais especializada, o que pode ser reflexo de uma menor plasticidade alimentar ou do uso de microhabitats mais restritos, quando comparado a *L. omospilus*. Ao analisar os itens alimentares em nível taxonômico de família, a sobreposição alimentar foi considerada moderada (Índice de Morisita-Horn = 0,4). Entretanto, análises mais refinadas, em nível de gênero, indicaram sobreposição muito baixa na dieta. Conclui-se que tal segregação alimentar pode favorecer a coexistência de *C.* aff. *declivirostre* e *L. omospilus*, mesmo que sejam espécies filogeneticamente próximas.

Palavras chaves: *Peixes de riacho, coexistência, sobreposição alimentar.*

Introduction

The co-existence of species can be affected by their ecological niche, characterized by their diet and feeding tactics, habitat preferences, reproductive strategy, and period of activity (Hutchinson 1957). However, species with similar ecological demands (e.g., phylogenetically closely related species) might not coexist during conditions of resource limitation, because the less competitive would inevitably become extinct as defended by the theory of limiting similarity (Mac Arthur & Levins 1967) and by the competitive exclusion principles (Hardin 1960). Schoener (1974) studied the trophic relationship between syntopic species and proposed three conditions that would allow the co-existence of related species: (1) species may explore distinct microhabitats, (2) they have different diel habits, or (3) they consume distinct food items. Thus, feeding studies can be used as a valuable data source for ecological modelling research, and for the better understanding of syntopic species interaction (Schoener 1974).

Fish feeding studies in small Amazonian streams began during the 1970s (regionally called "igarapés")(e.g., Knöppel 1970, Soares 1979), and were intensified after that (e.g., Silva 1993, Sabino & Zuanon 1998, Anjos 2005, Carvalho 2008, Zuanon & Ferreira 2008, Fernandes 2014; Barros et al. 2017). However, such information is still restricted to those streams with the typical regional geomorphology found in the Central Amazonia (i.e., with low declivity, sandy bed, the presence of stems, roots and a thick layer of humus) (Walker 1995, Mendonça et al. 2005). Moreover, few studies have been developed in areas of waterfalls and river rapids (Zuanon 1999), and to our knowledge, no studies were carried out about fish diet and resource partitioning in rapids with stony riverbed.

The region of Presidente Figueiredo is located at the southern boundary of the Guianas' shield and is characterized by rocky streams with long rapids and waterfalls (Nogueira & Sarges 2001). Moreover, studies that are being developed in rocky streams of this same region have already recorded a high co-occurrence of two rheophilic species of the subfamily Characidiinae: *Characidium* aff. *declivirostre* Steindachner, 1915 and *Leptocharacidium omospilus* Buckup, 1993 (R.P. Leitão, unpublished data) (Figure 1).To maintain their spatial position over the rocky substratum, to find and catch preys as well as interact with individuals of the same and other species, morphological and behavioral adaptations may occur in species that inhabit high-speed rapids. Thus, our aims were to determine the diet of *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus* as well as to investigate the degree of interspecific overlapping in this niche dimension.

Material and Methods

Study Area

This study was developed in Marupiara´ rocky stream (2° 3'15.41"S; 60° 6'21.72"W), a second-order stream of Urubu River, which flows into the left margin of Amazon River, municipality of Presidente Figueiredo (Amazonas, Brazil). Marupiara´ stream is characterized by long rapids with stony bed, low depth (3 – 70 cm), narrow width (4.8 m on average), clear and acidic waters with high levels of oxygen, and surrounded by partially intact old-growth forest (Figure 2).

Data collection

Specimens of *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus* were collected between June and December of 2009, and also in March of 2011 during the daytime, using small dip nets (1 mm mesh). The collected specimens were anaesthetized with Eugenol and fixed in 10% formaldehyde. Voucher specimens were deposited in the fish collection of the Instituto Nacional de Pesquisas da Amazônia (INPA33926, 33927, 33928, 33929), Brazil.

Data analysis

Stomach content of 20 adult individuals of *Characidium* aff. *declivirostre* (40.3 – 73.0 mm in standard length SL) and 23 of *Leptocharacidium omospilus* (40.0 – 68.5 mm SL) were analyzed using a stereoscopic microscope. Food items were identified to the lowest possible taxonomic level with the aid of specialised literature. Moreover, the degree of repletion was visually estimated (as the amount of food by stomach expressed in percentage) (adapted from Goulding et al. 1988).

Stomach contents were analyzed according to the methods of Frequency of Occurrence (FO%) (Hyslop 1980) and Relative Volume (Vol % = volume of a given food item/ volume of the stomach). To correct values with distinct amount of aliment, the relative volume was multiplied by the respective degree of stomach repletion (Soares 1979). Finally, the Feeding Index (IAi) was computed using the results previously provided from the determination of the frequency of occurrence as well as the percentage of volume (Kawakami & Vazzoler 1980). The Feeding Index determines the effective importance of each item found in species feeding and contributes to the analysis of the species feeding overlap (Kawakami & Vazzoler 1980).

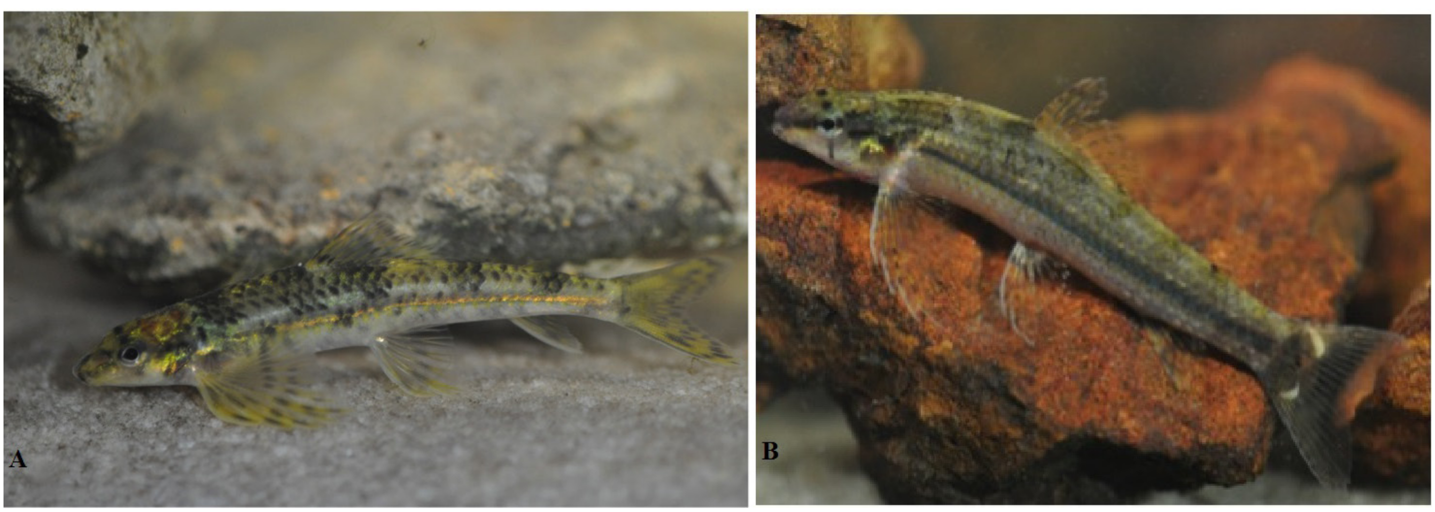

Figure 1: *Characidium* aff. *declivirostre* (A) and *Leptocharacidium omospilus* (B) specimens caught in Marupiara´rocky stream, Presidente Figueiredo – Amazonas, Brazil. Photos taken in aquaria by R. P. Leitão.

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The Feeding Index is determined by the equation: $IAi = Fi \times Vi/\sum(Fi \times Vi)$. Where, F_i = Frequency of occurrence (%) of item i; V_i = relative volume (%) of item *i*.

To assess the degree of diet overlap between the fish species, we used the Morisita-Horn Index (C_H; Horn 1966, Krebs 1998), determined by the equation: $C_H = 2 \sum^n_i pij \times pik / \sum^n_i p^2ij + \sum^n_i p^2ik$. Where, p_{ij} = proportion of item *i* relative to the total resources used by species *j*; p_{ik} = proportion of item *i* relative to the total resources used by species *k*; *n* = total number of resource items. C_H ranges from 0 (no food items shared) to 1.0 (complete overlap), with values > 0.6 indicating significant overlap (Richard & Wallace 1981). This analysis was based on the taxonomic levels of family and genus for the consumed items.

Results

Of the 43 individuals whose stomachs were examined, 13 had empty stomachs (three *Characidium* aff. *declivirostre* and 10 *Leptocharacidium omospilus*). We have registered 21 food items that were grouped into 12 taxonomic categories: larvae and pupae of Diptera (Chironomidade, Simulidae, Empidae, Tipulidae and Ceratopogonidae), larvae of Trichoptera (Hydropyschydae, Odontoceridae and Hydroptilidae), larvae and adults of Coleoptera (Elmidae), larvae of Lepidoptera (Pyralidae), nymphs of Ephemeroptera (Baetidae, Leptohyphydae, Leptophlebiidae and Caenidae), larvae of Megaloptera, nymphs of Odonata (Colopterygidae) and nymphs of Plecoptera (Perlidae), arachnids (aquatic mites), algae, organic debris (amorphous organic matter) and plant material (Table 1).

Figure 2: Marupiara´rocky stream, Presidente Figueiredo – Amazonas, Brazil. The samples were collected along stretch between less turbulent waters with dominance of released stones (left) and more turbulent waters with slabs of rock (right).

Table 1: Values of Frequency of occurrence (FO %), Relative volume (Vol %) and Feeding Index (IAi) for each of the food items consumed by *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus*. l = larvae, p = pupae, n = nymph, a = adult

Items	Origin	***Characidium* aff. *declivirostre* (n =17)**			***Leptocharacidium omospilus* (n = 13)**		
		FO%	**Vol%**	**IAi*100**	**FO%**	**Vol%**	**IAi*100**
Chironomidade (l+p)		100.00	33.68	**49.03**	69.23	2.40	3.80
Simulidae (l)		17.65	3.55	0.91	15.38	1.65	0.58
Empidae (l)		23.53	0.95	0.33	-	-	-
Tipulidae (l)		5.88	1.78	0.15	-	-	-
Ceratopogonidae (l)		5.88	0.57	0.05	7.69	0.24	0.04
Hydropyschydae (l)		17.65	2.44	0.63	38.46	28.57	**25.14**
Odontoceridae (l)		-	-	-	7.69	0.65	0.11
Hydroptilidae (l)		88.24	15.62	20.05	61.54	19.00	**26.76**
Elmidae (l+a)	autochthonous	17.65	0.95	0.24	23.08	2.29	1.21
Pyralidae (l)		41.18	11.33	6.79	38.46	22.82	**20.08**
Baetidae (n)		52.94	22.8	17.57	30.77	6.92	4.87
Leptohyphydae (n)		5.88	0.04	0.00	-	-	-
Leptophlebiidae(n)		-	-	-	15.38	0.90	0.32
Caenidae (n)		-	-	-	7.69	1.29	0.23
Megaloptera (l)		-	-	-	7.69	0.32	0.06
Colopterygidae (n)		-	-	-	7.69	0.39	0.07
Perlidae (n)		5.88	0.14	0.01	-	-	-
Algae		5.88	0.38	0.03	-	-	-
Plant material	alloctone	23.53	0.41	0.14	7.69	0.48	0.09
Acari		35.29	0.24	0.12	23.08	0.38	0.20
Organic debris	unidentified	52.94	5.12	3.94	61.54	11.68	16.45

The diet of *Characidium* aff. *declivirostre* was composed of 16 food items, and the most representative (i.e., with the highest IAi values) were: larvae and pupae of Chironomidae (49.02%), shelters and larvae of Hydroptilidae (20.05%), and nymphs of Baetidae (17.56%) (Table 1 and Figure 3). Moreover, *Leptocharacidium omospilus* also consumed 16 food items, and the most relevant items were: larvae of Hydroptilidae (26.75%), larvae of Hydropyschydae (25.13%), larvae of Pyralidae (20.07%) and organic debris (16.44%) (Table 1 and Figure 3). Overall, food items were more equally distributed in *L. omospilus* than in *C.* aff. *declivirostre*, as a clear predominance of one food category was observed for *C.* aff. *declivirostre* (Figure 3).

Based on the Morisita-Horn Index applied to the taxonomic level of family of the consumed items, a moderate degree of diet overlap was detected between the two syntopic species (C_H = 0.4). However, given the great importance of Hydroptilidae for the diet of both fish species, we conducted a more thorough analysis by identifying and quantifying the different genera of this family. This analysis revealed a clear difference between the proportions of each item consumed by the crenuchids. For example, the genus *Anchitrichia* composed almost half of Hydroptilidae (Vol = 47%) found in *C.* aff. *declivirostre* diet, but it was not observed in the diet of *L. omospilus* (Figure 4). On the other hand, the genus *Neotrichia* represented more than 70% of Hydroptilidae consumed by *L. omospilus*, but only 6% of Hydroptilidae consumed by *C.* aff. *declivirostre* (Figure 4).

Discussion

The diet of *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus* is mainly composed of aquatic or terrestrial insects in its aquatic phase. Therefore, we may categorize both species as autochthonous insectivores. Similar results have been found by other diet studies developed with species of the same sub-family such as, *Characidium* sp., *C. lanei*, *C. pterostictum* and *C. vidali* (in streams of Atlantic Forest: Sabino & Castro 1990, Aranha et al. 2000, Rezende et al. 2013), *C. pteroides* and *Characidium* sp. (in Amazonian streams: Anjos 2005, Carvalho 2008).

The great variety of preys ingested by both species suggests that they are generalist feeders. However, given the high proportion of a single family of insects (Chironomidade: 49.02%) in its diet, *C.* aff. *declivirostre* has also shown some tendency to specialization. This may be due to ecological causes associated with the Optimal Foraging Theory as many generalist populations could be composed by individuals with tendency to

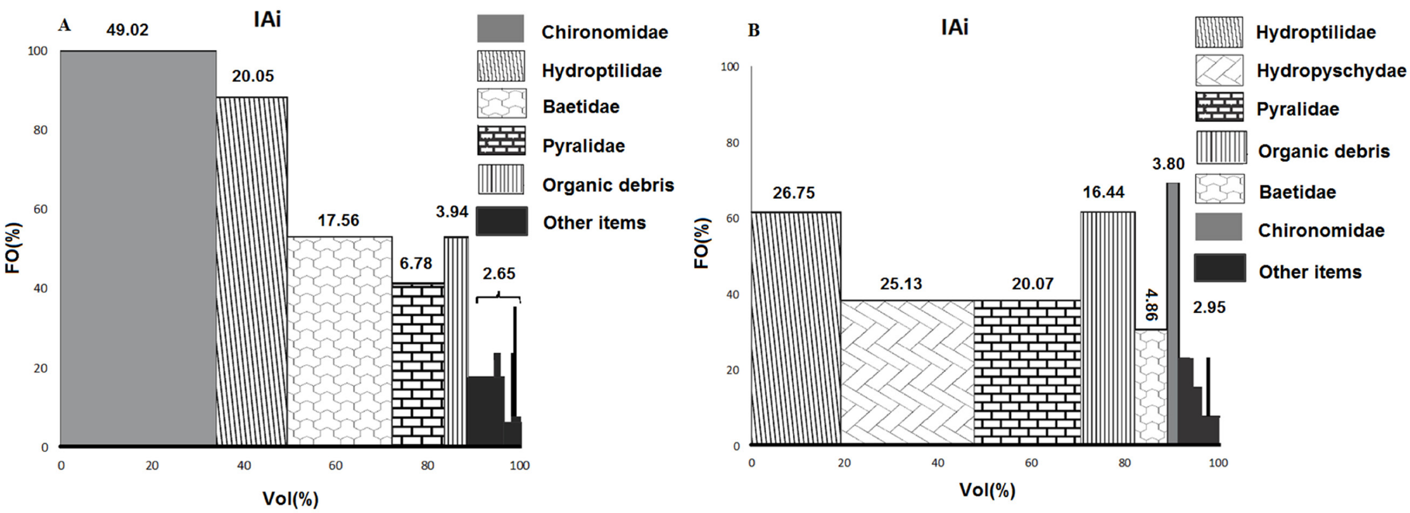

Figure 3: Frequency of occurrence (FO %) and Relative volume (Vol %), for the food items consumed by *Characidium* aff. *declivirostre* (A) and *Leptocharacidium omospilus* (B).

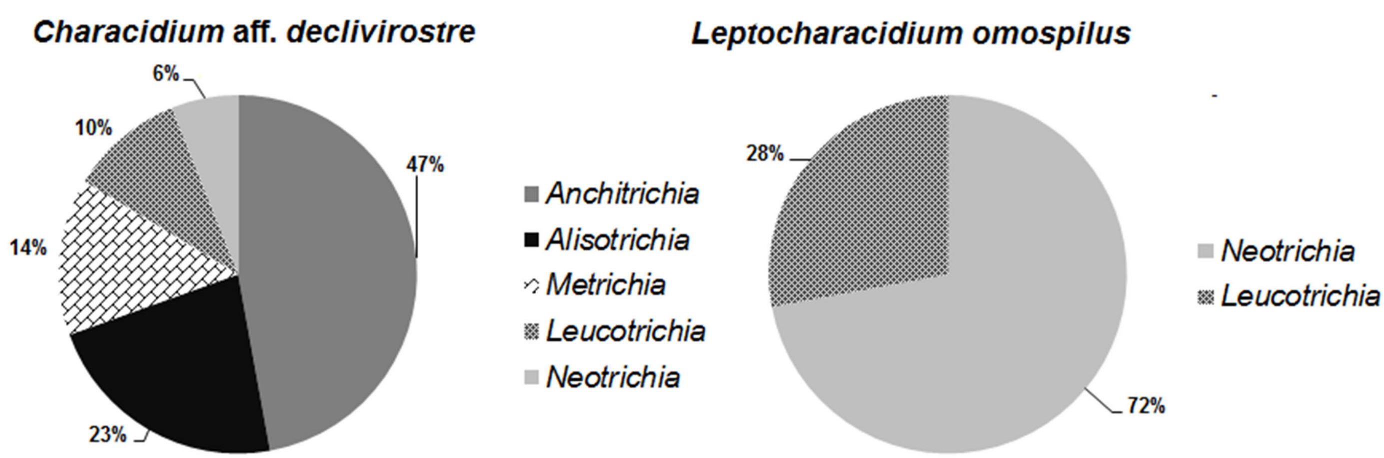

Figure 4: Family Hydroptilidae - Relative volume (%) of genera identified for *Characidium* aff. *declivirostre* and *Leptocharacidium omospilus*.

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specialization (Araújo et al. 2011). The generalist strategy here observed may be favored by the typical seasonality of prey availability found in Neotropical streams (Gerking 1994, Zuanon & Ferreira 2008). Indeed, many of the stream fishes have already been categorized as generalists, consuming the most abundant resources available in their habitats (Esteves & Aranha 1999). The results found in this study were not an exception, in fact, the vast abundance of larvae and pupa of Dipterans detected seem to support such information as they also use to be well represented in rapids (Carvalho 2008, Hamada et al. 2014). Great abundance and richness of aquatic insects such as larvae of Trichoptera (32 genera and 11 families) have also been recorded for the rocky streams of Presidente Figueiredo (Pes 2001). Therefore, and also according to the Optimal Foraging Theory, generalist strategy may be favored because the ingestion of the most available preys will represent a reduction in energy costs when searching and selecting them (Stephens & Krebs 1986).

Many aquatic insects found in the diet of both fish species (e.g., Simulidae, Hydroptilidae and Baetidae) have morphological and physiological characteristics that allow them to remain in fast-flowing waters (Hamada et al. 2014). For example, larvae of Simulidae have adhesive hooks that efficiently adhere to the substratum surface, and larvae of Hydroptilidae attach its shelters to large pieces of submerged wood (Hamada et al. 2014). Thus, here we show evidences that *C.* aff. *declivirostre* and *L. omospilus* are foraging in fast-flowing and turbulent rapids.

Based on the Morisita-Horn Index applied to the taxonomic level of family of the consumed items, a moderate degree of diet overlap was detected between the two syntopic species. This is probably explained by the similar overall composition of items, although the amount of each item is quite distinct between *C.* aff. *declivirostre* and *L. omospilus*. A more in-depth assessment of one of the most important prey families (the Hydroptilidae) has shown a very distinct contribution of genera between species diets. Therefore, despite *C.* aff. *declivirostre* and *L. omospilus* being phylogenetically related species, living in the same habitat, they seem to be, at least partly, segregating its niche dimension (i.e., diet).

Meanwhile, high feeding overlap has been extensively registered between closely related species, and the spatial segregation (e.g., meso or microhabitats) was considered as the most important partitioning mechanism (Schoener 1974, Aranha et al. 1998, Aranha et al. 2000, Mazzoni et al. 2012, Silva et al. 2012, Leitão et al. 2015, Barros et al. 2017).In Amazonian streams, high dietary overlap (60%) was registered between syntopic characids (*Bryconops inpai* and *B. giacopinii*), but they were occupying distinct positions in the water column (Barros et al. 2017). Furthermore, competition between species with similar dietary habits would also be reduced by the high amount of preys found in this same area (Knoppel 1970). However, the few studies developed in Amazonian streams were restricted to those streams with geomorphology typical of the region (i.e., low declivity and current velocity, sandy bottom with trunks and a dense layer of humus) (Santos 2005, Fernandes 2014, Barros et al. 2017). Streams with more homogenous and continuous rocky bottom (i.e., slab) with strong currents, as those of Presidente Figueiredo region, may offer less feeding opportunity and great difficulty of swimming and fish positioning along the water column. So, the co-existence of *C.* aff. *declivirostre* and *L. omospilus* may be favored by their partial partitioning of resources that probably is also reducing the competition between them (Pianka 1973, Schoener 1974). Notwithstanding, further studies on other niche dimensions, such as the use of microhabitats, are certainly desirable to a better elucidation of the mechanisms promoting their co-occurrence in these streams.

Fish feeding studies are crucial for several theoretical and practical aspects. Regardless of the vast biodiversity of Amazonia, such approach is still rare for stream systems. Particularly for rocky streams with rapids, this is the first study that aimed to investigate the fish diet and the importance of such niche dimension for explaining species co-existence, being an important basis for the knowledge of the natural history of the Amazonian ichthyofauna.

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Aquatic oligochaeta (Annelida: Clitellata) in extractive reserve Lake Cuniã, Western Brazilian Amazon

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Abstract: Oligochaeta is one of the most common and abundant taxon in continental aquatic fauna. However, knowledge of their distribution in Brazilian ecosystems is still incomplete and fragmented. Thus, the aim of this study was to develop an inventory of aquatic oligochaetes in the Extractive Reserve of Lake Cuniã in the State of Rondônia, Brazil. Collections of data were performed during the dry (August 2015) and rainy seasons (February 2016). The sediment samples were collected near the lakeside region using the kick sampling method and a kick-net sampler (mesh size of 0.25mm). This paper provides a catalog with 12 taxon from a total of 383 specimens, distributed into two families: Naididae (95.08%) and Opistocystidae (4.92%). The Pristininae subfamily was the most significant (85.68%), followed by subfamilies: Naidinae (8.36%), Tubificinae (0.52%) and Rhyacondrilinae (0.52%). In addition, some species such as *Allonais inaequalis*, *Aulophorus furcatus*, *Dero nivea*, *Pristina synclites*, *Pristina menoni* and *Opistocysta serrata*, were recorded for the first time in the Brazilian Amazon region. Therefore, the results of this study contribute to increase knowledge on the distribution of the Oligochaeta class in Brazil, particularly in the North of the country, which is so extensive and rich in water resources, but not extensively studied.

Keywords: *Biodiversity, aquatic macroinvertebrates, Amazon region, Microdrili.*

Oligoquetos aquáticos (Annelida: Clitellata) na reserva extrativista Lago do Cuniã, Amazônia Ocidental Brasileira

Resumo: Oligochaeta é um dos táxons mais comuns e abundantes na fauna aquática continental. No entanto, o conhecimento da sua distribuição nos ecossistemas brasileiros ainda é incompleto e fragmentado. Assim, o objetivo deste estudo foi desenvolver um inventário de oligoquetos aquáticos na Reserva Extrativista do Lago Cuniã no estado de Rondônia, Brasil. Foram realizadas duas coletas, cobrindo o período seco (agosto 2015) e chuvoso (fevereiro 2016). As amostras de sedimento foram coletadas perto da região marginal do lago pelo método de amostragem *Kick sampling* usando um amostrador *Kick-net* (malha de 0,25 mm). Nós catalogamos 12 táxons do total de 383 espécimes, distribuídos em duas famílias: Naididae (95,08%) e Opistocystidae (4,92%). A subfamília Pristininae foi a mais significativa (85,68%), seguida das subfamílias: Naidinae (8,36%), Tubificinae (0,52%) e Rhyacondrilinae (0,52%). Além disso, algumas espécies, como *Allonais inaequalis*, *Aulophorus furcatus*, *Dero nivea*, *Pristina synclites*, *Pristina menoni* e *Opistocysta serrata*, tiveram o primeiro registro na Amazônia brasileira. Assim, os resultados deste estudo contribuem para aumentar o conhecimento da distribuição da classe Oligochaeta no Brasil, particularmente no Norte do país, que é tão extenso e rico em recursos hídricos, porém pouco estudado.

Palavras-chave: *Biodiversidade, macroinvertebrados aquáticos, região amazônica, Microdrili.*

Introduction

Even though tropical regions are considered the most important in biodiversity, the biotas of these areas are rarely explored and still possess many undescribed species (Lewinsohn & Prado, 2005). In this context, Brazilian invertebrate diversity remains largely unknown, mainly because some groups receive more attention than others (Hortal et al. 2015) and the difficulties in taxonomic identification discourage the development of new studies. This results in fragmented and scarce information on the invertebrate fauna (Magurran, 2011), especially in continental aquatic ecosystems, where the information about this group is even more incomplete (Agostinho et al. 2005).

Aquatic Oligochaeta worms are one of the most abundant organisms in continental aquatic fauna and can be found in sediments, in water columns or associated to other organisms such as molluscs (Gorni & Alves, 2006), aquatic macrophytes (Alves & Gorni, 2007), insect larvae (Corbi et al. 2004), bryophytes (Gorni & Alves, 2007), sponges (Gorni & Alves, 2008a) and amphibians (Oda et al., 2015). It is important to highlight that these organisms are important for organic matter cycling of freshwater ecosystems, bioturbation processes, biomonitoring research, aquatic ecotoxicology and test organisms (Chapman, 2001, Corbi et al. 2015).

However, despite the importance of Oligochaeta in the dynamics of aquatic environments, there have been few scientific studies focused on registering these organisms in Brazilian ecosystems (Gorni & Alves, 2008b). Consequently, Christofferson (2010) affirms that studies on oligochaetes in South America are a pioneer phase.

In the Brazilian Amazon, Du Bois- Reymond Marcus (1947; 1949a; 1949b) and Marcus (1942; 1943; 1944) were the first researchers to register many of the species known in scientific literature. Eventually, some species of Oligochaeta were recorded by Imler (1989) and Collado & Schmelz (2000; 2001). Similarly, organisms of this class have been identified in the Ecuadorian Amazon (Turcotte & Harper, 1982) and Colombian Amazon (Carvajal et al., 2009). However, studies on the Amazon aquatic macrofauna have not evolved to present specific levels of species identification (Cleto-Filho & Walker, 2001, Lopes et al. 2011, Aviz et al. 2012).

This study provides a checklist of aquatic oligochaete in The Extractive Reserve of Lake Cuniã - Rondônia, in the Western Brazilian Amazon, and proposes a catalog of the local species found and their ecological information.

Materials and Methods

1. Area of Study

This study was conducted in the Extractive Reserve of Lake Cuniã (Figure 1), located about 130 kilometers from Porto Velho, on the left bank of the lower Madeira River. With an area of approximately 55.850 hectares, the Reserve has two distinct areas. The first is formed by a piece of land that has a diverse environment with high biodiversity and the second is a floodplain area with seasonal water flow throughout the year. The wet season occurs from November to April and the dry season from May to October.

2. Data collection

Two expeditions were conducted, covering the dry season (August 2015) and the rainy season (February 2016). Sediment samples were collected on seven sites that are near the lakeside region and have floodplain characteristics: S1 (08° 20' 15.5"S 63° 31' 38.5" W), S2 (08° 20' 08.9" S 63° 31' 04.8" W), S3 (08° 19' 44.3" S 63° 31' 23.4" W), S4 (08° 19' 37,3" S 63° 30' 29.2" W), S5 (08° 19' 10.9" S 63° 30' 01.9" W), S6 (08° 19' 22.8" S 63° 29' 29.5" W) and S7 (08° 18' 40.0" S 63° 29' 19.0" W). These sites are periodically inundated by the overflow of the lake and present a specific diversity of morphologically adapted vegetation that tolerate this seasonal flood pulse (Martinez & Le Toan, 2006). Furthermore, these sites are organically rich, due to the large amounts of organic substances, originated from local vegetation, that are dissolved in flood water (Junk, 1997). Sample collection was done using the kick sampling method and a kick-net sampler (mesh size of 0.25mm) as described by Alves et al. (2008) and Docile et al. (2016).

Samples were oxygenated with aquarium pumps to maintain the organisms living during the screening process. For organism selection, small portions of the samples were put into translucent trays containing water and subsequently fixed in 10% formalin and preserved in 70% alcohol (Alves & Gorni, 2007). Semi-permanent slides were prepared and the taxonomic criteria adopted by Righi (1984) and Brinkhurst & Marchese (1989) were followed in order to identify Oligochaeta. The identified specimens were deposited in the Zoological Collection of the University of Araraquara (UNIARA). Water temperature, dissolved oxygen, pH and electrical conductivity were measured in each site of the lake with a multiparameter sensor (YSI 5560). The concentrations of organic matter were determined

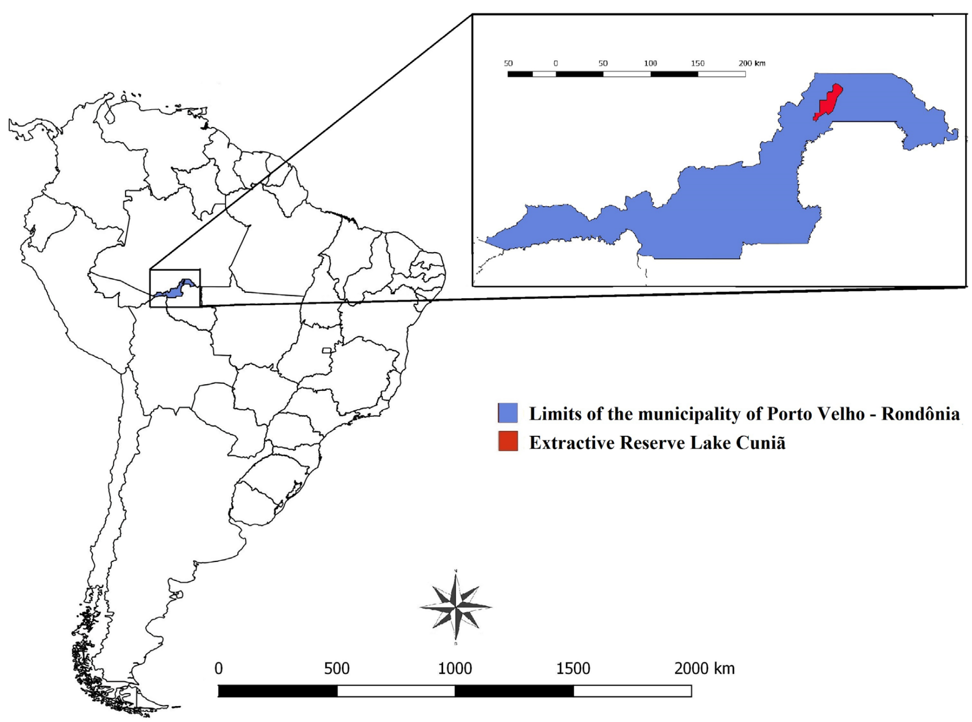

Figure 1. Location of Extractive Reserve Lake Cuniã in the state of Rondônia, Brazil.
Source: Own author (2016)

by mass loss. For this purpose, sediment samples were dried for 12 hours at 60 °C and then ignited at 550 °C for 5 h, according to Maitland (1979).

Information on oligochaeta species in Brazil were searched for in the following databases: Scopus, Google Scholar, and Scielo, with no date filter. Papers by Marcus (1942, 1943, 1944) and Christoffersen (2007) were also consulted for obtaining additional data.

Results

The dissolved oxygen in the rainy season reached a maximum of 5.250 mg/L in S5, while the minimum for the dry season was 1.950 mg/L in S6. The oxygen concentration measured in the Cuniã Lake followed a pattern previously found by Almeida & Melo (2009) in Amazon lakes. Temperature was high, electrical conductivity was low, especially during the dry season, and the pH was acid at all sites of the lake. Regarding organic matter, sites S6 and S7 presented the highest concentrations throughout the year and presented a small increase during the rainy season (Table 1).

This study provides a catalog with 12 taxa from a total of 383 specimens, distributed in two families: Naididae (95.08%) and Opistocystidae (4.92%). The subfamily Pristininae was the most significant (85.68%), followed by subfamilies: Naidinae (8.36%), Tubificinae (0.52%) and Rhyacondrilinae (0.52%) (Table 2).

The *Dero* sp. species was only collected in dry season samples, but we were not able to properly count the number of gills, making it impossible to identify the species level. Similarly, the *Bothrioneurum* sp. species and immature tubificinae were not identified at more specific levels. *Pristina* sp.1 showed a different dorsal needle setae compared to the species already described in literature, and may be a new species of the genus.

1. List and considerations about species

Allonais inaequalis (Stephenson, 1911) (Figure 2a)

A species common in freshwater ecosystems of tropical and subtropical regions (Timm, 1999, Suriano-Affonso et al. 2011). In Brazil, it has been found in the State of São Paulo associated with other organisms such as gastropods (Gorni & Alves, 2006), aquatic macrophytes (Alves & Gorni, 2007), and sponges (Gorni & Alves, 2008a). It has also been recorded in an urban stream by Alves et al. (2006).

Aulophorus furcatus (Müller, 1774) (Figure 2b)

A cosmopolitan species, characterized by the presence of gills and palps in the posterior region of its body (Brinkhurst & Marchese, 1989). Its occurrence in Brazilian aquatic environments has been registered in the States of São Paulo, Paraná, Minas Gerais, Pernambuco and Rio

Table 1 - Mean and standard deviation of the environmental variables (measured in the dry season in August 2015 and rainy season in January 2016). W.T.: Water Temperature (ºC); E.C.: Electrical Conductivity (µs/cm); D.O.: Dissolved Oxygen (mg/L) and pH; O.M.: Organic matter (%).

				Dry			
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
W.T (ºC)	28.20 (1.903)	29.28 (0.080)	29.21 (0.067)	29.52 (0.076)	29.21 (0.042)	29.70 (0.017)	29.44 (0.100)
E. C. (µs/cm)	10.00 (0.000)	10.00 (0.000)	11.00 (0.000)	26.33 (0.577)	19.00 (0.000)	41.33 (0.577)	25.00 (0.000)
D. O. (Mg/L)	3.540 (0.606)	3.450 (0.334)	3.830 (0.204)	3.880 (0.040)	4.780 (0.153)	1.430 (0.149)	2.350 (0.078)
pH	4.263 (0.042)	4.320 (0.185)	4.397 (0.045)	4.667 (0.032)	4.730 (0.017)	4.933 (0.075)	4.750 (0.044)
O.M (%)	8.6 (7.6)	10.3 (7.6)	9.5 (1.3)	19.8 (6.8)	5.7 (3.1)	19.4 (10.6)	15.1 (2.4)
				Rainy			
W. T. (ºC)	29.10 (0.091)	29.23 (0.031)	29.27 (0.070)	28.91 (0.119)	29.11 (0.292)	30.27 (0.363)	29.70 (0.605)
E. C. (µs/cm)	31.00 (1.000)	25.00 (1.732)	31.67 (1.528)	55.00 (1.000)	61.00 (0.000)	66.67 (1.155)	69.67 (1.155)
D. O. (Mg/L)	4.030 (0.085)	4.60 (0.254)	4.90 (0.023)	4.170 (0.178)	5.250 (0.131)	1.950 (0.116)	2.840 (0.055)
pH	4.883 (0.055)	4.817 (0.072)	4.937 (0.059)	5.060 (0.040)	5.187 (0.050)	5.317 (0.075)	5.400 (0.026)
O.M (%)	5.7 (2.0)	5.7 (2.8)	10.9 (7.3)	7.7 (6.8)	12.9 (9.3)	20.9 (10.2)	16.3 (10.7)

Table 2: Oligoqueta taxa registered in the Extractive Reserve Lake Cuniã.

Season				**Dry**							**Rainy**			
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Naididae Family														
Naidinae Subfamily														
Allonais inaequalis													x	x
Aulophorus furcatus		x		x										
Aulophorus costatus														x
Dero digitata								x						
Dero nivea								x		x		x		
Dero sp.				x	x	x								
Pristininae Subfamily														
Pristina synclites		x						x			x	x		x
Pristina menoni									x					
Pristina sp.1					x									
Tubificinae Subfamily														
Immature Tubificinae						x								
Rhyacodrilinae Subfamily														
Bothrioneurum sp.				x										
Opistocystidae Family														
Opistocysta serrata								x			x			

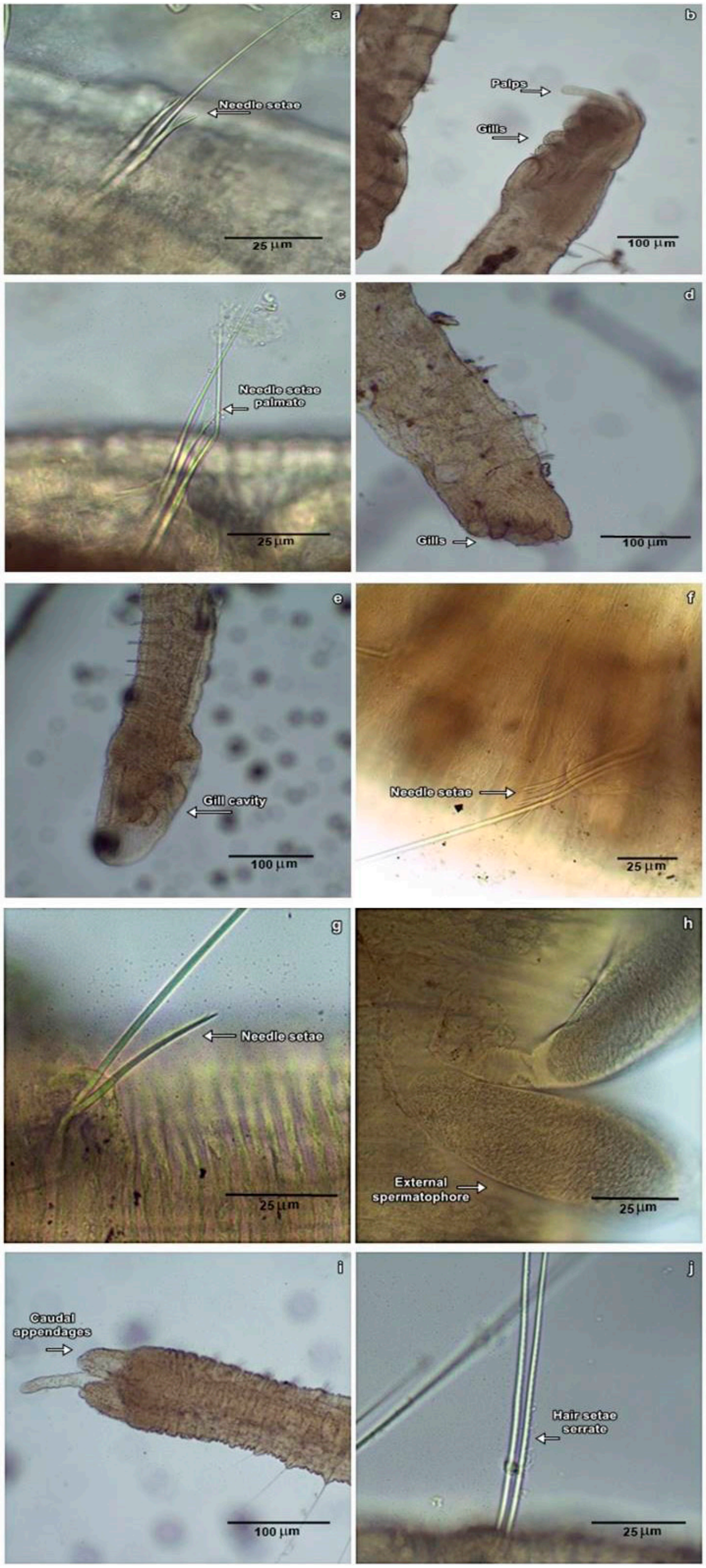

Figure 2. a: Needle setae of *Allonais inaequalis*; **b**: Gill cavity of *Aulophorus furcatus*; **c**: Needle setae palmate of *Aulophorus costatus*; **d**: Gills of *Dero digitata*; **e**: Gills of *Dero nivea*; **f**: Needle setae of *Pristina synclites*; **g**: Needle setae of *Pristina menoni*; **h**: External spermatophore of *Bothrioneurum* sp.; **i**: Caudal appendages of *Opistocysta funiculus*; **j**: Hair setae serrate of *Opistocysta funiculus*.

Grande do Sul. It also frequently lives in association with other organisms such as Nymphaea (Marcus, 1944), gastropods (Gorni & Alves, 2006), macrophytes (Montanholi-Martins & Takeda, 2001) and also to decomposing macrophyte leaves (Martins et al. 2011). It has been already detected in floodplain habitats (Ragonha & Takeda, 2014), in streams of preserved areas (Rodrigues et al. 2013) and in irrigated rice fields (Stenert et al. 2012).

Aulophorus costatus Du Bois-Reymond Marcus,1944 (Figure 2c)

Its main characteristic is the palmate dorsal needle seta and gill cavity with two pairs of gills (Brinkhurst & Marchese, 1989). Registration in Brazil has already occurred in the States of Amazonas and Pará, as well as in São Paulo, in association with other organisms such as macrophytes (Alves & Gorni, 2007) and sponge specimens (Gorni & Alves, 2008a).

Dero digitata (O. F. Müller, 1773) (Figure 2d)

In Brazil, this species has been registered in the State of São Paulo, in association with gastropods (Gorni & Alves, 2006) and macrophytes (Alves & Gorni, 2007), in reservoirs (Pamplin et al. 2005) and in urban streams (Alves & Lucca, 2000). In the Amazon region, it has been registered in many rivers in the State of Pará, such as Tapajós River, Cururu River, Acara River, Cupari River, Juruena River and São Samuel River (Marcus 1942, Marcus, 1944, Du Bois-Reymond Marcus, 1947, 1949a, 1949b). It has also occurred in areas of rice fields in Rio Grande do Sul (Stenert et al. 2012).

Dero nivea Aiyer 1930 (Figure 2e)

This species is cosmopolitan and is mainly characterized by its prolonged gill cavity (Brinkhurst & Marchese, 1989). Its incidence in Brazil is more significant in the State of São Paulo, but has also been recorded in Rio Grande do Sul. Like other species of Oligochaeta, it can live in association with other organisms, but is especially found on aquatic vegetation (Correia & Trivinho-Strixino, 1998, Trivinho-Strixino et al. 2000, Alves & Gorni, 2007).

Pristina synclites Stephenson, 1925 (Figure 2f)

The records of this species occurrence are in the State of São Paulo, where it was found in urban streams with low concentration of dissolved oxygen (Alves et al. 2006) and in organically enriched reservoirs (Fusari & Fonseca-Gessner, 2006).

Pristina menoni (Aiyer, 1929) (Figure 2g)

Species with records only in São Paulo and Paraná. It has been recorded that it associates with bryophytes (Gorni & Alves, 2007). It has also been found in urban stream waters in São Paulo (Alves et al. 2006) and in the Ivinhema River in Paraná (Montanholi-Martins and Takeda, 2001).

Bothrioneurum sp. (Figure 2h)

This genus is mainly characterized by the presence of a sensitive organ in the prostomium and the presence of an external spermatophore (Brinkhurst & Marchese, 1989). Its occurrence has been cataloged in the States of São Paulo, Minas Gerais, Amazonas, Pará and Rio Grande do Sul. It also occurs mainly in the sediments of degraded sites (Alves & Lucca, 2000; Suriani et al. 2007) and in the streams of preserved areas (Rodrigues et al. 2013). In the south of the country, it has been collected in rice fields (Stenert et al. 2012). In the Amazon region, it was found in Pará, near the County of Belterra, by Marcus (1942) and Du Boys-Reymond Marcus (1947, 1949a and 1949b) and in Central Amazon by Irmler (1989) and Collado & Schmelz (2000; 2001).

Opistocysta serrata Harman, 1970 (Figure 2i, j)

Serrate hair seta, the presence of proboscis and three (one median and two lateral) caudal appendages (Brinkhurst & Marchese, 1989) characterize this species. In Brazil it was collected only in the States of Paraná (Montanholi-Martins & Takeda, 2001) and Mato Grosso do Sul (Takeda et al., 2000).

Discussion

This study showed the occurrence of 12 taxa, representing approximately 13.95% of 86 species registered in Brazil (Christoffersen, 2007). In addition, this was the first registration of some species, such as *Allonais inaequalis*, *Aulophorus furcatus*, *Dero nivea*, *Pristina synclites*, *Pristina menoni* and *Opistocysta serrata*, in the Brazilian Amazon region. According to Christoffersen (2010) the catalogued oligochaetes from South America represent only a fraction of their true diversity, which emphasizes the need for more studies that contemplate species inventory in neotropical regions. Most of the global biodiversity data still comes from temperate regions and refer mainly to important and economically valuable taxa. These shortfalls in biodiversity information need to be recognized and quantified for more accurate conservation assessments and actions (Hortal et al. 2015).

In the aquatic community of Oligochaeta, the Naididae family stands out as the most abundant and diverse, consisting of eight subfamilies and comprising about 50% of the described species on the planet (Rodriguez & Reynoldson, 2011). Moreover, according to Martin (1996) Naididae is a cosmopolitan family, which is very common in lakes. In this study, this family was the most abundant (95.08%), mainly represented by the Pristininae subfamily (85.68%). Some species are periphytic naidids (*Pristina*), some have gills and respiratory appendages that allow inhabiting systems where oxygen is a limiting factor (*Dero*, *Aulophorus*) and others are more often found in sediment (Tubificinae, *Bothrioneurum*) (Martin, 1996).

From a regional perspective, the State of São Paulo has the highest species richness, with 75 species recorded (Gorni et al. 2015), but little is known about these species in other Brazilian States, which reinforces the importance of studies that seek to verify the occurrence of Oligochaeta in other places. Only recent inventories conducted in States, such as Minas Gerais (Rodrigues et al. 2013) and Rio Grande do Sul (Stenert et al. 2012) have started to change this scenario.

The results of this research will contribute to increase knowledge on Oligochaeta in tropical ecosystems, since some species were reported for the first time in the Western Brazilian Amazon. In this context, it is important to promote other biodiversity studies in areas with difficult access, because according to Hortal et al. (2007) species inventories are more frequent in places that offer more research infrastructure and logistics.

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New records of fruit trees as host for *Neosilba* species (Diptera, Lonchaeidae) in southeast Brazil

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GISLOTI, L. J., UCHOA, M. A., PRADO, A. **New fruit trees host for *Neosilba* (Diptera, Lonchaeidae) species in southeast Brazil**. Biota Neotropica. 17(1): e20160213. http://dx.doi.org/10.1590/1676-0611-BN-2016-0213

Abstract - Fruits of thirty-five cultivated native plant species (19 orders and 12 families) were sampled in farms of fruit production from two municipalities of São Paulo state, Brazil (January 2010 to March 2012) to evaluate species diversity of *Neosilba* flies. Thirty-one species of plants were the host for *Neosilba* species while four were not infested. Some aspects of the biology and patterns of species diversity, abundance, infestation rates, puparias viability and the interactions among species of frugivorous flies and their host plants were quantified. Seven species of *Neosilba* were reared: *Neosilba bella* Strikis & Prado (4 hosts), *Neosilba certa* (Walker) (4 hosts), *Neosilba glaberrima* (Wiedemann) (5 hosts), *Neosilba inesperata* Strikis & Prado (6 hosts) *Neosilba pendula* (Bezzi) (15 hosts), *Neosilba pradoi* Strikis & Lerena (8 hosts) and *Neosilba zadolicha* McAlpine (26 hosts). The association between the lance flies and the host fruit species is discussed.

Keywords: *Hosts; Lance Flies; Lonchaeid; Natural history.*

Novos registros de espécies de plantas hospedeiras para espécies de *Neosilba* (Diptera, Lonchaeidae) no sudeste do Brasil

Resumo: Foram coletados frutos de trinta e cinco espécies de plantas nativas (19 ordens e 12 famílias) de janeiro de 2010 a março de 2012, em pomares de produção de frutos em dois municípios do estado de São Paulo, Brasil, à fim de avaliar a diversidade de espécies de *Neosilba*. Trinta e uma espécies das plantas amostradas foram colonizadas por espécies de *Neosilba*, enquanto quatro não estavam infestadas. Foram quantificados alguns aspectos da biologia, dos padrões de diversidade de espécies, abundância, taxas de infestação, viabilidade pupal e aspectos da interação entre espécies de moscas frugívoras e suas plantas hospedeiras. Sete espécies de *Neosilba* foram criadas: *Neosilba bella* Strikis & Prado (4 plantas hospedeiras), *Neosilba certa* (Walker) (4 plantas hospedeiras), *Neosilba glaberrima* (Wiedemann) (5 plantas hospedeiras), *Neosilba inesperata* Strikis & Prado (6 plantas hospedeiras) *Neosilba pendula* (Bezzi) (15 plantas hospedeiras), *Neosilba pradoi* Strikis & Lerena (8 plantas hospedeiras) e *Neosilba zadolicha* McAlpine (26 plantas hospedeiras). A associação entre as espécies de *Neosilba* e as espécies de frutas hospedeiras é discutida.

Palavras-chave*:* *frutos hospedeiros; moscas das frutas; lonqueídeos; história natural.*

Introduction

The Neotropical genus *Neosilba* McAlpine (Diptera: Lonchaeidae) is comprised of fly species whose larvae feeds on many species of commercially important fruit species (Araújo & Zucchi 2002, Strikis & Prado 2005, Bittencourt et al. 2006). The genus is restricted to the Neotropical region, being known from Caribe, Mexico, and Colombia to Brazil. Forty species were described so far and at least more 60 species are waiting for descriptions (McAlpine & Steyskal 1982, Strikis 2011; Galeano-Olaya & Canal 2012).

Although of the economic importance of some species of *Neosilba* that occur as pests on fruit and vegetables in several countries, such as Colombia (Steyskal 1978, Peñaranda et al. 1986), Peru (Korytkowski & Ojeda 1971), Costa Rica (Sánchez et al. 1991), and Brazil, the knowledge of these dipterans is still very scarce (Uchoa et al. 2002).

Neosilba species obtains food resource in 113 plant species distributed in 39 families: Anacardiaceae, Annonaceae, Apocynaceae, Arecaceae, Bignoniaceae, Bombacaceae, Cactaceae, Caricaceae, Chrysobalanaceae, Combretaceae, Convolvulaceae, Cucurbitaceae, Ebenaceae, Euphorbiaceae, Fabaceae, Flacourtiaceae, Gnetaceae, Lauraceae, Lecythidaceae, Loganiaceae, Loranthaceae, Malpighiaceae, Malvaceae, Melastomataceae, Moraceae, Musaceae, Myrtaceae, Olacaceae, Oxalidaceae, Passifloraceae, Rhamnaceae, Rosaceae, Rubiaceae, Rutaceae, Sapotaceae, Solanaceae, Ulmaceae, Verbenaceae, and Vitaceae, confirming the relevance of this genus of fruit flies to tropical fruit and horticulture production (McAlpine

& Steyskal 1982, Raga et al. 1996, Raga et al. 2015, Araújo & Zucchi 2002; Uchoa et al. 2012).

Studies on the biology and ecology of economically important species of fruit flies have contributed for the management and control of agricultural pests (Carey 1993, Vargas et al. 2001, Papadopoulos et al. 2002). Integrated pest management has been more difficult by the lack of basic studies on taxonomy, biology, and ecology. In Brazil, records on the genera of Lonchaeidae associated with fruits are scarce. Regional surveys are very important because they can provide basic information for managing insect pest populations and their natural enemies (Uchoa et al. 2002).

Our hypothesis is that there are new associations between *Neosilba* species and native host fruit species in the southeast of Brazil.

The aim of this study was to provide original information on fruit infestation rates, puparia's viability and interactions with species of host plants by *Neosilba* species in fruits sampled in the southeast of Brazil.

Material and Methods

Characterization of the study area. These orchards are located in an important fruit producing area in the state of São Paulo, Brazil. According to Setzer (1976), the regions are inserted in a climatic transition between very humid subtropical with marked dry seasons (Mu-Cw), with mean temperatures around 24ºC and minimum temperatures around 16ºC in the summer. Mean annual precipitation is about 1,300 mm/year and the predominant soil type in the studied areas is Latosol with good aeration, permeability and drainage.

Collecting of the Host Fruits. Fruits from 35 species (Table 1) were sampled from January 2010 to March 2012, in fruit farms from the municipalities of Campina do Monte Alegre (23º 53' 37" S, 48º 51' 06" W, 612m) (site 1), and Paraibuna (23º 27' 94" S, 45º 42' 88" W, 647m) (site 2). On a monthly basis fruits were collected from each studied area. The mature fruits were collected directly from the plant and the amount of

Table 1. Indices of infestation and puparia's viability of *Neosilba* species (Diptera, Lonchaeidae) in 35 cultivated native host fruits from southeast Brazil (January 2010 to March 2012).

Plant Taxa						*Neosilba* male species							Indices/Rate		
	Collecting sites	Nº of fruits	Mass of fruits (g)	Pupae (n)	Emerged adults	*N. bella*	*N. certa*	*N. glaberrima*	*N. inesperata*	*N. pendula*	*N. pradoi*	*N. zadolicha*	Total fruit infestation[b]	Fruit infestation[c]	Puparia viability (%)
Anacardiaceae															
Spondias mombim L.	2	620	11,160	384	289	-	48	-	-	22	-	83	0.62	0.03	75.26
Spondias purpurea L.	2	460	3,220	189	102	-	-	-	-	17	-	50	0.41	0.06	53.97
Spondias tuberosa Arruda	2	1,280	19,200	412	302	-	-	-	-	-	-	127	0.32	0.02	73.30
Spondias venulosa Mart.	1	367	5505	113	81	-	-	-	-	-	-	54	0.31	0.02	71.68
Annonaceae															
Rollinia sylvatica (A. St.-Hil.)	1	67	5,360	64	50	-	-	6	-	-	-	12	0.96	0.01	78.13
Apocynaceae															
Hancornia speciosa Gomes	1	543	10,860	184	101	-	-	-	-	-	-	51	0.34	0.02	54.89
Arecaceae															
Bactris gasipaes Kunth	1	521	26,050	101	78	-	-	12	-	-	-	32	0.19	0.00	77.23
Cactaceae															
Selenicereus setaceus (Rizz)	2	62	7,740	37	25	-	-	12	-	-	-	-	0.60	0.00	67.57
Caryocaraceae															
Caryocar brasiliense Camb.	2	82	7,380	189	134	-	-	-	-	42	-	12	2.30	0.03	70.90
Fabaceae															
Inga vera Willd.	1	512	7,680	201	171	-	10	-	-	14	8	53	0.39	0.03	85.07
Malphigiaceae															
Byrsonima crassifolia (L.)	2	843	1,011	234	189	30	-	-	-	-	-	71	0.28	0.23	80.77
Malpighia emarginata DC.	2	1,384	4,152	389	302	11	-	37	53	21	12	33	0.28	0.09	77.63
Myrtaceae															
Acca sellowiana (Berg.)	2	100	5,178	42	21	-	-	-	-	-	-	7	0.42	0.01	50.00
Campomanesia aurea Berg.	2	189	4,725	47	19	-	-	-	-	-	8	-	0.25	0.01	40.43
Campomanesia guazumaefolia (Camb.)	1	112	2,688	45	21	-	-	-	-	-	3	6	0.40	0.02	46.67
Campomanesia phaea (Berg.)	1	229	8,450	79	54	-	-	-	-	-	9	12	0.34	0.01	68.35
Campomanesia sessiflora (Berg.)	1	340	6,667	-	-	-	-	-	-	-	-	-	-	-	-
Eugenia brasiliensis Lam.	2	670	1,150	231	120	-	-	-	-	12	-	31	0.34	0.20	51.95
Eugenia dysenterica (DC.)	2	578	8,670	289	201	-	-	-	43	20	11	40	0.50	0.03	69.55
Eugenia involucrata DC.	2	539	1,340	211	160	-	-	-	-	12	-	61	0.39	0.16	75.83

[a]Site 1: Campina do Monte Alegre; site 2: Paraibúna (São Paulo, Brazil);
[b]Number of puparia/number of fruit;
[c]Number of puparia/weight of fruits (in grams).

Table 1. Continued...

Plant Taxa						*Neosilba* male species							Indices/Rate		
	Collecting sites	Nº of fruits	Mass of fruits (g)	Pupae (n)	Emerged adults	*N. bella*	*N. certa*	*N. glaberrima*	*N. inesperata*	*N. pendula*	*N. pradoi*	*N. zadolicha*	Total fruit infestation[b]	Fruit infestation[c]	Puparia viability (%)
Eugenia matosii Legr.	1	501	8,020	-	-	-	-	-	-	-	-	-	-	-	-
Eugenia neonitida Sobral	1	1,279	3,837	467	321	-	-	-	-	27	-	102	0.37	0.12	68.74
Eugenia pitanga (Berg.)	1	1,021	3,063	312	204	-	-	-	-	-	-	81	0.31	0.10	65.38
Eugenia pyriformis Cambess	2	1,342	8,910	380	289	-	-	-	-	-	46	101	0.28	0.04	76.05
Eugenia stipitata McVaugh	2	234	3,880	98	69	2	-	-	-	31	-	-	0.42	0.00	70.41
Eugenia uniflora L.	2	2,295	4,704	467	332	8	-	-	119	-	-	22	0.20	0.10	71.09
Myrciaria dubia McVaugh	2	829	9,119	231	167	-	-	-	-	-	-	61	0.28	0.03	72.29
Myrciaria jaboticaba Baill.	2	801	8,604	-	-	-	-	-	-	-	-	-	-	-	-
Psidium cattleianum Sabine	1	876	5,310	465	398		22		34	17	27	101	0.53	0.09	85.59
Psidium guajava L.	1	335	33,890	189	89	-	10	-	5	4	-	21	0.56	0.01	47.09
Psidium guineense Swartz.	2	378	60,480	168	102	-	-	-	-	2	-	41	0.44	0.00	60.71
Rhamnaceae															
Ziziphus joazeiro Mart.	1	521	2,605	232	171	-	-	-	-	68	-	-	0.45	0.09	73.71
Rosaceae															
Rubus urticaefolius Poir	1	594	3564	281	188	-	-	-	33	31	-	-	0.47	0.08	66.90
Sapotaceae															
Pouteria caimito (Ruiz and Pav.)	2	549	1,6470	179	102	-	-	42	-	-	-	24	0.33	0.01	56.98
Pouteria macrophylla (Lam.)	2	443	15071	-	-	-	-	-		-	-	-	-	-	-
Total	-	21,496		6,910	4,852	51	90	109	287	340	124	1,289	-	-	-
Mean	-	-	-	-	-	-	-	-	-	-	-	-	0.46	0.05	67.23

[a]Site 1: Campina do Monte Alegre; site 2: Paraibúna (São Paulo, Brazil);
[b]Number of puparia/number of fruit;
[c]Number of puparia/weight of fruits (in grams).

fruits collected in each sample varies depending on the availability of fruit at the time of sampling. Were collected about 50 fruits per specie plant on average. The samples (fruits individualized) were kept at room temperature and humidity, stored in plastic boxes (25 × 50 × 10 cm) sealed with nylon organza and lined with moist, autoclaved fine sand (± 2 cm layer) as a substrate for larval pupation. After the larvae had pupated 15 to 25 d after the material was brought in from the field, the substrate was sieved to collect the puparia, which were counted and transferred to emergence boxes kept under the same environmental conditions. Daily over a period of 40 d, substrate humidity was checked and the emergence of flies and parasitoids was monitored. The emerged parasitoids were fed with honey and water for 3 d, to fix the coloring that would allow their correct identification. They were then killed and preserved in 85% ethanol in labeled flasks for subsequent counting and species identification (Uchoa & Zucchi 1999).

Fly Identification. The adults were identified in the Departamento de Biologia Animal, Instituto de Biologia, Universidade Estadual de Campinas (Unicamp), Campinas-SP, by the first author. *Neosilba* species were identified using keys and original descriptions (Korytkowski & Ojeda 1971, McAlpine & Steyskal 1982, Strikis 2011, Galeano-Olaya & Canal 2012). Only males were used since the group taxonomy is based on analysis of male genitalia (McAlpine & Steyskal 1982). Plant species were identified by botanists at the Departamento de Botânica, Universidade Estadual de Campinas (Unicamp), Campinas, SP, Brazil. Voucher specimens of the insects (stored in 85% alcohol) were deposited at Coleção Zoológica (ZUEC), Universidade Estadual de Campinas (UNICAMP), Campinas, SP, Brazil.

Quantitative variables, infestation indexes, and viability of the puparia. Two quantitative variables were evaluated: the number of puparia and of emerged flies. The fruit infestation levels were evaluated by two indexes: number of puparia/fruit, and the number of puparia/mass (g) of fruit. The viability of the puparia was calculated by the equation: %V = No. of EA/ PUP) X 100, where: %V = Percent of viability), No. of EA = number of emerged adults, and PUP =A total number of puparia; and the quotient was multiplied by 100.

Faunistic analysis. The faunistic analysis of *Neosilba* species was conducted according to Silveira-Neto et al. (1976). The following parameters were estimated: frequency, dominance, abundance, and constancy, using the Anafau software developed by the Departamento de Entomologia, Fitopatologia e Zoologia Agrícola, ESALQ/USP (Moraes et al. 2003):

Frequency: Number of individuals of one species divided by the total number of individuals in the sample.

Dominance: The ratio given by the number of individuals of a given species divided by the number of individuals of all collected species. A species is considered dominant when its frequency is higher than 1/S. (S = total number of species in the community). Species classification according to dominance: Super-dominant (sd): number of individuals is higher than the upper limit of the 5% confidence interval (CI). Dominant (d): number of individuals is within the range of the 5% CI. Non-dominant (nd): number of individuals is lower than the lower limit of the 5% CI.

Abundance: Refers to the number of individuals of a given taxonomical category per unit of surface or volume. Species abundance was classified into five categories, as follows: Super-abundant (sa): number of individuals is higher than the upper limit of the 1% CI; Very abundant (va): number of individuals is situated between the upper limits of the 5% and 1% confidence intervals; Common (c): number of individuals is within the

5% CI range; Dispersed (d): number of individuals is situated between the lower limits of the 5% and 1% confidence intervals, and Rare (r): number of individuals is lower than the lower limit of the 1% CI.

Constancy: Percentage of samples in which a given species is present. Species constancy was classified into three categories: Constant (w): when the species was present in more than 50% of collections; Accessory (y): when the species was present in 25% to 50% of collections; Accidental (z): when the species was present in less than 25% of the collections.

Results

Host Fruit Species. Seven *Neosilba* species were reared: *N. bella* Strikis & Prado, *N. certa* (Walker), *N. glaberrima* (Wiedemann), *N. inesperata* Strikis & Prado, *N. pendula* (Bezzi), *N. pradoi* Strikis & Lerena and *N. zadolicha* McAlpine. The highest number of individuals belong to *N. zadolicha* (1,289 individuals), followed by *N. pendula* (340). Both species are generalists, infesting 26 species of host fruits. *N. bella* was less abundant (51 individuals) (Table 1).

Out of 35 fruit species sampled, belonging to 12 different families, were obtained 2290 frugivorous flies from 31 plant species. No adult of *Neosilba* emerged from four host fruit species: *Campomanesia sessiflora* (Berg.), *Eugenia matosii* Legr., *Myrciaria jaboticaba* Baill. (Myrtaceae), and *Pouteria macrophylla* (Lam.) (Sapotaceae) (Table 1).

N. bella (n = 51) was the only species that occurred in only one site (site 2), and this species had the lowest number of host fruit (4 species) from two plant families: *Byrsonima crassifolia* (L.), *Malpighia emarginata* DC. (Malphigiaceae), *Eugenia stipitata* McVaugh and *Eugenia uniflora* L. (Myrtaceae).

N. certa (90 individuals) also had four species of host fruits, occurring in three plant family: *Spondias mombin* L. (Anacardiaceae), *Inga vera* Willd. (Fabaceae), *Psidium cattleianum* Sabine, and *Psidium guajava* L. (Myrtaceae).

N. glaberrima (n = 109) hosted five fruit species from five families: *Rollinia sylvatica* (A. St.-Hil.) (Annonaceae), *Bactris gasipaes* Kunth (Arecaceae), *Selenicereus setaceus* (Rizz) (Cactaceae), *M. emarginata* (Malpighiaceae), and *Pouteria caimito* (Ruiz & Pav.)(Sapotaceae) (Table 1).

N. inesperata (n = 287) was found in six host fruit from three families: *Malpighia emarginata* (Malpighiaceae), *Eugenia dysenterica* (DC.), *E. uniflora*, *P. cattleianum*, *P. guajava* (Myrtaceae), and *Rubus urticaefolius* Poir (Rosaceae) (Table 1).

N. pendula (n = 340) infested 15 host fruit species, from seven plant family: *S. mombin*, *Spondias purpurea* L. (Anacardiaceae), *Caryocar brasiliense* Camb. (Caryocaceae), *I. vera* (Fabaceae), *M. emarginata* (Malpighiaceae), *Eugenia brasiliensis* Lam., *E. dysenterica*, *Eugenia involucrata* DC.,*Eugenia neonitida* Sobral, *E. stipitata*, *P. cattleianum*, *P. guajava*, *Psidium guineensis* Swartz. (Myrtaceae), *Ziziphus joazeiro* Mart. (Rhamnaceae), and *R. urticaefolius* (Rosaceae)

N. pradoi (n = 124) was recovered from eight fruit species, from three families: *I. vera* (Fabaceae), *M. emarginata* (Mapighiaceae), *Campomanesia aurea* Berg.,*Campomanesia guazumaefolia* (Camb.), *Campomanesia phaea* (Berg.), *E. dysenterica*, *Eugenia pyriformis* Cambess and *P. cattleianum* (Myrtaceae).

N. zadolicha (n = 1,289) occurred in 25 fruit species from seven plant family: *S. mombim*, *S. purpurea*, S. purpurea, *Spondias tuberosa* Arruda, *Spondias venulosa* Mart. (Anacardiaceae), *Rollinia silvatica* (Annonaceae), *Hancornia speciosa* Gomes (Apocynaceae), *B. gasipaes* (Arecaceae), *C. brasiliense* (Caryocaraceae), *I. vera* (Fabaceae), *B. crassifolia*, *M. emarginata* (Malpighiaceae), *Acca sellowiana* (Berg.), *C. guazumaefolia*, *C. phaea*, *E. brasiliensis*, *E. dysenterica*, *E. involucrata*, *E. neonitida*, *Eugenia pitanga* (Berg.), *E. pyriformis*, *E. uniflora*, *Myrciaria dubia* McVaugh, *P. cattleianum*, *P. guajava*, *P. guineensis* (Myrtaceae), and *P. caimito* (Sapotaceae).

Malpighiae marginata was the host fruit species with the highest *Neosilba* diversity: six species, being all of them found in this work, with the exception of *N. certa.* Strikis et al. (2011) also found no association record between *N. certa* and *Malpighia* sp. in his survey about frugivorous flies in the Amazon Rain Forest. *P.cattleianum* was the host with the largest number of *Neosilba* specimens, from which emerged 398 individuals (males + females).

Infestation Indexes and Puparia's Viability. The mean of infestation was: 0.46 (puparia / total fruit) and 0.05 (puparia / fruit weight in grams). The mean of the viability of the puparia was 67.23%. *C. brasiliensis* was the plant species that showed the highest infestation indexes based on the number of fruit (2.30 puparia / fruit) and, *B. crassifolia* was the plant species presented the highest infestation indexes based on the mass of fruit (0.23 puparium/ mass in grams).

Faunistic analysis. The faunistica analysis is shown in Table 2**.** The Shannon diversity index (H), Equitability index (E) and Margalef (α) indexes were similar between the two areas. *Neosilba zadolicha* was the most frequent, most dominant and the most abudant specie. *Neosilba bella* was the less frequent specie. Faunistic indices classified all species as "accidental". (Table 2).

Table 2. Faunistic analysis of *Neosilba* spp. (Diptera, Lonchaeidae) in southeast Brazil (January 2010 to March 2012).

Species	Area 1	Area 2	Total	F[1]	C[2]	Do[3]	A[4]
Neosilba bella	51	0	51	Lf	Z	D	d
Neosilba certa	42	48	90	f	Z	D	c
Neosilba glaberrima	55	54	109	f	Z	D	c
Neosilba inesperata	101	186	287	Vf	Z	D	a
Neosilba pendula	159	181	340	Vf	Z	D	va
Neosilba pradoi	67	57	124	f	Z	D	c
Neosilba zadolicha	789	500	1289	Sf	Z	Sd	sa
Total	1264	1026	2290				
H[5]	1.6755	1.4248					
α[6]	0.8113	0.7211					
E[7]	0.9351	0.7952					

[1]F= Frequency - Lf: little frequent, F: frequent, Mf: Vf frequent; Sf: super frequent;

[2]C= Constancy - Z: Accidental

[3]Do= Dominance – D: dominant; Sd: super dominant;

[4]Abundance= d: dispersed; c: common; a: abundant; va; very abundant; sa: super abundant

[5] H'= Shannon-Wiener diversity index

[6] α = Margalef diversity index.

[7] E= Equitability index

Discussion

Seven *Neosilba* species were recorded (Table 1), and this species richness is within the range reported in other inventories carried out in the state of São Paulo. In a previous study carried out in the municipality of Monte Alegre do Sul, Souza-Filho et al. (2009) reported eight species during a sampling period of one year. Silva et al. (2006) carried out a two-year study in two locations in the Southern Brazil, and reported five *Neosilba* species. In the south in the east-west direction across the state of Mato Grosso do Sul, nine species were registered (Nicácio & Uchoa 2011).

Neosilba zadolicha was the most predominant, frequent, dominant and abundant species indicating its importance in the region (Table 2). This species is a very common in citrus orchards reaching high rates of infestation (Uchoa et al. 2002, Raga et al. 2006, Raga et al. 2011). State of São Paulo has approximately 600,000 hectares of sweet oranges [*Citrus sinensis* (L.) Osbeck], with different varieties fruiting all year long. This fact may contribute to the abundance, dominance and constancy of *N. zadolicha* (Raga et al. 2015).

In our study, all species captured presented low constancy being considered accidental. This result suggests that adults of the low constancy species were not resident on the orchards, but they came from other hosts nearby the farm and/or the surrounding forest area. The sampled areas are surrounded by one of the few and largest remnants of the highly endangered mature coastal rainforest in Brazil (Faria et al. 2006). The Brazilian Atlantic rainforest is considered one of the richest biomes on earth, and southwest São Paulo harbors high species richness, high levels of endemism and local sites of diversity of trees in families that comprise species which are known hosts of *Neosilba*, such as Fabaceae, Malpighiaceae, Myrtaceae, Rutaceae, and Sapotaceae (Thomas et al. 1998, Faria et al. 2006, Martini et al. 2007). Thus, the forest areas surrounding the orchards can provide an important reservoir for lonchaeid populations that probably migrate to the orchards. The movement of fruit flies from the adjacent native vegetation, particularly forest fragments, into orchards was demonstrated by Vargas et al. (2001), and Kovaleski et al. (1999).

Uchoa et al. (2002) found *Neosilba* species infesting fruits of *C. sessiflora* and *M. jaboticaba* in the cerrado of Mato Grosso do Sul, Brazil, and Strikis et al. (2011) found *N. glaberrima* and *N. zadolicha* in *P. macrophylla* fruits, but in this paper, we did not record these associations. Many different biotic and abiotic stimuli can account for the presence of the lesser abundant fruit flies species in environments that do not provide optimal host plants, such as commercial orchards (Aluja et al 1996). The authors suggest that the odor of ripening fruit, shelter conditions of perennial trees, and emission of volatiles by certain tree species that are similar to those found in the sexual pheromones of fruit flies could draw adult fruit flies into the orchard.

Adaime et al. (2012), recorded *N. bella* in *B. crassifolia* in the Amazon region. Bittencourt et al. (2013) reared *N. bella* from *E. stipitata* fruits in northeastern Brazil. In previous surveys, *N. bella* have been found in twelve plant species from nine plant families (Table 2).

According to Bittencourt et al. (2013), *N. bella* has a wide geographical distribution in Brazil, ranging from Atlantic Forest, Amazon Rain Forest, and Cerrado. Its plasticity in occupying such different biomes, and attacking different host plants, makes this species a candidate in becoming an important pest, once it is found in environments occupied by crops plantation, especially coffee crops. However, *N. bella* was one of the species with the lowest number of host fruit recorded in this research.

McAlpine & Steyskal (1982) also found *N. certa* in *I. vera*, and Souza-Filho et al. (2009) recorded this species in orchards of *P. guajava*. *N. certa* has already been registered in fifty host fruits, belonging to seventeen plant families (Table 2).

Strikis et al. (2011) recorded *N. glaberrima* in *M. emarginata*, and Raga et al. (2003), Strikis et al. (2011), and Fernandes et al. (2013) found this species in *P. caimito*. *N. glaberrima* has already been registered in thirty-eight fruit species in sixteen botanical families (Table 2).

N. inesperata had already been reported in twenty-eight host fruits in sixteen plant families (Table 2). This species was previously reported by Nicácio & Uchoa (2011) in *P. guajava*. Raga et al. (2015) also found this species in *M. emarginata*.

In the literature *N. pendula* had already been reported infesting fifty-five host fruit species from twenty-five plant families. Seventeen plant species from nine families have been reported previously as hosts of *N. pradoi* (Table 1). *N. zadolicha* has already been recorded in ninety-four host species from thirdy-two plant families (Table 1).

Malpighia emarginata was the host fruit species with the highest *Neosilba* diversity: six species, being all of them found in this work, with the exception of *N. certa.* Strikis et al. (2011) also found no association record between *N. certa* and *Malpighia* sp. in his survey about frugivorous flies in the Amazon Rain Forest. *P. cattleianum* was the host with the largest number of *Neosilba* specimens, from which emerged 398 individuals (males + females).

Adaime et al. (2012) found lower infestation indexes to that found in this work: 0.06 puparia/fruit to *N. bella* hosted in *B. crassifolia*. Aguiar-Menezes et al. (2004) reported to *N. zadolicha* hosted in *P. alata* infestation index (2.1 puparia/fruit) higher than all indexes found herein.

Souza et al. (2012) reported infestation indexes to *Neosilba* species hosted in *P. guajava* (0.03 puparia/ g of fruit) similar than mass fruit infestation that was found here (0.01 puparia/ g of fruit). However to *Neosilba* species hosted in *Z. joazeiro*, Souza et al. (2012) found higher infestation indexes (3.28 puparia/ g of fruit) than the herein obtained (0.09 puparia/ g of fruit).

Araújo & Zucchi (2002) found similar infestation indexes to *N. pendula* hosted in *S. purpurea*, *M. emarginata* and in *P. guajava* (0.06, 0.1 and 0.1 puparia/ g of fruit, respectively), however to *Z. joazeiro* (0.2 puparia/ g of fruit) those authors found infestation indexes higher than reported in this paper (0.09).

Conclusion - New Records

In this work were reported the following new records of *Neosilba* fruit trees host in southeast Brazil:

N. bella in *E. uniflora.*
N. certa in *P. cattleianum and S. mombin*.
N. glaberrima in *B. gasipaes, R. sylvatica,* and *S. setaceous*.
N. inesperata in *E. dysenterica*, *E. uniflora*, *P. cattleianum*, and *R. urticaefolius*.
N. pendula in *E. brasiliensis, E. dysenterica*, *E. involucrate, E. neonitida, E. stipitata, I. vera, P. guineensis, R. urticaefolius,* and *S. mombin.*
N. pradoi in *C. aurea, C. guazumaefolia*, C. *phaea*, and *E. dysenterica*.
N. zadolicha in *A. sellowiana*, *B. gasipaes, C. brasiliense, C. guazumaefolia*, *C. phaea*, *E. brasiliensis*, *E. dysenterica*, *E. involucrata*, *E. neonitida*, *E. pitanga*, *E. pyriformis*, *I. vera*, *M. emarginata*,*M. dubia*, *P. guineensis*, *P. alata, S. mombim*, *S. tuberose,* and *S. venulosa.*

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Live-trapping Ocelots (*Leopardus pardalis*): traps, baits, injuries, immobilization and costs

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***Abstract*:** The capture of wild animals can provide important information on community structure, population dynamics, home range size, activity patterns, habitat use, denning, social behavior and health status. The objective of this study was to describe the method of capture with details on baits, injuries, non-target captures, anesthesia and costs, to evaluate its success as part of a health evaluation program of ocelots in a Brazilian Atlantic Forest Reserve. From a total of 1,011 trap-night effort in 86 days, we had 68 capture events composed of ocelots (22%, n=15) and non-target species (78%, n = 53). We captured 10 individual ocelots in 15 capture events, corresponding to 5.7 days to capture one ocelot. Capture efficiency was 14.8 ocelots/1,000 trap-nights effort. We suggest capture methods should be selected and implemented based on the following criteria: (i) high capture efficiency; (ii) high selectivity; (iii) low injury rate; (iv) high immobilization suitability; and (v) low costs, in order to enable comparisons of studies from different research groups and from different study areas, allowing a deliberate choice of the best method.

***Keywords*:** *Atlantic forest, Brazil, capture cost, capture efficiency, capture selectivity, injury rate.*

Captura de jaguatiricas (*Leopardus pardalis*): armadilhas, iscas, ferimentos, imobilização e custos

Resumo: A captura de animais selvagens é capaz de proporcionar informações importantes acerca da estrutura da comunidade, dinâmica populacional, tamanho das áreas de vida, uso dos hábitats, locais de toca, comportamento social e estado de saúde. Este estudo teve como objetivo descrever o método de captura enfatizando as iscas utilizadas, ferimentos, capturas de espécies não-alvo, anestesia e custos, para avaliar o sucesso de captura como parte de um programa de avaliação de saúde de jaguatiricas numa reserva de Mata Atlântica no Brasil. De um de esforço total de 1.011 armadilhas-noite em 86 dias, nós tivemos 68 eventos de captura compostos de jaguatiricas (22%, n= 15) e espécies não-alvo (78%, n= 53). Nós capturamos 10 indivíduos diferentes em 15 eventos de captura, correspondendo a 5,7 dias para capturar uma jaguatirica. A eficiência de captura foi de 14,8 jaguatiricas/1.000 armadilhas-noite. Nós sugerimos que os métodos de captura deveriam ser selecionados e implementados com base nos seguintes critérios: (i) alta eficiência de captura; (ii) alta seletividade; (iii) baixa taxa de ferimentos; (iv) alta adequação de imobilização; e (v) baixos custos, de forma a viabilizar comparações de estudos de diferentes grupos e diferentes áreas, permitindo a escolha do melhor método.

***Palavras-chave*:** *Brasil, custo de captura, eficiência de captura, Mata Atlântica, seletividade de captura, taxa de ferimentos.*

Introduction

The ecological importance of wild felids as top predator and therefore as ecosystems regulators is recognized worldwide (Terborgh et al. 2001). However, due to their secretive behavior, these animals are difficult to capture and its capture is expensive (Barea-Azcón et al. 2006; McCarthy et al. 2013), which may explain the increasing use of remote camera-trapping in recent carnivore ecological research (Dillon & Kelly 2008). Despite being useful and cost-effective for a wide range of objectives, non-invasive techniques may provide limited information on community structure, population dynamics, home range size, activity patterns, habitat use, denning, social behavior and health status (Deem et al. 2001; Kolbe et al. 2003; Michalski et al. 2007). Considering the advantages of capturing and handling carnivore species to perform ecological and health survey, humane and efficient live-trapping are the best methods to be used (Mowat et al. 1994).

Despite a proliferation of studies evaluating mammal trap efficiency and related injuries in the 1990s (Mowat et al. 1994), very few have been published targeting capture methods of carnivores in tropical forests (Michalski et al. 2007; McCarthy et al. 2013). With few exceptions (i.e. McBride-Jr & McBride 2007), most peer-reviewed ecological studies on neotropical carnivores that used live-trapping methods focused on monitored animals, without providing details on the methods of trapping, injuries and anesthetic doses and responses (i.e. Emmons 1988; Crawshaw & Quigley 1989; Harveson et al. 2004; Haines et al. 2005; Dillon & Kelly 2008). This is also true for epidemiological studies, which focus mainly on pathogens surveys (i.e. Filoni et al. 2006; Fiorello et al. 2007; Metzger et al. 2008; Jorge et al. 2010; Widmer et al. 2011).

Most carnivore species occur at low densities and require a large amount of capture effort for success (Logan et al. 2009). In tropical forests, carnivores are often listed as threatened, as well as the non-target species that eventually may be also captured (McCarthy et al. 2013). Therefore, before considering the capture of these animals, efforts should be made towards increasing efficiency and selectivity, reducing injuries and finding a suitable immobilization protocol at reasonable cost-effectiveness (Mowat et al. 1994; Austin et al. 2004; McBride-Jr &McBride 2007; McCarthy et al. 2013)

The ocelot (*Leopardus pardalis*) is a medium sized felid (adult weight ranges from 7 to 16 kg), distributed from Mexico to Northeast Argentina (IUCN 2015) that is listed on CITES´s Appendix I (CITES 2013) and considered Least Concern by IUCN (2015). Across its distribution, ocelots are often considered an abundant carnivore, reaching densities as high as 95 ocelots/100 km^2 (Kolowski & Alonso 2010). To date there is a paucity of published data on capturing and handling free-ranging ocelots and no study has been performed focusing specifically on the evaluation of methods of trapping and the capturability of this wild felid. The objective of this study was to describe the method of capture with details on trapping methods, baits, injuries, non-target species captures, anesthesia and costs, in order to evaluate its success. This research was part of a health evaluation program of ocelots in a Brazilian Atlantic Forest Reserve (Widmer et al. 2016).

Material and Methods

1. Study area

This study was conducted at Rio Doce State Park (RDSP, 19º29´24” - 19º48´18”S and 42º28´18” - 42º38´30”W), the largest Atlantic Forest patch of Minas Gerais State, Brazil (Lima-Bittencourt et al. 2011). RDSP is composed by 360 km^2 of semi-deciduous seasonal tropical forest and is surrounded by Eucalyptus plantations, pastures and cities (da Silva Júnior et al. 2009). Three different areas of RDSP were surveyed during this study using the following criteria: easy access, reports of park rangers about the presence of wild carnivores and distance away from cities.

2. Trap and baits

Trap effort was calculated multiplying the number of traps by the number of days that traps were opened in the field. Between 2012 and 2013, we conducted eight periods of captures of approximately 10 days each. The number of traps set per night varied between two and 15, with an average of 12.5. We set the traps along dirt roads, well-traveled trails or inside the forest, with a mean distance of 396 meters between traps in each area (Figure 1). We used 15 foldable galvanized mesh cage traps with single door, triggered by treadle, size 115 x 40 x 60 cm and 12.2Kg weight (Gabrisa Ambiental, Cafelândia – SP, Brazil) (Figure 2). We used nylon mason line between trigger and treadle instead of the provided steel cable, and inserted plastic seals between the back and superior walls, and between lateral and superior walls to steady the trap. We covered the top of all traps with a black plastic sheet to protect bait and captured animals from sun, rain and wind. All traps were checked daily from 6 to 8 a.m. We used seven different baits. In the first capture season we used bacon, bacon and sardine and roasted chicken. For live-baits, we used chicks and quails from nearby agricultural suppliers. We adapted a plastic mesh in the back of the trap to provide a bait compartment. Occasionally, dead chicks and quails were also used as a bait. Trapped animals were allowed to feed on the bait to reduce capture stress (Crawshaw & Quigley 1989). Drinking water and food for the bait were checked and replaced at least once a day. We recorded the amount of food eaten by live baits in a daily basis. We calculated bait death rates as the number of live baits dead divided by the total time effort done using that type of bait. We classified an event as a bait theft when a bait was removed from the trap with no animal captured inside.

3. Handling and anesthesia

After caught in the trap, a biologist or a veterinary evaluated general health, body condition and estimated body weight. A blowpipe and 3cc nylon darts (Telinject, Romerberg, Germany) were then prepared with an estimated 10mg/kg dose of Tiletamine Hipocloride and Zolazepam Hipocloride association (Zoletil™, Virbac do Brasil, São Paulo, Brazil). One person would approach the trap to attract the animal´s attention while another would use the blowpipe to anesthetize it. After intramuscular injection in the upper thigh area, the animal was observed from a distance until immobilized. When needed, supplemental doses were applied intramuscularly by hand injection. Once under anesthesia, ocelots were treated with an ophthalmic ointment, had their eyes covered, and were checked by a veterinarian for vital parameters. We implanted a subcutaneous microchip in all ocelots (Animal Tag, São Carlos-SP, Brazil) between the shoulder blades.

We recorded each animal´s response to Zoletil and time of those responses. We defined cataleptic anesthesia as the interval (min) from Zoletil injection to first signs of ocelot´s recovery. Incapacity time was defined as the interval from Zoletil injection until total recovery. Following immobilization, we weighed, measured, determined age class based on tooth wear and morphological measurements, and took blood and ectoparasite samples. Ocelots were aged as adults (> 24 months), subadults (12 to 24 months) and juveniles (< 12 months). We also recorded information on trap injuries, heart and respiratory rates (every 15 min.), sex, and other parameters (Appendix A). During anesthesia, we recorded rectal temperature using a digital thermometer at 15-min intervals. We recorded hypothermia (rectal temperature below 37.0 °C), hyperthermia (rectal temperature above 40.0 °C), dehydration, excessive salivation and muscle tremors during anesthesia. After first signs of anesthesia recovery, animals were put back into the trap and the door closed until total recovery and release by the biologist in charge. Capture and handling procedures were approved by the Ethics Committee on the Use of Animals in Research of

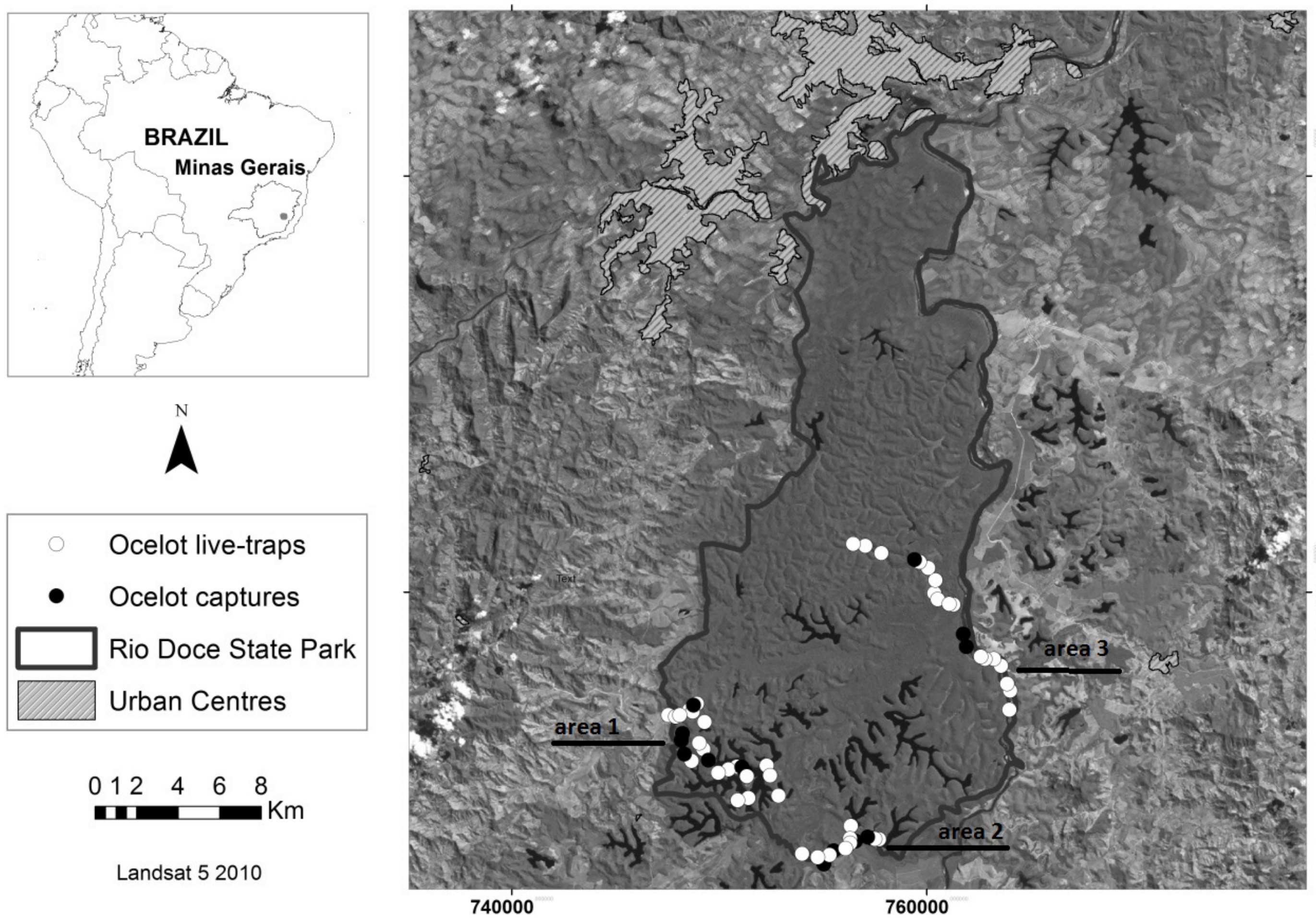

Figure 1. Map of Rio Doce State Park showing ocelot live-trap and capture sites in 2012 and 2013. Three different areas of RDSP were surveyed during this study using the following criteria: easy access, reports of park rangers about the presence of wild carnivores and distance away from cities.

Figure 2. Cage trap utilized for ocelot trapping at Rio Doce State Park, Minas Gerais, Brazil, in 2012 and 2013.

Escola Superior de Agricultura Luiz de Queiroz (ESALQ) – University of São Paulo (USP), Sistema de Autorização e Informação em Biodiversidade (SISBio, permit 34284-1), Instituto Estadual de Florestas de Minas Gerais (IEF, permit UC053/12) and Ethics Committee on the Use of Animals in Research of Universidade Federal de São João del Rei (UFSJ -003/20013, CEUA/UFSJ).

4. Costs

During field work, we kept receipts and took notes of all relevant expended money for accountability. Costs of vehicle and personnel time were calculated from market prices and scholarships values of experienced graduate students involved in the project. In addition, field assistants were provided by RDSP to help during captures and their daily cost was calculated based on current market prices for the Minas Gerais State in Brazil. All costs were calculated based on 2012 United States Dollars (US$).

5. Analysis

Capture efficiency for our data and for available literature data (see Appendix B) was calculated based on the number of ocelot capture events per 1,000 trap-nights effort (Mowat et al. 1994). Bait success was measured by dividing the number of ocelot captures by the total trap-night effort of that specific bait. Each live bait cost was calculated by the sum of the bait and food costs divided by the total trap-night effort using that bait. We also evaluated the influence of environmental factors such as moonlight, average temperature and precipitation on capture success. To evaluate the effect of moonlight, data on moon phase, number of hours the moon was exposed and the fraction of moon illuminated were used to calculate a moonlight index. The index was calculated as the fraction of moon illuminated (%) multiplied by the number of hours the moon was exposed and visible during each night of trapping. Data on moonlight phases was obtained from Time and Date AS (2014). Data on average temperature and precipitation were obtained from Instituto Nacional de Pesquisas Espaciais (INPE 2014) specifically for the year 2012 when all the first captures of adult ocelots were realized. We used logistic regression to evaluate the influence of moon phase, temperature, precipitation and moonlight index on ocelot capture success. A successful capture was scored 1 and an unsuccessful 0. Moon phase was entered as a categorical variable scored 1 full, 2 half and 3 dark. Temperature and precipitation were entered as continuous variables. Capture selectivity was calculated based on ocelot capture events divided by the total captures (ocelots and non-targets) (modified from Fleming et al. 1998).

Injury rate, the proportion of injuries that might affect or even cause death of target and non-target individuals divided by total capture events (adapted from McBride-Jr & McBride 2007, McCarthy et al. 2013), was divided into three categories: (i) Very minor injuries (very minor cuts, abrasions, claw damage, rubbing), (ii) Minor injuries (not likely to cause animal´s death – i.e. superficial lacerations and abrasions), and (iii) Major injuries (likely to cause animal´s death – i.e. deep lacerations, severe bleeding lesions; adapted from Mowat et al. 1994). We calculated injury rates for ocelots (injuries/total ocelot capture events) and for all captures (all injuries/all total captures).

We used Pearson correlations to examine relationships between body weight and dosage to cataleptic anesthesia, incapacity time, and minimum or maximum body temperature. We did not included induction time in our analysis. We used the Kolmogorov-Smirnov test to evaluate normality of data. Ocelot capture cost was calculated by the total cost of the project divided by the number of ocelot capture events (adapted from Austin et al. 2004; Mowat et al. 1994).

Results

In 86 days of captures, we had 1,011 trap-night effort, 68 capture events (6.7% success) and 24 bait thefts (2.4% bait loss). We detected thefts by examining hairs left inside the trap. Capture events were composed of ocelots (22%, n=15) and non-target species (78%, *n* = 53) such as tayra (*Eira Barbara*) and jaguarundi (*Puma yagouaroundi*) (*n* = 2), big-eared opossums (*n* = 26) and other mammals, birds and reptiles (n=25) (Table 1). We captured 10 individual ocelots being nine adults (5 males, 4 females) and one subadult female in 15 capture events (6 males, 9 females events), corresponding to 5.7 days with 15 trap-nights per ocelot capture. Capture efficiency was 14.8 ocelots/1,000 trap-nights effort. Mean values of body mass for male ocelots (11.3 ± 2.52 S.D.) did not differ from mean values for females (9.3 ± 1.07) (*df* = 7, *t* = 1.452, *P* = 0.19). Ocelot capture selectivity was 22%. Regarding the environmental factors that could have affected capture success, logistic regression analysis showed that moon phase, moonlight index, temperature and precipitation were not significant predictors of capture success of ocelots during 2012 (Table 2).

Among the baits tested, only the two live baits were successful in capturing ocelots. We captured six ocelots in 323 trap-nights using live chicks (18.5 ocelots/1,000 trap-nights) and nine ocelots in 562 trap-nights using live quails (16.0 ocelots/1,000 trap-nights). Other baits comprised 126 trap-night effort (Table 3). The main difficulties with bait use were thefts, especially from black capuchins, ants (once ants discovered the trap, it was moved away) and trap-happy opossums (after the second capture of the same individual in the same trap we would move the trap). Quails consumed 206 gr of food/quail and chicks consumed 455 gr of food/chick. Death rates for quails and chicks were 0.53% and 2.16% per day respectively.

In general, animals that fought to escape the trap targeted the door, back, or lateral walls near door and the pedal. In the case of ocelots, only three animals presented very minor injuries, which represents 20% of all ocelots captured and 4.4% when all captures are considered. Two ocelots presented minor gum lesions and one presented a superficial abrasion on the nose. Another ocelot had an uncomplicated crown fracture on its cheek tooth that could not be proven to be due to trapping.

Considering all captures, injuries in other species represented only 3.0% (n = 2). Other injured animals were a yagouaroundi and a tayra. The yagouaroundi had abrasive lesions and swelling on the face (minor injury; Figure 3a) as a result of the animal jumping and beating its head against the trap. This might also have been a consequence of too much time inside the trap, since it is a diurnal species and most traps were checked only once a day between 6:00 to 8:00 am. The tayra had a deep punctual lesion in the gum resulting in severe bleeding, which, after chemical immobilization, was reversed by lesion compression for some minutes (major injury). The tayra´s injury was a result of the animal trying to chew its way out of the trap after noticing the presence of people near the trap and having success on breaking the rods of lateral wall near the door (Figure 3b).

Ocelots were considered calm during trap approach in eight capture events and alert in the other seven. They were considered healthy in 11 capture events and under mild disease associated with weight loss in another four (see Widmer et al. 2016).

Table 1. Capture events per group and respective proportion to total capture events at Rio Doce State Park, Minas Gerais, Brazil, 2012 and 2013.

Captured animal			Capture events	Proportion (%)
Order	**Family**	**Species**		
Carnivora	Felidae	Ocelot (*Leopardus pardalis*)	15	22.0
	Felidae	Yagouaroundi (*Puma yagouaround*)	1	1.5
	Mustelidae	Tayra (*Eira barbara*)	1	1.5
	Canidae	Domestic dog (*Canis familiaris*)	2	2.9
Didelphimorphia	Didelphidae	Big-eared opossum (*Didelphis aurita*)	26	38.2
		Brown four-eyed opossum (*Metachirus nudicaudatus*)	3	4.4
Primates	Cebidae	Black capuchin monkey (*Sapajus nigritus*)	1	1.5
Lagomorpha	Leporidae	Tapeti (*Sylvilagus brasiliensis*)	1	1.5
Cingulata	Dasypodidae	Nine-banded armadillo (*Dasypus novemcinctus*)	1	1.5
Accipitriformes	Accipitridae	Roadside hawk (*Rupornis magnirostris*)	6	8.8
		White hawk (*Leucopternis* sp.)	1	1.5
Falconiformes	Falconidae	Collared forest falcon (*Micrastur semitorquatus*)	3	4.4
Cariamiformes	Cariamidae	Red legged siriema (*Cariama cristata*)	1	1.5
Squamata	Teiidae	Tupinambis (*Tupinambis* sp.)	6	8.8
Total			68	100.0

Table 2. Logistic regression model testing the effects of environmental factors on ocelot capture success at Rio Doce State Park, 2012.

Variable	*B*	*S.E.*	*Wald*	*df*	*P*
Moon phase (categorical variable)	-0.927	0.795	1.358	1	0.244
Index (continuous variable)	-0.251	0.211	1.417	1	0.234
Temperature (continuous variable)	-0.226	0.134	2.864	1	0.091
Precipitation (continuous variable)	0.022	0.050	0.202	1	0.653

Hand injection was tried in calm animals, but was unsuccessful since the animals move and turn around too fast. Data from two of the 15 capture events were excluded because we could not be sure of the dosage of first injection due to dart problems. Mean initial dose was 10.4 mg/kg (±1.63 S.D., range = 7.74 to 14.28), which resulted in 10 cataleptic anesthesia, one sedation, and two light sedations. The three sedations required a supplemental dose of anesthetic. Induction time for animals that reached cataleptic anesthesia with first dose varied between three to 10 minutes. Data for doses and effects are shown in Table 4. We recorded hypothermia, hyperthermia, dehydration, excessive salivation and muscle tremors during anesthesia. Besides excessive salivation, one ocelot presented muscle tremors during

Table 3. Bait success and bait losses at Rio Doce State Park 2012 and 2013. The success of each bait was measured dividing the number of captures by the total trap-night effort of that specific bait.

Bait success (number of captures or thefts)						
Species captured (effort in trap-nights)	Sardine + Bacon (36)	Bacon (10)	Roast chicken (47)	Live chicks (323)	Dead chick (25)	Live quail 562
Ocelots	0	0	0	1.85 (6)	0	1.6 (9)
Other carnivores	0	0	0	0.31 (1)[Y]	4.0 (1)[T]	-
Domestic dogs	0	10.0 (1)	0	0	0	0.17 (1)
Big-eared opossums	0	0	4.25 (2)	2.47 (8)	8.0 (2)	2.49 (14)
Hawks (4 spp.)	0	0	0	1.85 (6)	4.0 (1)	0.53 (3)
Tupinambis	0	0	0	0	0	1.06 (6)
Others	0	10.0 (1)	4.25 (2)	0	4.0 (1)	0.53 (3)
Total captures	0	20.0 (2)	8.51 (4)	6.5 (21)	20.0 (5)	6.4 (36)
Thefts	5.5 (2)	0	4.25 (2)	3.09 (10)	4.0 (1)	1.6 (9)

[Y] Yagouaroundi (*Puma yagouaroundi*). [T] Tayra (*Eira Barbara*)

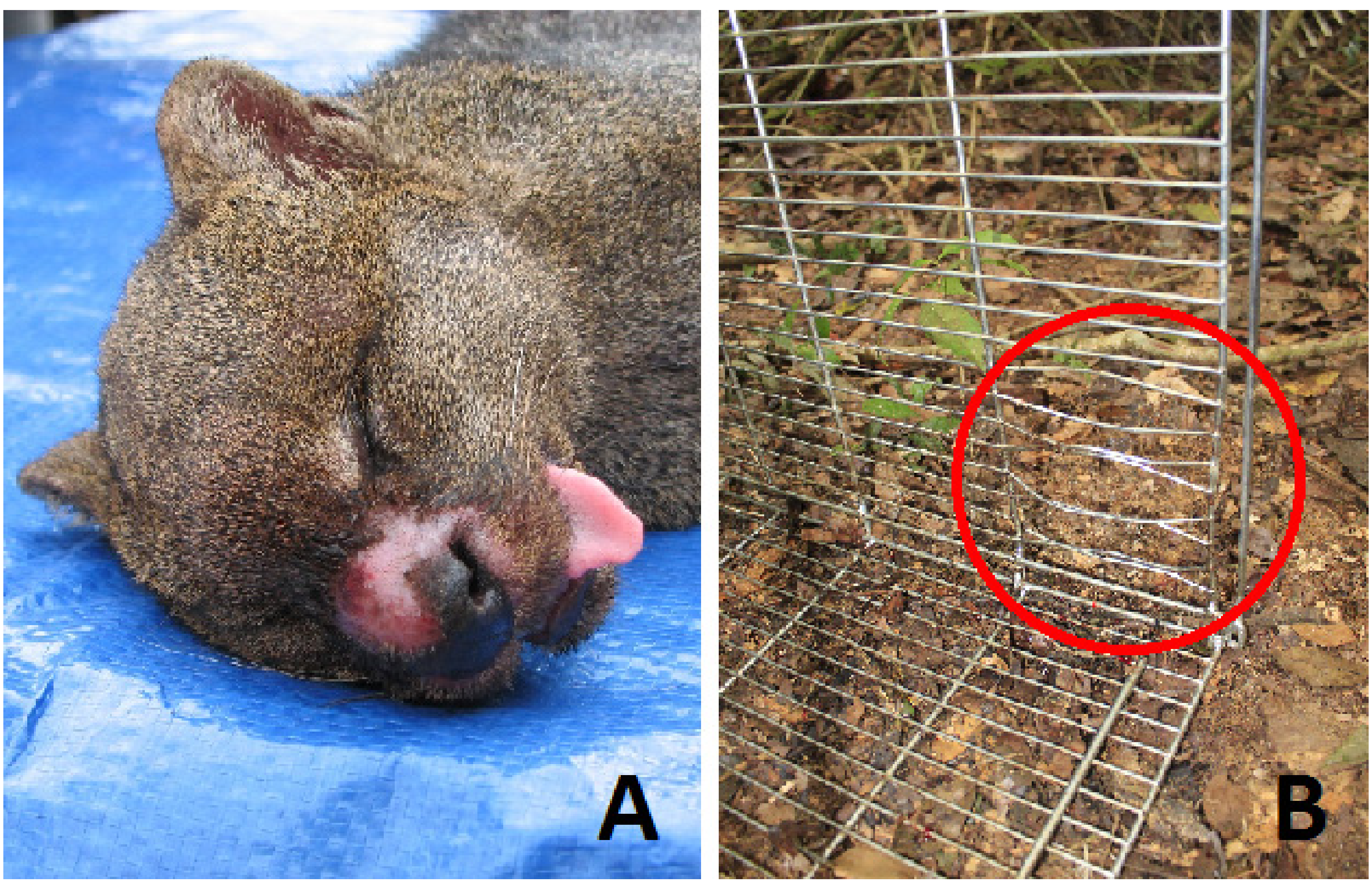

Figure 3. A) Yagouroundi (*Puma yagouaroundi*) presenting abrasive lesions and swelling of face. B) Bent rods near trap door that were chewed by a tayra (*Eira barbara*) at Rio Doce State Park during 2012 capture campaign.

Table 4. Zoletil™ doses and effects on 13 wild ocelots captured at Rio Doce State Park, Minas Gerais, Brazil, 2012 and 2013.

	Variables measured	Mean (± SD)	Range
Animals that received single dose (n = 10)	Body weight (kg)	10.08 (2.30)	7.0 - 15.5
	Zoletil ™ dose (mg/kg)	10.30 (1.88)	7.74 - 14.28
	Cataleptic anesthesia (min)	136.0 (57.43)	50 - 210
	Incapacity (min)*	257.2 (59.16)	170 - 350
	Minimum rectal temperature (°C)	36.98 (0.85)	36.1 - 38.9
	Maximum rectal temperature (°C)	38.96 (0.77)	38.1 – 40.5
Animals that received supplement dose (n = 3)	Body weight (kg)	9.3	6.8 – 11.0
	1rst Zoletil ™ dose (mg/kg)	10.4	10.0 – 10.9
	2nd Zoletil ™ dose (mg/kg)	2.9	1.0 – 5.1
	Interval between injections (min)	16.6	12 – 24
	Cataleptic anesthesia (min)[a]	28.0	25 -31
	Incapacity (min)[a]	156.6	90 – 220
	Minimum rectal temperature[b] (°C)	37.5	37.2 – 37.9
	Maximum rectal temperature[b] (°C)	38.8	38.1 - 39.5

Cataleptic anesthesia = period from injection to first signs of recovery. Incapacity = period from injection to total recovery. *1 animal was withdrawn from this analysis - it was released earlier because it was fighting against the trap. [a]time after second injection. [b]data from one animal was missing

Table 5. Anesthesia complications, their frequencies, and field treatments of ocelots captured at Rio Doce State Park, Minas Gerais, Brazil, 2012 and 2013.

Complication	Frequency	In field treatment
Hypothermia - (rectal temperature below 37.0 °C)	5	Paw bandage, cotton blanket, emergency blanket, car´s hot air
Hyperthermia - (rectal temperature above 40.0 °C)	4	3 lowed spontaneously during anesthesia, 1 was cooled with water in paws and flank
Dehydration - (mild loss of cutaneous elasticity)	4	Subcutaneous saline solution (30 to 60 ml/animal)
Excessive salivation	3	0.01mg/kg subcutaneous atropine sulfate
Muscle tremors	1	Avoid sound stimulus

Table 6. Total costs of 1,011 trap-night effort at Rio Doce State Park, 2012 and 2013, in 86 days.

Item (quantity)	Approximate cost (2012 US$)
Vehicle	50,000.00
Experienced Field Veterinarian	12,000.00
Experienced Field Biologist	10,000.00
Field Assistant	1,000.00
Vehicle maintenance (2)	3,500.00
Tomahawk traps (15)	2,500.00
Microchip kit (1)	700.00
Trap extras (wires, canvas, etc) -	100.00
Veterinary items (anesthetics, stethoscope, medications, etc)	1,300.00
Handling equipment (scales, table, etc)	800.00
Daily wage - team maintenance	1,500.00
Fuel (1800lts of Diesel)	2,000.00
Personal protective equipment	100.00
Waterers (26)	36.00
Feeders (46)	80.00
Chick food (35kg)	25.90
Quail food (15kg)	10.40
Chicks (77)	83.70
Quails (73)	114.30
Total	85,850.30

recovery when under sound stimulus. All hyperthermic ocelots were alert during trap approach. The main anesthesia complications and applied treatments are shown in Table 5. We found no significant relationship between body weight and cataleptic anesthesia (r = -0.485, P = 0.186), incapacity time (r = 0.052, P= 0.903), minimum temperature (r = -0.087, P = 0.825), and maximum temperature (r = -0.438, P = 0.239). Similarly, the dosage of Zoletil™ was not correlated to cataleptic anesthesia (r = 0.384, P = 0.308), incapacity time (r = 0.083, P = 0.845), minimum temperature (r = -0.171, P = 0.660) nor maximum temperature (r = 0.130, P = 0.739).

The cost of using quails was US$0.26/trap-night while chicks cost was US$0.39/trap-night. Calculated cost for each ocelot capture was US$ 5,723.35 and the average for each of the 8 periods of captures was US$ 11,106.29. General costs of 8 periods of captures are shown in Table 6.

Discussion

Previous reports in the peer-reviewed literature lack detail regarding ocelot capture and handling methods (see Appendix B). Many of these publications do not mention bait used, just a few report trap effort and only one reports trap-related injuries (Emmons 1988). Although some of authors mention anesthesia protocols, just the papers on anesthesiology report responses and side-effects (Beltran & Tewes 1995; Shindle & Tewes 2000). We found only one peer-reviewed literature reporting the costs of capturing non-target species (Newsome et al. 1983) and none specifically about costs of capturing ocelots.

Our capture efficiency using box-traps (14.84 ocelots/1,000 trap-nights) was higher than that reported for ocelots in forested areas of Belize, in the Chaco of Bolivia, and in the Cerrado of Brazil (Fiorello et al. 2006; Dillon & Kelly 2008; Rocha et al. 2013) but lower than reported from forests of Venezuela (Ludlow & Sunquist 1987). We found the model of traps used advantageous as they could be moved to different sites inside study area, contrary to what has been previously reported for box-traps (Mowat et al. 1994). Moreover box-traps were especially useful to house ocelots during anesthetic recovery (Mowat et al. 1994). We captured felids exclusively with live-baits (similar to Michalski et al. 2007), although there are reports of occasional ocelots captured using dead baits (i.e. Rocha et al.

2013). Furthermore, our ocelot capture efficiency would be even higher if calculated considering only live-baits effort (17 ocelots/1,000 trap-nights). Among the baits, quails were cheaper, cleaner to handle, died less often than chicks and could be used indefinitely as baits, contrary to chicks that in 30-60 days would grow too large for the bait compartment.

We found no relationships between capture success and environmental factors probably because of our small sample size of ocelots captured. In addition, we would need to increase our capture effort during more years/seasons in order to increase the chances of finding any possible significant relationship.

Our selectivity of live-trapping ocelots (22.06%) was similar to the selectivity for box-trapping felids (22.58%) of the only available published data from Brazil (Michalski et al. 2007). This rate can be considered low, as expected for box-trapping (McCarthy et al. 2013), when compared to other methods such as leg hold traps for pumas (70.13%, calculated from Logan et al. 1999). However, box-traps are considerably safer and cause less injuries to both target and non-target species (i.e. Mowat et al. 1994).

We consider our injury rate for ocelots acceptable. Even it was not possible to compare our result with other ocelot box-trapping data due to the lack of this information reported, other similar felid species such as the Canadian lynx had 32% minor injuries (Mowat et al. 1994). To avoid non-target species injuries, the method we used needs to be improved, once their capture appears inevitable.

Our results were contrary to the expectations for tropical environments, because hypothermia was the main anesthesia side effect (McCarthy et al. 2013). Our dose of 10mg/kg of the association of tiletamine and zolazepam (Rabinowitz 1990) when compared to the literature´s 5 mg/kg dose (Shindle & Tewes 2000) resulted in a longer period of cataleptic anesthesia but surprisingly a very similar time of motor incapacity. Despite the small number of animals captured, the great variation of response using similar doses for all ocelots supports the hypothesis of individual response to anesthetics being the main factor influencing response to the association of tiletamine and zolazepam (Shindle & Tewes 2000).

Besides considering efficiency, selectivity and injury rate for capture success, we believe evaluating capture costs is also important when selecting live-trapping methods for research (Austin et al. 2004). When costs of capturing carnivores are mentioned, they are restricted to capture devices (i.e. Mowat et al. 1994; Austin et al. 2004), and do not consider other logistical costs which may be influenced by the capture method itself and the duration of the project.

In order to improve the trap´s safety for other species we suggest a reduction to less than 10 cm in the spaces between the 4 vertical rods near the door and back of the trap, and to increase the number of horizontal rods on the door to reduce to 5cm the distance between the 6 lower rods, therefore preventing animals chewing on the rods. We also suggest shutting the traps during the day or performing more than one check per day (i.e. by the use of trap-transmitters). Concerning the use of live baits, we recommend choosing locally raised animals, evaluating the need of eventual preventive medical treatment/tests on live baits to avoid disease transfer to the study area and that sick birds are promptly replaced.

Because of the great variation in the response to the anesthetics and the long recovery period and adverse effects on some individuals, we recommend that other anesthesia protocols be tested for free-ranging ocelots. Based on the results presented here and authors´ previous experiences, we highlight the importance of having anesthesia protocols for all non-target species that might be captured and also to promptly release or anesthetize mustelids as soon as they are found trapped in order to avoid possible injuries.

Regarding selectivity, it would be especially useful to develop strategies to avoid the capture of opossums, for instance, reinforcing the mesh wire between the bait and the trapped animal and using a less sensitive treadle, since they represent the majority of capture events. Because live-trapping and immobilizing carnivores is expensive and requires an experienced team (McCarthy et al. 2013), we advocate that substantial and long term funding would decrease the cost of each capture, since most expensive items (for instance vehicle) could be used for a longer period without a significant increase in the total costs and, furthermore, would allow the persistence of trained personnel and the conduction of long-term health and ecological research projects targeting threatened species.

Considering the inherent risks of live-trapping, the endangered status of species that might get trapped and the insufficiency of data, we recommend

Appendix A. Parameters evaluated from captured ocelots captured at Rio Doce State Park, Minas Gerais, Brazil, 2012 and 2013.

Behaviour	General Health	Body condition	Anesthesia	Hydration status	Anesthesia complications
Calm	Normal	Normal	No effect	Normal	None
Alert	Mild disease	Underweight	Mild sedation	Mild dehydration	Excessive salivation
Agressive	Severe disease	Cachectic	Sedation	Moderate dehydration	Hypothermia
Apprehensive	High anesthetic risk	Overweight	Cataleptic anesthesia	Severe dehydration	Hyperthermia
			Overdose		Respiratory alterations
					Cardiac alterations
					Other

Appendix B. Data on ocelot capture method from peer-reviewed reports.

Country	Captured ocelots (capture events)	Study objective	Trap used	Bait used	Duration (trap-night effort)	Anesthesia protocol (dose)	Capture efficiency[1]	Injuries	Non-target captures	Costs	Reference
Belize	7 (13)	Ecology	Box-trap	Live chicken and lures	1 year (1040)	Tiletamine-zolazepam-xylazine-butorphanol (25:15:1) and ketamine (supplementation)	12.5	-	-	-	(Dillon and Kelly 2008)
Bolivia	10	Epidemiology	Box-trap	-	2 years (1,514)	Ketamine(5.8mg/Kg)-medetomidine (0.06mg/Kg)	6.60	-	-	-	(Fiorello et al. 2006)

Shows details on induction time, immobilization time and handling procedures with statistical analysis on anesthetic response. [1] Calculated based on reported information – ocelot capture events / 1,000 trap-nights effort. [2] Data from animals on the blood collection group. - Data not available.

Appendix B. Continued...

Country	Captured ocelots (capture events)	Study objective	Trap used	Bait used	Duration (trap-night effort)	Anesthesia protocol (dose)	Capture efficiency[1]	Injuries	Non-target captures	Costs	Reference
Brazil	3 / 1	Ecology	Box-trap and Leg hold trap/ Trained dogs	Live chicken	-	Tiletamine-zolazepam (7 mg/Kg) / Ketamine-xilazine (3.3mg/ kg:0.3mg/Kg)	-	-	-	-	(Crawshaw and Quigley 1989)
Brazil	6	Ecology/ Epidemiology	-	-	6 years	-	-	-	-	-	(Labruna et al. 2005)
Brazil	1	Ecology/ Epidemiology	Box trap	-	6 years	Tiletamine-zolazepam	-	-	-	-	(Filoni et al. 2006)
Brazil	2	Epidemiology	Box trap / Trained dogs	-	6 years	Tiletamine-zolazepam	-	-	-	-	(Nava et al. 2008)
Brazil	1	Epidemiology	Box trap	Sardine and boiled chicken	4 years (3,819)	Tiletamine-zolazepam (8.3mg/Kg)	0.26	-	-	-	(Rocha et al. 2009)
Brazil	10	Ecology/ Epidemiology	-	-	6 years	-	-	-	-	-	(Jorge et al. 2010)
Mexico	17 (21)	Epidemiology	Box-trap	-	8 years	Ketamine-xilazine	-	-	-	-	(Rendón-Franco et al. 2012)
Panama	12	Epidemiology	Box-trap	-	3 years	Tiletamine-Zolazepam/ Ketamine-xilazine	-	-	-	-	(Franklin et al. 2008)
Panama	3	Ecology	Box-trap	Live chicken		Ketamine-xylazine		-	-	-	(Mares et al. 2008)
Peru	9 (8 / 10)	Ecology, Behaviour	Box-trap / Leg snares	Live chicken	-	Ketamine-chlorpromazine	-	Swollen foot from snare	-	-	(Emmons 1988)
Venezuela	12 / 1	Ecology	Box-trap	Live chicken / Unbaited	2 years (334 / 30)	Ketamine (23-28mg/Kg)	35.93 / 33.33	-	-	-	(Ludlow and Sunquist 1987)
USA	10	Anesthesiology	Box-trap	Live chicken	8 months	Ketamine-xilazine (14.1mg/kg:1.1mg/kg)[#]	-	-	-	-	(Beltran and Tewes 1995)
USA[2]	20 (25)	Toxicology	Box-trap	Live chicken	3,5 years	Ketamine-acepromazine (19mg/Kg/0,19mg/Kg)	-	-	-	-	(Mora et al. 2000)
USA	11 (13)	Anesthesiology	Box-trap	Live chicken	1 year	Tiletamine-zolazepam (5mg/Kg)[#]	-	-	-	-	(Shindle and Tewes 2000)
USA	33	Ecology	Box-trap	Live chicken	8 years	Ketamine-xilazine (14.1mg/kg:1.1mg/kg)	-	-	-	-	(Harveson et al. 2004)
USA	80	Ecology (survival and sources of mortality)	Box-traps	Live chicken	19 years	Ketamine-acepromazine (9:1 ratio, 20mg/Kg)	-	-	-	-	(Haines et al. 2005)
USA	15	Theriogenology	Box-trap	Live chicken	15 years	Ketamine-acepromazine (9:1, 20mg/kg)	-	-	-	-	(Laack et al. 2005)
USA	1	Ecology	Box-trap	-	-	Tiletamine-Zolazepam (10-15mg/Kg)	-	-	-	-	(Haines et al. 2006)

Shows details on induction time, immobilization time and handling procedures with statistical analysis on anesthetic response. [1] Calculated based on reported information – ocelot capture events / 1,000 trap-nights effort. [2] Data from animals on the blood collection group. - Data not available.

the implementation of studies designed to test and compare different methods of capturing neotropical carnivores. To enable comparisons of studies from different research groups and from different study areas, allowing a deliberate choice of the best method, we suggest capture methods should be selected and implemented aiming the following criteria: (i) high capture efficiency; (ii) high selectivity; (iii) low injury rate; (iv) high immobilization suitability (safe for the animal, suitable to time to procedures, fast recovery and predictable effects, adapted from Austin et al. 2004, Mowat et al. 1994); and (v) low costs.

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Morphological variation of *Philodryas patagoniensis* (Girard, 1858) (Serpentes, Dipsadidae) from Brazil, based on the study of pholidosis, coloration and morphometric features

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Abstract: The current study aimed to verify the relationship between the patterns of coloration, the morphometrical features and pholidosis of specimens of *Philodryas patagoniensis* from Brazil, with the sexual dimorphism of this species. We studied specimens of *P. patagoniensis* deposited in several herpetological collections from Brazil. A total of 355 specimens were analyzed, of these 145 were males (87 adults and 58 juveniles) and 210 were females (134 adults and 76 juveniles). Adult specimens exhibit sexual dimorphism in snout-vent length, tail length, head length, number of ventral shields, and number of subcaudal shields. The analysis of variance showed that the adult females are significantly longer than adult males, both at snout-vent length and tail length. Females have a greater number of ventral shields (138-210) than males (151-200). The specimens studied also exhibit two distinct patterns of coloration unrelated to geographical or sexual variations.

Keywords: *Morphology, sexual variability, Philodryadini, Brazilian snakes.*

Variação morfológica de *Philodryas patagoniensis* (Girard, 1858) (Serpentes, Dipsadidae) no Brasil, com base em estudos das características de folidose, coloração e morfometria

Resumo: O presente estudo objetivou verificar a relação entre os padrões de coloração, as características morfométricas e a folidose de espécimes de *Philodryas patagoniensis* existentes no Brasil, com o dimorfismo sexual dessa espécie. Foram estudados espécimes de *P. patagoniensis* depositados em várias coleções herpetológicas do Brasil. Um total de 355 espécimes foram analisados, destes 145 eram machos (87 adultos e 58 filhotes) e 210 fêmeas (134 adultos e 76 filhotes). Espécimes adultos apresentam dimorfismo sexual no comprimento rostro-cloacal, comprimento da cauda, comprimento da cabeça, número de escudos ventrais e número de escudos subcaudais. A análise de variância mostrou que fêmeas adultas são significativamente maiores que machos adultos, tanto no comprimento rostro-cloacal quanto no comprimento da cauda. Fêmeas possuem maior número de escudos ventrais (138-210) que machos (151-200). Os espécimes estudados apresentam dois padrões distintos de coloração não relacionadas a variações geográficas ou sexuais.

Palavra-chave: *Morfologia, variabilidade sexual, Philodryadini, serpentes Brasileiras.*

Introduction

External morphological features as pholidosis, measures of body regions and patterns of coloration of specimens are fundamental for taxonomic studies of snakes (Thomas 1976, Di-Bernardo & Lema 1990, López & Giraudo 2008). Pholidosis is the arrangement or pattern of scales and shields on the body surface of reptiles and is one of features for identification of snakes, and is a feature that may have variations within the same species (Dowling 1951a, b). The differences between the phenotypes such as folidose, morphology and physiology reflect the functional capacity of the specimen, which in turn are adaptive to different environments (Arnold 1983, Greene 1986, Coddington 1988, Emerson & Arnold 1989, Arnold 1994, Garland & Losos 1994). Differences in behavior may influence evolutionary changes, for example, the occurrence of populations or species different from the usual habitat, possibly exhibiting different behaviors, which may lead to selection of phenotypes that maximize the effectiveness of these behaviors and the animal's relationship to its habitat (Mayr 1963).

Research on sexual dimorphism performed in several species of snakes of the families Colubridae and Dipsadidae demonstrate that the females have a highest snout-vent length compared to males (Solorzano & Cerdas 1989, Rivas & Burghardt 2001, Zug et al. 2001), and males have a highest tail length than females (King 1989, López & Giraudo 2008, Matias et al. 2011).

The Patagonia green racer, *Philodryas patagoniensis* (Girard, 1858) (Dipsadidae), occurs in Argentina, Bolívia, Brazil, Chile, Paraguay and Uruguay (Thomas 1976, Peters et al. 1986, López & Giraudo 2008). This species was described by Girard (1858) as *Callirhinus patagoniensis*, based on two specimens collected in coastal region of Patagonia, Argentina. These two specimens were described as having mainly an olive-green coloration with black spots, 19 midbody rows of dorsal scales, divided cloacal and subcaudal shields, a divided nasal scale, a pair of loreal scales, two post-ocular scales, and one pre-ocular scale, with the third and fourth supralabial scales in contact with the orbit. This species was included into the genus *Philodryas* Wagler, 1830 by Hoge (1964), and redescribed by Thomas (1976).

The study of López & Giraudo (2008) with specimens of *P. patagoniensis* from Argentina is the only one that reveals significant differences in pholidosis of males and females of this species. Other studies with *P. patagoniensis* have addressed for ecological and natural history approaches (see Thomas 1976, Cei 1993, Achaval & Olmos 1997, Fowler & Salomão 1994a, b, 1995, Hartmann & Marques 2005, López & Giraudo 2008). Not only in specimens of *P*. *patagoniensis*, as in the whole tribe Philodryadini, from Brazil the studies with sexual dimorphism associated with pholidosis and morphometric features of this species are still scarce (Fowler & Salomão 1994a, b, 1995, Pontes 2007, López & Giraudo 2008, Zaher et al. 2008).

Therefore, the current study aimed to analyze the differences between sexual dimorphism and the patterns of coloration, the morphometrical features and pholidosis of *P*. *patagoniensis* from Brazil.

Material and methods

The current study was performed with specimens of *P. patagoniensis* from several locaties in Brazil, and deposited in the following herpetological collections from Brazil: Museu de Zoologia João Moojen (MZUFV), from Universidade Federal de Viçosa, Viçosa; Fundação Ezequiel Dias (FUNED), Belo Horizonte; Museu de Ciências Naturais from Pontifícia Universidade Católica de Minas Gerais (MCNR), Belo Horizonte; Laboratório de Zoologia de Vertebrados from Universidade de Federal de Ouro Preto (LZV), Ouro Preto; Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília; Universidade Federal de Juiz de Fora (UFJF), Juiz de Fora; Centro Universitário de Lavras (UNILAVRAS), Lavras; Museu Nacional (MNRJ), Rio de Janeiro; and Museu de Zoologia da Universidade de São Paulo (MZUSP), São Paulo.

The specimens were analyzed between May 2011 and September 2012. A total of 355 specimens of *Philodryas patagoniensis* were analyzed, of which 145 were males (87 adult specimens and 58 juveniles) and 210 were females (134 adults and 76 juveniles) (see appendix). The speciemens analyzed in the current study were identified according to morphological diagnostic features proposed by Girard (1858), and Thomas (1976).

The features of pholidosis analyzed or quantified are: number of dorsal scales (NDS), number of ventral shields (NVS), number of subcaudal shields (NSS), supra-labial scales (SLS), pre-ocular scales (PRS), post-ocular scales (POS), supra-ocular scales (SOS), nasal scale (NS), rostral scale (RS), loreal scale (LS), pre-frontal scales (PFS), parietal scales (PS), temporal scales (TS), infra-labial scales (ILS) and cloacal shields (CS), when possible was used the "first wider than long" system. The measurable features analyzed are: head length (HL), head width (WH), inter-ocular width (IOW), snout-vent length (SVL), tail length (TL), and total length (TBL). The measurements were made using analogical calliper and metric rule, according to Dowling (1951b), Thomas (1976), and Francini et al. (1990).

The sexual size dimorphism (SSD) was calculated according to the index adapted from Gibbons & Lovich (1990): SSD = mean SVL of the larger sex divided by the mean SVL of the smaller sex, where negative values indicate that males are larger than females. Individuals missing all or part of the tail were not used in the analyses that employed SVL and TL values. Females with SVL < 670 mm and males with SVL < 525 mm were classified as juveniles, according to Pontes (2007).

The relationship of sex and/or sexual maturity with the NVS, NSS, SVL, and TL was determined by the Wilcoxon test. The same test was used to relate the HL with the sex of the specimens and to relate the NVS, NSS, SVL, TL, TBL, HL, WH and IOW to the patterns of coloration.

An analysis of covariance (ANCOVA) was used to relate the SVL to the TL; three models were tested: model 1 ($SVL=\beta_0 + \beta_1 Sex + \beta_2 TL + \beta_3 Sex:TL$), model 2 ($SVL=\beta_0 + \beta_1 Sex + \beta_2 TL$) and model 3 ($SVL=\beta_0 + \beta_1 TL + \beta_2 Sex:TL$). The same tests were used to relate the SVL with the HL using the following models: model 1 ($SVL=\beta_0 + \beta_1 Sex + \beta_2 HL + \beta_3 Sex:HL$), model 2 ($SVL=\beta_0 + \beta_1 Sex + \beta_2 HL$) and model 3 ($SVL=\beta_0 + \beta_1 HL + \beta_2 Sex:HL$). The proportions between TBL and TL, TBL and HL and NVS and NSS were tested by linear regression.

The data was tested for homogeneity of the variances by the Levene test before other statistical analyses. The significance level in all the tests was $\alpha = 0.05$. The statistical analyses were carried out with the R for Windows program.

To determine the sex, in all specimens analyzed was verified the absence or presence of hemipenis by eversion, or through a small median longitudinal section in the region of the first subcaudal shields according to Yuki (1994).

Results

In the current study the specimens analyzed exhibited an unmodified rostral-scale, two inter-nasal scales, two prefrontals, a single frontal scale, two parietals, a divided nasal scale, one loreal, one pre-ocular, two post-oculars, one supra-ocular, 1+2 temporals, 7 or 8 supralabials (with the third and fourth contacting the orbit), 9 or 10 infralabials, 19-19-15 dorsal scale rows with one apical pit, a divided cloacal shield, 138 to 210 ventral shield, and 40 to 123 divided subcaudal shields.

The juvenile and adult females have a higher NVS that than males (Table 1; W = 3179.5, p = 0.005 for juveniles; W = 10062, p < 0.001 for adults). Adult females have a longer SVL than adult males (Table 1; W = 10606.5, p < 0.001) and the SVL of juvenile snakes does not differ between the sexes (Table 1; W = 2390, p = 0.330).

Males have a greater NSS than females, according to the analysis of covariance (Table 1; W = 748, p = 0.001 for juveniles; W = 1923.5, p = 0.001 for adults), however the adult females have a greater TL than males (Figure 1) (Table 1; W = 6995, p = 0.007). The TL of the juvenile specimens did not present a statistical difference between the sexes (Figure 1) (Table 1; W = 1981.5, p = 0.381).

The sexual size dimorphism (SSD) index in *P*. *patagoniensis* was positive in this study, confirming that females are larger than males (1.004).

The analysis of covariance between the TL and SVL of males and females ($R^2 = 0.7848$; F = 638.3) shows that the TL is influenced both by SVL and by sex, with a good approximation between the sexes based on the TL and SVL values (Figure 1).

The HL of the females (n=208) ranged between 11.3 to 44.2 mm, while for the males (n=144) it ranged between 7.7 to 33.1 mm. The HL of the females was significantly greater that than HL of males (W = 20925, p = 0.001).

Three models were also tested in the analysis of covariance between the HL and SVL of males and females. Model 3 presented the highest significance ($R^2 = 0.8867$; F = 2740), with the following coefficients (Intercept: -280.7827, p = <0.001; HL: 38.6754, p = < 0.001). Therefore, the head length is closely related to the snout-vent length of the specimens, however, there was no influence of sex in any of the models (Figure 1).

According to the R^2 value ($R^2 = 0.006$) found in the regression analysis, there was no correlation between the variables NVS, NSS, and TBL, indicating that these variables are not closely related.

The regression analysis also showed that the TL of these specimens corresponds to 24% ($R^2 = 0.840$) of the TBL. This means that the TL value

Table 1. Variation of morphometric and pholidosis characteristics measures separated by sex and age range in the specimens of the *Philodryas patagoniensis*.

Morphometric	Females				Males			
	N	Means (cm)	SD	Variation (cm)	N	Means (cm)	SD	Variation (cm)
Juveniles								
Ventral	75	185.21	12.69	147-207	58	177.53	7.82	155-194
Subcaudal	74	97.62	12.60	40-117	56	108.38	7.94	73-119
SVL	75	349.03	137.48	144-669	58	325.10	107.94	177-517
TL	75	129.35	71.06	57-439	58	128.60	51.26	66-241
Adults								
Ventral	134	188.28	8.65	138-210	86	178.06	6.7	157-196
Subcaudal	131	94.97	12.08	40-116	85	106.13	9.24	66-123
SVL	134	923.81	149.1	679-1345	86	688.38	97.2	539-1010
TL	134	298.43	56.24	90-429	86	285.84	42.43	181-396

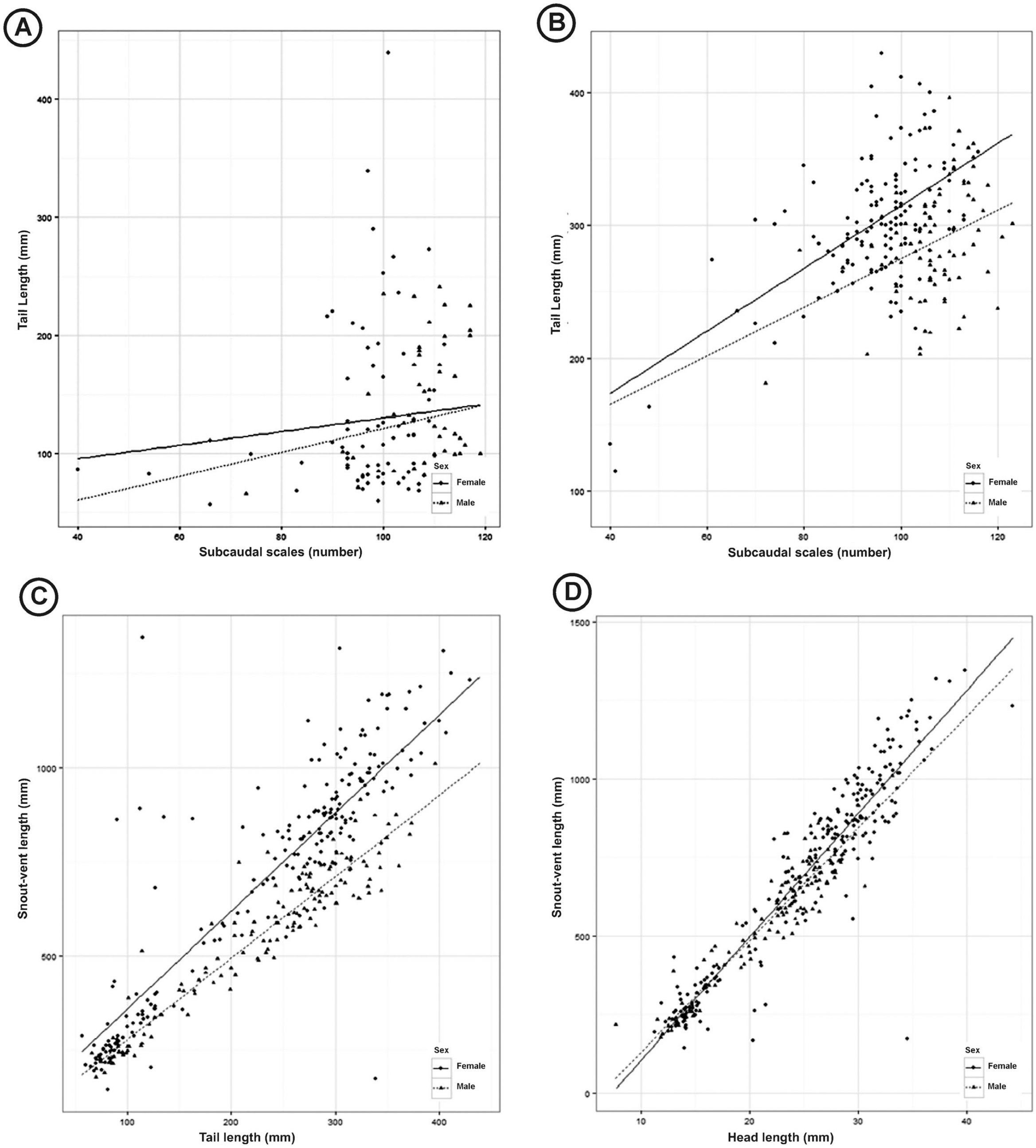

Figure 1. (A) Relation between the tail length and number of subcaudal shield of juveniles females and males. (B) Relation between the tail length and number of subcaudal shield of adults females and males. (C) Relation between the tail length and snout-vent length of females and males. (D) Relationship of the snout-vent length, head length and sex of the specimens.

can be predicted from the TBL value. The head length is corresponds to 1.8% (R^2 = 0.903) of the TBL. Thus it is possible to obtain any value of TBL or HL from the other pair.

We observed two patterns of coloration in the specimens studied herein: (1) dorsum dark brown, with black spots only on the posterior region of the scales, a clear and delineated black belly, and orange coloration on the head, and (2) uniform brown head and dorsum, light greenish-brown without black spots on the posterior portion of the dorsal scales, a light belly. These patterns (with or without spots) were used for comparisons of the all specimens studied herein (Figure 2) (Table 2).

The correlations of these two patterns of coloration between the NVS, NSS, TBL, SVL, TL, WH, HL, and IOW were performed according to the Wilcoxon test. We observed that only the NVS and NSS variables were not significant. Among the variables that showed significant results, only the variable IOW showed higher mean for the patterns of coloration 2. For all other variables, the mean was higher for the patterns of coloration 1 (Table 3).

Discussion

The wide range observed in the NVS and NSS in the specimens studied herein was also observed by Thomas (1976) and D'Agostini (1998) in other species of the genus *Philodryas*, such as *P. aestiva* (Duméril, Bibron & Duméril, 1854), *P. olfersii* (Lichtenstein, 1823) and *P. viridissima* (Linnaeus, 1758). Thomas (1976) studied *P. patagoniensis* also found a large variation in the NVS and NSS (151 to 194 ventral shields and 68 to 120 subcaudal shields) than that observed in the current study.

The results obtained on the NSS and NVS of the adults of *P. patagoniensis* are in accordance with observed by López & Giraudo (2008) in the same species from Argentina. These authors observed that the adult males of *P. patagoniensis* have a higher NSS than the adult females, while the adult females have a higher NVS than the adult males. The higher body size and NVS in females of this species can be a reflective of the optimization of reproductive potential, since the females needs a higher body volume to produce a higher number of eggs or large size of eggs (Mebert 2011). In the current study we observed a significant difference in the NSS between males and females (Table 1).

We observed that the adult females of *P. patagoniensis* have a largest body size that than adult males (Table 1). A similar pattern of females were reported in several other species of snakes (Solorzano & Cerdas 1989, Shine 1993, Rivas & Burghardt 2001, Gregory 2004, Pinto & Fernandes 2004, Matias et al. 2011, Mesquita et al. 2011, Henao-Duque & Ceballos 2013).

Several authors observed that the fertility is considered one of the main selective agents for the larger size of females for some species of snakes; however, the largest size of males in another species of snakes can be considered as a significant feature of males of species that possesses the behaviour of competition for territory and for females in reproductive period (Shine 1978, 1993, 1994, 2000, Zug et al. 2001, Matias et al. 2011, Henao-Duque & Ceballos 2013).

We found a high value of sexual size dimorphism index (SSD), which can possibly indicate the absence of territorial behaviour in *P. patagoniensis*, since the species of snakes that exhibiting the behaviour of territorial and reproductive dispute usually have SSD < 0.05 or with negative values of this index, according Shine (1978, 1994) and Oliveira (2008). The same applies to the species *P. agassizii* and *P. nattereri*, since they have SSD >0.05 and there are no indications of territorial behavior (Marques et al. 2006, Mesquita et al. 2011).

Shine (1978) says that the late maturity in female snakes can represent a trade off in which the sexual maturity is delayed until their body size allows the production of sufficiently numerous and large eggs, and highest fertility. Sex differences in snake body size adjustments are considered that may lead to a higher success reproductive (Pizzatto et al. 2007). Therefore, the sexual dimorphism probably exists due to the benefits of larger body size of female so that, according to Pizzatto et al. (2007), the natural selection has favored females with larger body size. Studies of snakes have shown that on the average females are larger than males (approximately 15%) in species where males do not compete for mates, Shine (1994). The sexual dimorphism occurring in the SVL of *P. patagoniensis* indicates than the sexes reach maturity at different sizes; however, according to Fowler & Salomão (1995) it is known that growth is not a continuous process.

Table 2. Percentage of specimens from different Brazilian states according to the color pattern, sex and sexual maturity in the specimens of the *Philodryas patagoniensis*.

Localities	Female				Males			
	Juveniles		Adults		Juveniles		Adults	
	Pattern 1	Pattern 2	Pattern 1	Pattern 2	Pattern 1	Pattern 2	Pattern 1	Pattern 2
Bahia	0%	0%	2%	0%	0%	3%	10%	8%
Distrito Federal	20%	23%	10%	15%	16%	0%	0%	17%
Espírito Santo	0%	0%	4%	0%	0%	0%	0%	0%
Goiás	7%	0%	5%	9%	2%	18%	6%	8%
Maranhão	0%	0%	0%	0%	0%	0%	4%	0%
Mato Grosso	0%	0%	0%	0%	0%	0%	4%	0%
Mato Grosso do Sul	0%	0%	3%	0%	0%	0%	0%	0%
Minas Gerais	35%	38%	30%	30%	49%	29%	33%	34%
Pará	0%	0%	1%	0%	0%	0%	0%	0%
Paraíba	0%	0%	0%	3%	2%	13%	0%	0%
Paraná	0%	0%	1%	3%	2%	6%	0%	0%
Pernambuco	0%	0%	0%	0%	0%	6%	0%	0%
Rio de Janeiro	17%	15%	16%	9%	10%	3%	16%	8%
Rio Grande do Sul	2%	8%	5%	3%	4%	0%	0%	0%
Roraima	0%	0%	0%	0%	0%	0%	0%	0%
Santa Catarina	4%	0%	1%	0%	4%	0%	0%	1%
São Paulo	9%	8%	20%	9%	7%	23%	23%	17%
Sergipe	4%	0%	0%	6%	0%	3%	0%	0%
Tocantins	2%	8%	2%	13%	4%	6%	4%	0%

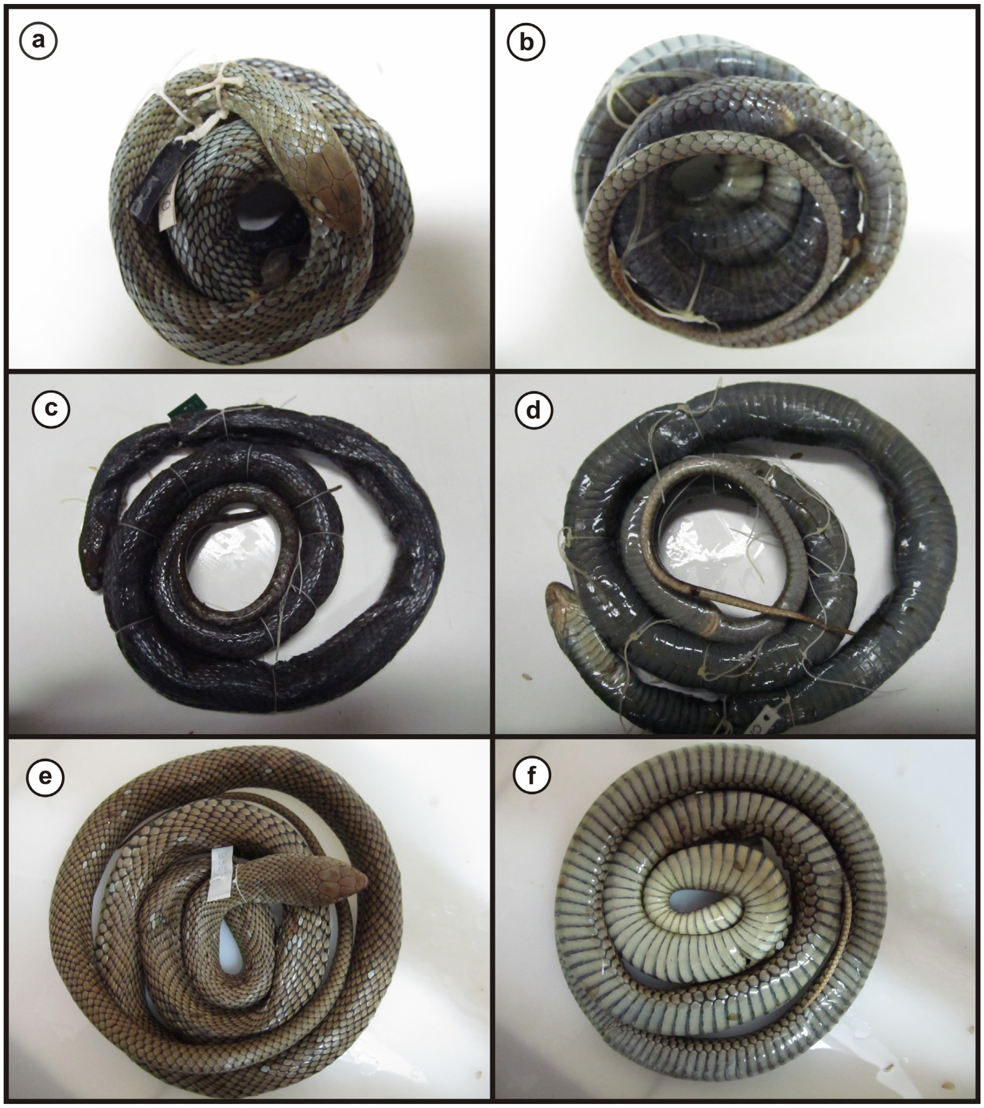

Figure 2. (A) Pattern of coloration 1 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Universidade de Brasília (CHUNB), dorsal view. (CHUNB 3779, Brasília - DF). (B) Pattern of coloration 1 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Universidade de Brasília (CHUNB), ventral view. (CHUNB 3779, Brasília - DF). (C) Pattern of coloration 2 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Universidade de Brasília (CHUNB), dorsal view. (CHUNB 19337, Brasília - DF). (D) Pattern of coloration 2 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Universidade de Brasília (CHUNB), ventral view. (CHUNB 19337, Brasília - DF). (E) Pattern of coloration 1 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Museu de Zoologia João Moojen of Universidade Federal de Viçosa (MZUFV), dorsal view. (MZUFV 1369, Tocantins - MG). (F) Pattern of coloration 1 of specimen of *Philodryas patagoniensis* deposited in the Herpetological Collection of Museu de Zoologia João Moojen of Universidade Federal de Viçosa (MZUFV), ventral view. (MZUFV 1369, Tocantins - MG).

Table 3. Total number, means, standard deviation, variation and statistical results between the coloration patterns with variable: number of ventral and subcaudal shields (NVS and NSS), total length (TBL), snout-vent length (SVL), tail length (TL), head length (HL), head width (WH) and inter-ocular width (IOW) of *Philodryas patagoniensis*. Statistical significance represented by * in the table.

Scales	Patterns of coloration								Test Wilcoxon
	Parttens 1				Parttens 2				
	N	Means (cm)	SD	Variation (cm)	N	Means (cm)	SD	Variation (cm)	p-value
NVS	256	183.73	9.81	138-210	95	183.05	10.65	154-207	W = 12893.5, p < 0.385
NSS	251	100.25	11.45	40-123	93	102.12	11.95	41-120	W = 10205.5, p < 0.073
TBL	256	907.12	397.57	247-1713	95	807.21	284.64	225-1460	W = 14612, p < 0.004*
SVL	256	670.09	304.86	168-1317	95	587.38	219.36	144-1345.4	W = 14566, p < 0.004*
TL	256	237,03	103.03	60-439	95	219.83	78.35	57-360	W = 14251, p < 0.013*
HL	256	24.45	7.37	11-44.2	94	22.62	5.64	7.7-39.8	W = 14320, p < = 0.006*
WH	256	10.03	3.59	3.3-29.6	95	9.5	3.44	4.4-32.8	W = 13854, p < 0.045*
IOW	256	7.98	2.41	2.2-21.1	95	8.24	7.79	3.1-81.3	W = 14000.5, p < 0.019*

According to Mesquita et al. (2010), in specimens of *Oxybelis aeneus* (Wagler in Spix, 1824) (Colubridae) from Brazil the growth rate declines after sexual maturity and therefore females reach maturity at a larger average size than males; although adult males with larger tails that adult females are most commonly pattern found in snakes, due to the presence of hemipenis and retractors muscles (Shine 1993). However, we observed that this pattern of tail sizes of males and females in *P. patagoniensis* (Table 1) not correspond with reported in most species of snakes. We can not find arguments that could support a hypothesis about females having a larger tail than males. Perhaps in the future, with the increase of studies on morphometric patterns in other species of neotropical snakes, not yet studied, could elucidate this fact. López & Giraudo (2008) found out that there is difference in the count and size of the shields of females and adults males of *P. patagoniensis*. Females have more ventral shields and males have more subcaudal shields. Vanzolini & Brandão (1944, 1945) found the occurrence of that dimorphism in *Bothrops alternatus* Duméril, Bibron & Duméril, 1854, with the same pattern (females have a higher number of dorsal scales and ventral shields, and males have a higher number of subcaudal shields), however, as in the current study these authors also do not find a justification that would explain this fact.

Although sexual dimorphism in HL is related to dietary differences as suggested by Pough & Groves (1983), Forsman (1991), Shine (1986, 1991), Houston and Shine (1993), Forsman (1996), King (2002), Shetty & Shine (2002), Nogueira et al. (2003), Aubret et al. (2004), Vincent et al. (2004), and López et al. (2013). The differences found in HL between males and females of *P. patagoniensis* are not related to diet (Hartmann & Marques 2005, López & Giraudo 2008). This result is consistent with the observations of Mesquita et al. (2011) for the species *P. nattereri*, indicating that the sexual dimorphism observed is due to reproductive characteristics (Luiselli et al. 2002).

The sexual dimorphism can be observed in different ways in snakes (Rivas & Burghardt 2001). For several species the sexual dimorphism is related to volume and size of body (Shine 1993, 1994); the shape and size of head is another feature for diagnostic of sexual dimorphism (Camilleri & Shine 1990, Vincent et al. 2004); the size of visceral mass and glands also is a important feature for detection of sexual dimorphism (Kissner et al. 1998); but some authors affirms that the patterns of coloration rarely is related to sexual dimorphism (Shine 1993, Marques & Sazima 2003). In the current study we can affirm that not exist a significant difference in the pattern of coloration between males and females of *P. patagoniensis*.

The specimens that we examined from the state of Roraima have not been reported previously in the literature; therefore the current study provides data that expands geographic distribution of this species in Brazil (Figure 3).

We can affirm that the specimens of *P. patagoniensis* studied herein had a great variation in the folidosis. Sexual dimorphism in relation to

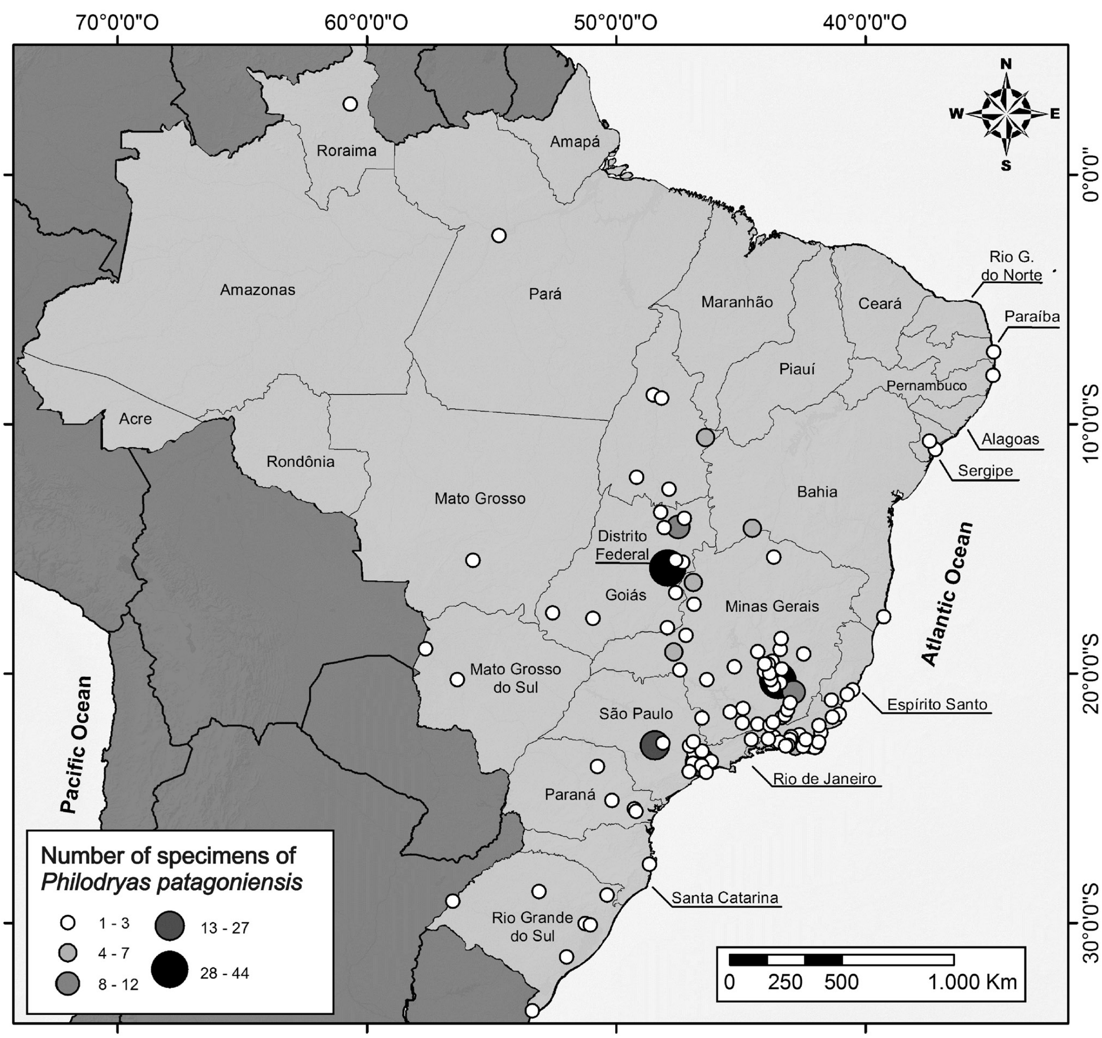

Figure 3. Distribution of studied specimens of *Philodryas patagoniensis* in 18 states of Brazil and the Federal District.

SVL, TL, NVS, NSS, and HL was observed. Adult females are larger than adult males in SVL and TL. However, in juveniles these differences do not occur. Females have a greater number of ventral shields than males, and males have a greater number of subcaudal shields. Also we can affirm that the specimens of *P. patagoniensis* studied have two distinct patterns of coloration, however without geographic and sexual specificity for these patterns.

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Appendix

Localities of collection of specimens analyzed, and respectives codes of voucher specimens at the zoological collection of reference.

Bahia: Caravelas (MNRJ 9336), Cocos (CHUNB 51369; CHUNB 51370; CHUNB 51371; CHUNB 51372; CHUNB 51373), Sem procedência (MUZUSP 10082). Brasília: Brasília (CHUNB 139; CHUNB 3620; CHUNB 3640; CHUNB 3773; CHUNB 3774; CHUNB 3775; CHUNB 3776; CHUNB 3777; CHUNB 3778; CHUNB 3779; CHUNB 3781; CHUNB 3783; CHUNB 3784; CHUNB 3785; CHUNB 3787; CHUNB 3788; CHUNB 3789; CHUNB 3790; CHUNB 3837; CHUNB 14181; CHUNB 18473; CHUNB 19328; CHUNB 19330; CHUNB 19334; CHUNB 19337; CHUNB 19339; CHUNB 19340; CHUNB 19341; CHUNB 24445; CHUNB 24563; CHUNB 24596; CHUNB 25355; CHUNB 28948; CHUNB 30380; CHUNB 30843; CHUNB 40793; CHUNB 44139; CHUNB 49608; CHUNB 50788; CHUNB 56858; CHUNB 56862; CHUNB 56880; CHUNB 57464; CHUNB 65799). Espírito Santo: Guarapari (MNRJ 20045), Piúma (MCNR 2611). Goiás: Alto Paraíso de Goiás (MNRJ 9351; CHUNB 3751; CHUNB 19331; CHUNB 19342; CHUNB 59128; CHUNB 59129; CHUNB 59131; CHUNB 59132; CHUNB 59523), Catalão (MNRJ 7440), Cristalina (CHUNB 19335), Colinas do Sul (MNRJ 21197), Formosa (CHUNB 27640), Mineiros (CHUNB 18440; CHUNB 23729), Minaçu (CHUNB 3768), Planaltina de Goiás (CHUNB 19332), Rio Verde (CHUNB 50288), Teresinha de Goiás (MCNR 3187), Sem procedência (MUZUSP 1908; MUZUSP 15047; MUZUSP 17773). Mato Grosso: Chapada dos Guimarães (CHUNB 20421), Corumbá (MNRJ 20943; MNRJ 20994). Maranhão: Perdizes (MNRJ 11342). Mato Grosso do Sul: Miranda (FUNED 1942). Minas Gerais: Belo Horizonte (FUNED 973, MNRJ 4849), Bom Despacho (FUNED 195), Cachoeira Escuro (FUNED 2206), Caxambú (UFJF 17), Chácara (UFJF 875; UFJF 883), Conceição do Mato dentro (FUNED 1637; FUNED 1695), Contagem (MCNR 81), Cordisburgo (FUNED 198), Coromandel (MNRJ 8405; MNRJ 8406), Ingaí (UNILAVRAS 157; UNILAVRAS 216; UNILAVRAS 241), Irapé (MCNR 1707; MCNR 2913), Itabirito (LZV 94), Itaperuna (FUNED 1913), Jaboticatubas (MNRJ 8879), Jaíba (FUNED 1466), Juiz de Fora (UFJF78; UFJF79; UFJF500), Km 813, sentido Juiz de Fora, BR-040, RJ (MNRJ 19878), Lagoa Santa (MNRJ 1323), Liberdade (MNRJ 8408), Mariana (MUZUSP 15726; MUZUSP 15727; FUNED 1142), Mina do Serro (MCNR 3390), Nova Lima (LZV 177), Nova Ponte (FUNED 725; FUNED 772; FUNED 774; FUNED 775), Ouro Branco (LZV 285; LZV 493), Ouro Preto (LZV 16; LZV 17; LZV 18; LZV 36; LZV 61; LZV 62; LZV 69; LZV 93; LZV 148; LZV 161; LZV 162; LZV 172; LZV 250; LZV 262; LZV 273; LZV 286; LZV 287; LZV 293; LZV 294; LZV 327; LZV 357; LZV 358; LZV 391; LZV 424; LZV 442; LZV 443; LZV 454; LZV 455; LZV 481; LZV 596; LZV 654; LZV 666; LZV 670; LZV 671; LZV 736; LZV 773; LZV 787; LZV 807; LZV 886; LZV 902; LZV 989; MUZUSP 14096; MUZUSP 14212; MUZUSP 15149), Paracatu (MCNR 3158), Pedro Leopoldo (MCNR 95), Poços de Caldas (MNRJ 4511, MUZUSP 14072; MUZUSP 14093), Queimados, entre os municípios de Cabeira Grande - MG, Unaí - MG, Cristalina – GO (MNRJ 10930), Rio novo (MNRJ 9584), Rio Preto (MCNR 4338), Sacramento (MZUFV 1077), Santa Bárbara do Monte Verde (MNRJ 7068), São Gonçalo do Rio abaixo (MNRJ 9047), São Roque de Minas (MZUFV 1755; MZUFV 1761; MZUFV 1826), São Simão (MNRJ 4919), Serra do Cipó (MCNR 3530), Tocantins (MZUFV 1369), Transição ente Paracatu e Pirapora (MCNR 3286), Unaí (CHUNB 3618; CHUNB 24389; CHUNB 24477; CHUNB 29420), Varginha (MUZUSP 15155), Viçosa (MZUFV 62; MZUFV 155; MZUFV 336; MZUFV 719; MZUFV 1149; MZUFV 1203; MZUFV 1611; MZUFV 1615), Sem procedência (MUZUSP 15732; MUZUSP 17984; MUZUSP 17991; MUZUSP 18656). Pará: Santarém

(CHUNB 6665). Paraíba: João Pessoa (MUZUSP 8267; MUZUSP 8999; MNRJ 9717). Paraná: Curitiba (MUZUSP 3685; MUZUSP 3686), Ponta Grossa (MUZUSP 5787), São Jerônimo da Serra (MUZUSP 11520), São José dos Pinhais (MUZUSP 13868). Pernambuco: Sem procedência (MNRJ 620; MUZUSP 975). Rio de Janeiro: Arraial do Cabo (MNRJ 21774; MNRJ 21775; MNRJ 22924), Barra de São João (MNRJ 7684; MNRJ 8376), Búzios (MNRJ 10182), Cabo Frio (MUZUSP 4203; MUZUSP 5870; MUZUSP 10802; MNRJ 8401; MNRJ 8373), Cachoeira do Macacu (MNRJ 12350; MNRJ 12351; MNRJ 20923), Campos dos Goytacazes (MNRJ 16400; MNRJ 17765), Conceição de Macabu (MNRJ 16438), Guapimirim (MNRJ 14303), Iguaba (MNRJ 8403), Iguaba Grande (MNRJ 17340; MNRJ 17926; MNRJ 18128; MNRJ 18129; MNRJ 18130; MNRJ 18521), Macaé (MUZUSP 7486; MNRJ 15660; MNRJ 19240), Magé (MNRJ 16470), Maricá (MNRJ 4739; MNRJ 8402; MNRJ 13165, MNRJ 13166; MNRJ 14087), Nova Iguaçu (MNRJ 21776), Niterói (MNRJ 8359), Paulo Frantin (MNRJ 8377), Piraí (MNRJ 8375), São João da Barra (MNRJ 17436), Saquarema (MNRJ 7269; MNRJ 14122), Silva Jardim (MNRJ 19861), Sem procedência (MUZUSP 2338; MNRJ 3992). Rio Grande do Sul: Itaqui (MUZUSP 1385; MUZUSP 1852), Jaquirana (CHUNB 49965), Porto 15 de Novembro (MUZUSP 11604; MUZUSP 11605), São Lourenço (MUZUSP 248; MUZUSP 250), Taím (MUZUSP 7435), Tupaceretan (MUZUSP 2576), Viamão (MUZUSP 5745), Sem procedência (MUZUSP 270; MUZUSP 7335). Roraima: Boa Vista (CHUNB 6650). Santa Catarina: Palhoça (MNRJ 20169; MNRJ 20172; MNRJ 20173), Sem procedência (MUZUSP 5182; MUZUSP 9425; MUZUSP 9426). São Paulo: Anhembi (MNRJ 21753), Atibaia (MUZUSP 245), Botucatu (MUZUSP 2426; MUZUSP 2644; MUZUSP 2645; MUZUSP 3458; MNRJ 18545; MNRJ 18546; MNRJ 19336; MNRJ 20480; MNRJ 21131; MNRJ 21745; MNRJ 21748; MNRJ 21751; MNRJ 21908; MNRJ 21919; MNRJ 22079; MNRJ 22136; MNRJ 22140; MNRJ 22142; MNRJ 22143; MNRJ 22144; MNRJ 22147; MNRJ 22841; MNRJ 22903; MNRJ 22904; MNRJ 22905; MNRJ 22906; MNRJ 22907), Campinas (MUZUSP 244), Cotia (CHUNB 6124), Divisa do Campos dos Goytacazes - SP e Mimoso do Sul – ES (MNRJ 15817; MNRJ 15847), Jundiaí (MUZUSP 12810; MNRJ 17120), Juquitiba (MUZUSP 16495), Mogi das Cruzes (CHUNB 24490), Pedreira (CHUNB 6123), São Bernardo do Campo (MUZUSP 4024), São Vicente (MUZUSP 4572), Serra da Bocaina (MUZUSP 4103; MUZUSP 4649; MUZUSP 4911), Sem procedência (MUZUSP 239; MUZUSP 1689; MUZUSP 1955; MUZUSP 2640; MUZUSP 2860; MUZUSP 2861; MUZUSP 2979; MUZUSP 3457; MUZUSP 4068; MUZUSP 4069; MUZUSP 4605; MUZUSP 4651; MUZUSP 5519; MUZUSP 8531; MUZUSP 12355; MUZUSP 12832; MUZUSP 16529). Sergipe: Itabaiana (MUZUSP 15840), São Cristovão (MUZUSP 15838; MUZUSP 15839), Sem procedência (MUZUSP 17454; MUZUSP 17455). Tocantins: Figueirópolis (CHUNB 62806), Guaraí (MUZUSP 1269), Mateiros (CHUNB 33800; CHUNB 41108, CHUNB 41109; CHUNB 41110; CHUNB 41111), Paraná (CHUNB 38287), Pedro Afonso (CHUNB 52431), Sem procedência (MUZUSP 14396; MUZUSP 14601; MUZUSP 15508; MUZUSP 15509; MUZUSP 15510; MUZUSP 15511).

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Aquatic invertebrates associated with bromeliads in Atlantic Forest fragments

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Abstract: Forest fragments in the state of Minas Gerais contain a large number of Bromeliaceae genera, whose cisterns accumulate water and organic matter, providing shelter and food for a wide range of organisms. However, these fragments often consist only of small patches of vegetation, making the species more vulnerable to the effects of this landscape changes. This study aimed to test the effect of the distance to the edge and of the matrix type on the structure of aquatic invertebrate communities in four morphospecies of bromeliads. Samples were collected in Atlantic Forest fragments adjacent to pasture and planted forest areas, in a region of Serra da Mantiqueira, Brazil. Of the 147 bromeliads investigated, we found 35 taxa, among which the most abundant groups were Ostracoda (4,962 individuals), Culicidae (2,358), Tanypodinae (1,164) and Scirtidae (1,043). The richness of taxa and Shannon diversity of invertebrates were similar at different distances from the edge, with variation of richness between 17 and 23 taxa and diversity between 1.25 and 1.52. The composition of the fauna in the cisterns changed between some collection sections (A vs. C, C vs. D and D vs. F), irrespective of being close to or far from the edge. There was no variation of richness (t=-1.145, df=106, p= 0.341) and diversity (t= 1.376, df= 106, p= 0.429) among samples collected from fragments next to planted forest and pasture, likely because the bromeliads studied were located on hillsides above the canopy of planted forests, and subject to similar conditions to those found in the fragments next to pastures. The results demonstrate the importance of bromeliads in maintaining the richness and diversity of the invertebrates they harbor, even in altered landscapes exposed to extreme conditions such as fragment edges.

Keywords: *Edge effect, phytotelma, invertebrates, remaining forest*

Invertebrados associados a bromélias em fragmentos de Mata Atlântica

Resumo: Remanescentes florestais em Minas Gerais concentram grande número de gêneros de Bromeliaceae, cujos taques acumulam água e a matéria orgânica que servem de abrigo e fonte de alimento para uma grande variedade organismos. Entretanto, tais áreas têm sido reduzidas a pequenas manchas de vegetação, tornando as espécies mais vulneráveis aos efeitos decorrentes dessa modificação da paisagem. O presente estudo visou caracterizar a estrutura das comunidades de invertebrados aquáticos em quatro morfoespécies de bromélias. As amostras foram coletadas em fragmentos de Mata Atlântica em matriz de pastagem e floresta plantada, em uma região da Serra da Mantiqueira, Brasil. Nas 147 bromélias investigadas foram encontrados 35 táxons, entre os quais os grupos mais abundantes foram Ostracoda (4,962 individuals), Culicidae (2,358), Tanypodinae (1,164) e Scirtidae (1,043). A riqueza de táxons e diversidade de Shannon foram semelhantes em diferentes distâncias da borda, com variação entre 17 e 23 táxons e diversidade entre 1.25 e 1.52. A composição da fauna nos tanques variou entre algumas faixas de coleta (A vs. C, C vs. D e D vs. F), independentemente de estarem próximas ou distantes da borda. Não houve variação da riqueza (t=-1.145, df=106, p= 0.341) e diversidade (t= 1.376, df= 106, p= 0.429) entre as amostras coletadas em fragmentos próximos à floresta plantada e pastagem, provavelmente devido às bromélias estudadas estarem localizadas em encostas acima do dossel das florestas plantadas, e sujeitas a condições semelhantes às encontradas nos fragmentos ao lado de pastagens. Foi possível demonstrar a importância das bromélias na manutenção da riqueza e da diversidade de invertebrados que abrigam, mesmo em paisagens alteradas e expostas a condições extremas, tais como nas bordas dos fragmentos.

***Palavras-chave*:** *Efeito de borda, fitotelma, invertebrados, remanescentes florestais.*

Introduction

Bromeliads in neotropical forests serve as habitats for bacteria (Haubrich et al. 2009), algae (Brouard et al. 2011), fungi (Sophia 1999), protozoa (Foissner et al. 2003), arthropods and anuran amphibians (Benzing 1990, Silva et *al*. 2011). Some invertebrates, such as oligochaetes and ostracods, spend their entire lives inside the cisterns of bromeliads (Montero et al. 2010, Pinto & Jocqué 2013), while others are only found in such habitats in immature forms, mainly beetles and flies (Mestre et al. 2001). These organisms play an important role (Sodré et al. 2010), by helping to degrade the organic matter falling from the forest canopy, accelerating the release of nutrients to the bromeliad (Benzing 1990, Richardson 1999, Armbruster et al. 2002, Araújo et al. 2007, Brouard et al. 2012). Furthermore, Martinelli et al. (2008) mentioned the ecological importance of bromeliads due to their interaction with fauna, making a considerable contribution of the biodiversity of the communities in which they live. Some endangered insect species in the Atlantic Forest use bromeliads as habitat for reproduction (De Marco-Júnior e Furieri 2000).

The reduction of forest habitats by human activities leads to landscapes formed of isolated fragments (Fahrig 2003), and consequently causes the isolation of populations, making species more vulnerable to hunting, competition with invasive species, predation and alterations in physical and chemical parameters (Laurance 1991, Murcia 1995, Dale et al., 2000, López-Barrera et al. 2005). This fragmentation interferes in the composition and distribution of the species along the edge gradient towards the center of the fragment (Ewers & Didham 2006). Such alteration of forest habitats often occurs in short time intervals and impairs the evolutionary processes of species.

Although studies of bromeliads have shown high potential to understand the ecology of forested landscapes and the different compositions of the invertebrate communities in distinct landscapes (Yanoviak et al. 2006), information about the influence of habitat fragmentation on invertebrates that live inside bromeliads is still scarce in the literature. Minas Gerais, especially in the Mantiqueira and Serra mountain ranges, contains various remnant areas of Atlantic Forest and Cerrado, respectively, with a rich diversity of epiphytes, including some bromeliads considered in danger of extinction (Versieux & Wendt 2007), whose tanks serve as habitat for diverse fauna. Hence, there is a need for studies in this region that can generate information to support measures to preserve bromeliad communities.

Animals that live in plants can be influenced by factors such as geography, environment and human activities (Kitching 2008). In the local context, the edge effect can lead to changes in the structure, composition and dynamics of populations of plants (Lima-Ribeiro 2008) and animals (Ewers & Didham 2007). Based on these patterns, the aim of this study was to test the hypothesis that the populations of invertebrates that live in bromeliad tanks located near the edges of forest fragments have lower richness and diversity than those living in bromeliads within such fragments. Additionally, we tested whether the fauna in bromeliads located in native fragments near planted forests is less susceptible to the edge effects than those in a pasture matrix.

Material and methods

1. Study area

The study was carried out in 12 forest fragments located in the Serra da Mantiqueira range in the municipality of Bom Jardim, located in the state of Minas Gerais, southeastern Brazil (Figure 1), between the coordinates 21º54'S; 44º05'W and 22º04'S; 44º13'W. In each fragment (In this study identified with the letter F) the number of bromeliads sampled varied according to the availability of these plants: F1 (12 sampled bromeliads), F2 (8 bromeliads), F3 (15 bromeliads), F4 (20 bromeliads), F5 (9 bromeliads), F6 (13 bromeliads), F7 (16 bromeliads), F8 (9 bromeliads), F9 (15 bromeliads), F10 (7 bromeliads), F11 (15 bromeliads) and F12 (8 bromeliads), The two fragments nearest together (F9 and F10) are separated by approximately 120 m, while the two furthest apart (F1 and F12) are about 21 km from each other. The altitude in the area ranges from 1,200 to 1,450 m.

The remaining Atlantic Forest vegetation in the study area is composed of phytophysiognomies classified as dense high-montane ombrophilous forest or cloud forest, and by some rocky field areas. The matrixes adjacent to the fragments are composed of plantations of *Pinus* sp. or *Eucalyptus* sp., designated in the present study as planted forests, and pastures of *Brachiaria* sp. and molasses grass, *Melinis minuflora* Pall de Beauv.

2. Sampling and identification of invertebrates

The study was carried out between November 2012 and March 2013 (rainy season). The invertebrate specimens were sampled by suction, using a syringe with capacity of 50 ml, coupled to a hose with length of 50 cm and diameter of 5 mm, from the lateral and central tanks of 147 bromeliads belonging to four morphospecies of the genus *Vriesea* Lindl (Bromeliaceae, Tillandsioideae). The size of the invertebrates collected was limited by the diameter of the hose attached to the syringe. The invertebrates that remained in the hose or did not pass through the syringe were disregarded. The most efficient way to overcome this limitation is to collect the bromeliad, but this is unsuitably invasive for these species, considering their slow growth, delay in flowering and scarcity.

Of the invertebrates sampled, 123 were located in fragments adjacent to pastures and 24 in fragments next to planted forest areas. To analyze the effect of distance from the edge on the bromeliad communities, we chose four fragments next to pastures: F1, F4, F7 and F11, whose widths in collection points varied from 100 (F4) to 1,700m (F11). In each of these, we established six sections according to the distance from the edge toward the center of the fragment: section A (0 to 13 m), section B (14 to 24 m), section C (25 to 35 m), section D (36 to 56 m), section E (80 to 117 m) and section F (150 to 230 m). We then randomly chose nine bromeliads in each of these sections.

The influence of the matrix (pasture or planted forest) was analyzed using the bromeliads found between 0 and 100 m from the edge of each fragment. We chose 16 bromeliads from the fragments neighboring planted forests (F3, F9 and F10) and 16 from the fragments next to pastures (F3, F4 and F8).

Water samples, varying in volume from 60 to 100 ml, were obtained from bromeliads whose bases were located between 0 and 1.9 m from the soil, with the use of a syringe connected to a hose. The specimens found in the water samples were fixed in 98º GL ethanol and were sorted and identified under a stereomicroscope. The invertebrates were identified to the family and subfamily level (except Annelida, Crustacea and Acari) using the keys in the literature: Merritt and Cummins (1996) for insects; Epler (2001) for Chironomidae, and Domínguez and Fernández (2009) for arthropods. Then the invertebrates were further analyzed at the Benthic Invertebrate Laboratory of Juiz de Fora Federal University (LIB/UFJF). After sorting the organisms to obtain the amount of organic material present in the samples, they were dried at 60 °C for 72 hours and then the material was weighed on an analytical balance (precision of 0.001g). Next, the samples were burned in a muffle furnace at 550 °C for 4 hours and again weighed. The amount of organic matter was obtained from the difference between the weight of the samples before and after burning (Suguio, 1973).

3. Data analysis

The abundance, taxon richness, Shannon diversity and Pielou evenness were calculated for the specimens obtained from each of the six bands in relation to the edges of the fragments and for the fragments next to pasture and planted forest. For this purpose we used the PAST program, version 2.10 (Hammer et al., 2001).

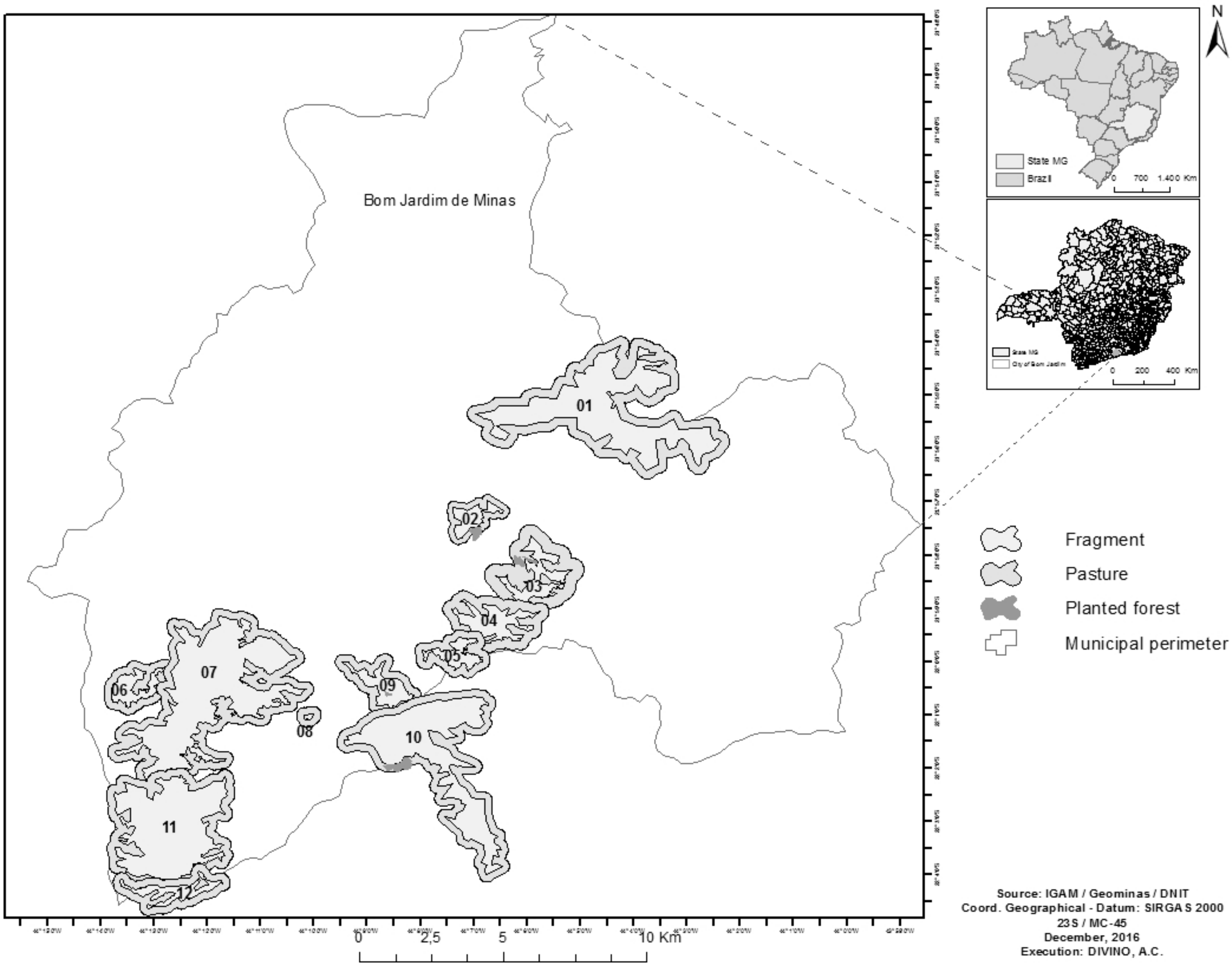

Figure 1: Map of Brazil indicating the Atlantic Forest fragments studied (F1 to F12) in the southeast of the state of Minas Gerais. The fragments shown have different sizes: F1(400 ha), F2 (120 ha), F3 (100 ha), F4 (150 ha), F5 (50 ha), F6 (50 ha), F7 (700 ha), F8 (0.5 ha), F9 (130 ha), F10 (350 ha), F11 (500 ha), F12 (100 ha).

The Kruskal-Wallis test ($p<0.05$) was used for comparison of the metrics among the distance bands from the edge, followed by multiple pairwise comparisons of the p-values. The Mann-Whitney test or t-test ($p<0.05$) was used to test differences in the metrics between the fragments located next to planted forest and pasture. Factorial ANOVA ($p<0.05$) was employed to test for effects of interaction between distance from edge (0 to 30 m or 50 to 100 m) and adjacent matrix (planted forest or pasture) on the structure of the invertebrate fauna. Differences in the fauna composition among the four bromeliad morphospecies were checked by the multi-response permutation procedure (MRPP), since the intrinsic traits of each species can influence the differences in invertebrate fauna (Marino et al. 2013). This analysis was carried out with the PC-ORD 5.15 program (McCune & Mefford, 2006).

Simple linear regression was used to analyze if the abundance, taxa richness and Shannon diversity were related directly to the distances of the bromeliads in relation to the fragment edge. This analysis was also used to determine if these same metrics had any relation with the organic matter content found in the tanks of each bromeliad. This analysis was performed with the Statistica 7.0 program (Statsoft, Inc. 2004).

The ordination analysis was performed to visualize whether the fauna composition changed as the location of the bromeliads became further from the edges of the fragments. For this purpose, the data were log-transformed [(log (x+1)] and submitted to Detrended correspondence analysis (DCA). This same analysis was used to order the fauna according to the adjacent area (pasture or planted forest), using the log-transformed abundance data. Differences in the fauna composition between the sections sampled were also analyzed by MRPP. These analyses were performed using the PC-ORD 5.15 software (McCune & Mefford, 2006). Analysis of similarity (ANOSIM) was used to test for significant differences in the taxa composition between the fragments next to planted forest and pasture. This analysis was carried out with the R program (R Foundation for Statistical Computing 2011).

Results

In the 147 sampled bromeliads, 12,918 invertebrates were identified, represented by 35 taxa. The most abundant were Ostracoda (4,962 individuals), Culicinae (2,358), Tanypodinae (1,164) and Scirtidae (1,043). The number of organisms per taxon is shown in Table 1.

Table 1: Abundance of bromeliad invertebrates sampled in forest fragments in the municipality of Bom Jardim de Minas, Minas Gerais.

Nematoda	7
Annelida	
Oligochaeta	46
Hirudinea	8
Crustacea	
Copepoda	195
Ostracoda	2663
Arachnida	
Acari	59
Hexapoda	
Collembola	8
Insecta	
Odonata	
Zygoptera	
Coenagrionidae	2
Dicteriadidae	41
Coleoptera	
Dytiscidae	2
Noteridae	5
Hydraenidae	6
Scirtidae	721
Elmidae	2
Diptera	
Tipulidae sp1	23
Tipulidae sp2	2
Sciaridae	5
Ceratopogonidae	61
Chironomidae	
Tanypodinae	755
Chironominae	190
Orthocladiinae	173
Culicidae	
Anophelinae	336
Culicinae	1498
Psychodidae	179
Tabanidae	4
Stratiomyidae	9
Empididae	52
Syrphidae	3
Muscidae	5
Total	7218

No significant changes were detected ($p > 0.05$) in the abundance, taxon richness and diversity of invertebrates in bromeliads located different distances from the edges (Table 2).

Although not statistically significant ($p > 0.05$), the greatest abundance of invertebrates was recorded in the fragments next to pasture areas and the highest richness was found in fragments adjacent to planted forest areas (Table 3).

The factorial ANOVA did not reveal a significant effect of the interaction between matrix (pasture or planted forest) and distance from the edge on the community of invertebrates found in the bromeliads: abundance ($F=0.145$; $p=0.745$), taxon richness ($F=0.001$; $p=0.998$) and Shannon diversity ($F=0.290$; $p=0.915$). Regarding the fauna composition, no differences were detected among the bromeliad morphospecies (MRPP, $p>0.05$).

The simple regression analysis did not reveal any relationship between the distance from the edge and abundance ($R^2=0.006$; $F=0.078$; $p=0.779$), taxon richness ($R^2=0.005$; $F=1.712$; $p=0.193$) and Shannon diversity ($R^2=0.005$; $F=0.413$; $p=0.521$). This same analysis also did not reveal any relation between the organic matter content of the cisterns and the community metrics: abundance ($R^2= 0.034$; $F=0.031$; $p=0.860$), richness ($R^2=0.035$; $F=0.003$; $p=0.953$) and diversity ($R^2=0.034$; $F=0.039$; $p=0.843$).

The DCA did not reveal any distinction of the fauna composition between the sections located at different distances from the fragment edge (Figure 2). The MRPP analysis distinguished the fauna composition between only some of these sections, regardless of distance from the edge (Table 4).

The detrended correspondence analysis (DCA) did not show any distinction in the fauna composition between the samples from fragments next to planted forest and pasture (Figure 3). This result was confirmed by the analysis of similarity (ANOSIM), which did not show a significant variation in the taxon composition ($p>0.05$) between the two matrixes.

Discussion

In general, the composition of invertebrates found in this study was similar to those found in other studies investigating the fauna of bromeliads in different regions of Brazil (Lopez 1998, Araújo et al. 2007, Marques et al. 2008, Sodré 2010). This result corroborates Mestre et al. (2000), who indicated the possible existence of a characteristic bromeliad fauna associated with these plants, permanent and transitory, but with a predominance of some groups adapted to changes in the chemical composition and input of nutrients to the bromeliads (Schuttz et al. 2012). According to Franck (1983), many endemic invertebrate species are specialized in this micro-environment. On the other hand, some organisms only are accidentally found in bromeliads, using the plants as temporary refuge (Armbruster et al., 2002), especially in areas where the external

Table 2: Fauna metrics of invertebrates found in bromeliads sampled in sections at different distances from the edge of Atlantic Forest fragments in southeastern Brazil.

	Distance from the edge (m)						Statistic
	Up to 13	14 to 24	25 to 35	36 to 56	80 to 117	150 to 230	
Abundance	667	1578	593	249	502	605	F= 0.794; p=0.558
Richness	19	22	23	17	19	17	H= 7.985; p=0.157
Equitability	0.74	0.69	0.7	0.75	0.74	0.74	F= 0.475; p=0.793
Shannon	1.42	1.25	1.51	1.32	1.49	1.52	F= 0.608; p=0.693

Table 3: Richness total number of taxa, mean and standard deviation (SD) of abundance, diversity and evenness of fauna found in bromeliads sampled in Atlantic Forest fragments in southeastern Brazil, according to neighboring matrix (planted forest or pasture).

	Planted Forest SD		Pasture SD		Statistic
Richness total	24		23		t= -1.145; p=0.341
Mean abundance	88.13	±67.39	123.31	±124.48	t= -0.744; p=0.458
Shannon	1.42	±0.29	1.35	±0.45	Z= -0.705; p=0.480
Equitability	0.69	±0.17	0.69	±0.20	Z= 1.586; p=0.112

environment is less favorable to the development and survival of these organisms (Oliveira et al., 1994).

Unlike our initial hypothesis that the richness and diversity of bromeliad fauna of the fragment edge are more vulnerable to the effects of environmental conditions at these sites, our results showed no significant differences in the composition, richness and diversity of the communities near the edge or inside the fragments. This result can be a reflection of the low stature of the high-montane vegetation, (Meireles et al. 2008) and of the elongated shape of the fragments (whose width in collection points varied from 100 m (F1) to 1,700 m (F11), making the environmental conditions (e.g., wind, sunlight, input of plant material) in the interior sections similar to those near the edges, and therefore insufficient to promote dissimilarities in the structure of the invertebrate community found in bromeliads situated at different distances from the edges.

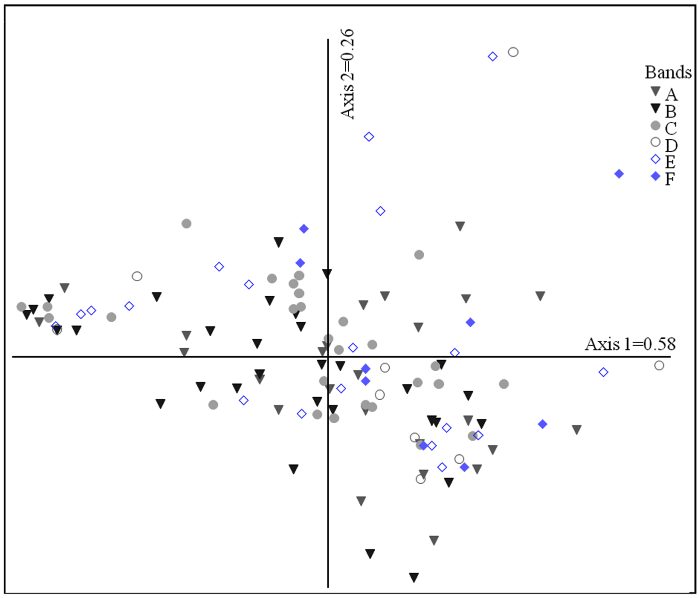

Figure 2: Results of the detrended correspondence analysis (DCA) of invertebrate fauna in bromeliads collected in sections at different distances from the edge of Atlantic Forest fragments in southeastern Brazil. A= up to 13 m, B= 14 to 24 m, C= 25 to 35 m, D= 36 to 56 m, E= 80 to 117 m and F= 150 to 230 m.

Table 4: Results of the MRPP analysis of fauna found in bromeliads sampled in sections at different distances from the edge of Atlantic Forest fragments in southeastern Brazil.

Sections			T	A	p
A	vs.	C	-1.815	0.028	0.050
C	vs.	D	-2.656	0.043	0.016
D	vs.	F	-2.491	0.035	0.017

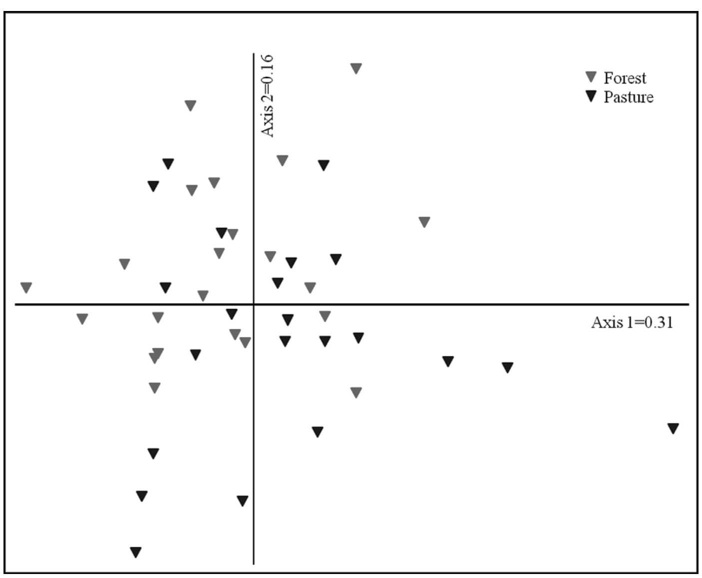

Figure 3: Results of the detrended correspondence analysis (DCA) of invertebrate fauna found in bromeliads sampled in Atlantic Forest fragments in southeastern Brazil, next to pasture or planted forest.

Similar environmental conditions can also explain the absence of significant differences in the composition, richness and diversity of invertebrates in the fragments adjacent to both landscapes (planted forest or pasture). The terrain, marked by high declivity, could have influenced this result, since the bromeliads were often located on hillsides above the canopy of planted forests, and thus were subject to similar conditions to those found in the fragments next to pastures. On the other hand, it is possible that the habitat of the bromeliad itself softens the adverse effects of location near the edge of fragments, by providing protection against external factors such as wind and heat. Oliveira et al. (1994), for example, indicated that bromeliads in coastal marshland are used by meso and microscopic fauna due to the moisture and protection against heat of the biome, besides the shelter from predators. In turn, Marques et al. (2008), comparing the fauna of Culicidae (Diptera) from terrestrial bromeliads, found similar richness and species diversity among urban, semi-urban and forest environments.

Considering bromeliads ‘per se’, among the factors that influence the fauna that find shelter in these plants is the quantity to organic detritus (food) and volume of water, both of which contribute to increase the abundance and richness of morphospecies of invertebrates, as found by Armbruster et al. (2002). The food that is available has a strong effect on the richness of morphospecies within the plant, while the volume of water in the tanks favors the presence of organisms that are not suited to living in dry plants, besides increasing the heterogeneity of the habitat. However, we did not detect any relation between the quantity of detritus in the bromeliads and the abundance and richness of invertebrates. This might have occurred because those authors considered all the material accumulated in the bromeliads, while we set a maximum limit on the material collected. In turn, the absence of differences in the fauna composition between the morphospecies of bromeliads probably occurred because they had similar architecture with respect to cistern size. This aspect can result in similar quantities of food resources for the fauna (Marino et al., 2013), explaining the absence of differences in the composition of invertebrates.

With respect to the predominance of some groups in the bromeliads, Ostracoda was the most abundant taxon, as also observed by Lopez et al. (1999) in bromeliads from Atlantic Forest fragments in the state of Rio de Janeiro. Among the insects collected, the majority belonged to the sub-order Nematocera (Diptera), which is associated with moist environments (Grimaldi & Engel, 2005) because their reproduction depends on the availability of water reservoirs for larval development. Culicidae was the most abundant family in all the bromeliads, corroborating the findings of Hilsenhoff (1991), Araújo et al. (2007), and Parker et al. (2012), all of whom observed this family, along with the Chironomidae, to be among the most abundant phytotelma insects. In this study, Chironomidae was represented mainly by the sub-family Tanypodinae, a result that differs from that reported by Sodré (2010) in Atlantic Forest areas of Rio de Janeiro. In both of these cases the authors found the greatest abundance of larvae of the sub-family Orthocladiinae in bromeliads. In turn, Scirtidae (Coleoptera), also abundant in this study, was the most numerous insect family found by Mestre et al. (2001) among insects from bromeliads in an Atlantic Forest area of the state of Paraná. According to Oliveira et al. (1994), the different predominance of some groups in bromeliads reflects local differences in the organization of bromeliad communities

Conclusions

In conclusion, in the forest fragments investigated, bromeliads proved to be a favorable habitat for invertebrate fauna, so we suggest they be included in policies to protect biodiversity, not only for their floristic richness, but also their importance in maintaining the richness and diversity of invertebrates that use them for shelter, even in altered landscapes exposed to adverse conditions, such as the borders of forest fragments.

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Inventory

Phanerogamic flora and vegetation of Itacolomi State Park, Minas Gerais, Brazil

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Abstract: Located in the municipalities of Ouro Preto and Mariana, in the *Quadrilátero Ferrífero* (QF) of Minas Gerais, the Itacolomi State Park (ISP) shelters more than 7,000 ha of remnants of Atlantic Forest and *campos rupestres*. The QF region has high biodiversity and is being highly impacted, mainly by mining activities. Aiming to organize the available floristic information and to support related research, this study presents the list of phanerogamic species of the ISP and the major vegetation types. This survey was carried out from October/1992 to July/2006, by monthly field trips. Besides authors´ personal collections, other records were assembled from herbarium databank. A total of 1623 taxons belonging to 122 families were listed. The families with higher species richness were Asteraceae, Fabaceae, Melastomataceae, Poaceae and Orchidaceae. The vegetation is represented by *campos rupestres* (51% of the total area), followed by montane forests (40%) and anthropogenic disturbed areas (9%). The greatest species richness occurs in *campos rupestres*. Several species are threatened and/or endemic. The knowledge of phanerogamic flora of ISP can help the Management Plan of this Unit of Conservation. It is also a contribution for future-related studies of the flora of ISP, Minas Gerais and Brazil.

Keywords*: Brazilian flora, campos rupestres, Iron Quadrangle flora, montane forests, rock outcrop flora, Neotropics.*

Flora Fanerogâmica e Vegetação do Parque Estadual do Itacolomi, Minas Gerais, Brasil

Resumo: O Parque Estadual do Itacolomi (PEIT) localiza-se nos municípios de Ouro Preto e Mariana, no Quadrilátero Ferrífero de Minas Gerais e abriga mais de 7.000 ha de remanescentes de mata atlântica e campos rupestres. A região do QF possui alta biodiversidade vegetal e está sobre alto impacto, principalmente pela mineração. O objetivo deste estudo foi apresentar a lista das espécies fanerogâmicas e os principais tipos vegetacionais desse Parque, no intuito de organizar a diversidade florística existente, além de servir como suporte para pesquisas correlatas subsequentes. O levantamento florístico foi realizado de outubro/1992 a julho/2006 por excursões mensais ao campo. Foram também incluídas na listagem registros ocorrentes em banco de dados de herbários. Foram identificados 1623 táxons, pertencentes à 122 famílias. As famílias mais representativas foram Asteraceae, Fabaceae, Melastomataceae, Poaceae e Orchidaceae. A vegetação do PEIT é constituída por campos rupestres (51% da área do parque), seguido pelas florestas montanas (40%) e por formações antrópicas (9%). A maior riqueza de espécies ocorre nos campos rupestres. Diversas espécies figuram em listas de espécies ameaçadas de extinção e outras são endêmicas. O conhecimento da flora fanerogâmica do Parque Estadual do Itacolomi pode subsidiar o Plano de Manejo dessa Unidade de Conservação e contribuir para futuros trabalhos correlatos nesta área, além de suprir conhecimentos para a flora de Minas Gerais e do Brasil.

Palavras-chave: *campos rupestres, flora do Quadrilátero Ferrífero, flora do Brasil, florestas montanas, Neotropicos.*

Introduction

Minas Gerais is the Brazilian State with the highest richness and endemism of Angiosperm species and harbors important phytogeographical domains (BFG 2015). Among them, the Atlantic Forest and the *Cerrado* are the largest, richest and more threatened ones (Fundação SOS Mata Atlântica 1998, Coutinho 2006). Atlantic Forest is one of the most biologically rich and diverse domain in the world, with around 20,000 plant species, representing 8% of the Earth's flora (Fundação SOS Mata Atlântica 1998). In spite of the Atlantic Forest being considered a biodiversity hotspot (Myers et al. 2000), it was reduced to less than 12% of its original size, with most of the remaining areas restricted to Conservation Units (Ribeiro et al. 2009).

With regard to Brazilian vegetation, the *campos rupestre*s stand out due to the peculiar and rich biodiversity. They occur from an altitude of 900m above sea level and upwards, mainly along the *Espinhaço* Range, in the States of Minas Gerais and Bahia, and with disjunct parts of the same chain, between latitudinal limits of 21º10’ and 10º S (Giulietti & Pirani 1988). Phytogeographically, the *campos rupestres* are classified into the *Cerrado* domain (Coutinho 2006, Batalha 2011), a kind of savanna, where plants grow in a wide variety of substrates including rock outcrops of quartzite, sandstone or ironstone, along with sandy, stony and waterlogged grasslands (Silveira et al. 2016). *Campos rupestres* also harbor several endemic and threatened species from different groups of organisms (Echternacht et al. 2011, BGF 2015, Silveira et al. 2016). Among the 538 threatened plant species of the Minas Gerais State, 358 (66.5 percent) occur in this kind of physiognomy (Costa et al. 1998). Among the Brazilian vegetation types, the *campos rupestres* have the highest percentage of endemism (1,951 endemic out of 4,928 species) (BGF 2015)

The *Quadrilátero Ferrífero* (Iron Quadrangle) Region (QF) covers an area of 7,000 km^2, in the central portion of the Minas Gerais State (Dorr 1969). This region is considered to be of extreme biological importance (Costa et al. 1998). The QF landscape is a mosaic under the influence of two Brazilian hotspots, Cerrado and Atlantic Forest, giving evidence of the ecotonal character of the region (Echternacht et al. 2011). The Atlantic Forest in the QF Region is mainly represented by a seasonal semideciduous type (Morellato & Haddad 2000) and the *Cerrado* occurs in different phytophysiognomies, including the *campos rupestres* (Silveira et al. 2016). The *campos rupestres* in the *Quadrilátero Ferrífero*, occur frequently in areas with quartzite rocks, interspersed mainly with banded iron formation (BIF´s), also known as *cangas* (Messias et al. 2011, 2012, 2013). *Cangas* are one of the most diverse, threatened and least studied ecosystems of southeast Brazil (Jacobi et al. 2007). In spite of the great biodiversity, mining, as well as metallurgical industries, urban expansion, among other activities, have provoked a high environmental impact in the *Quadrilátero Ferrífero* Region (Jacobi et al. 2007).

Itacolomi State Park is one of the few full protection conservation units in this region (IEF 2016) and shelters more than 7,000 ha of remnants of Atlantic Forest and *campos rupestres*. Most of the *campos rupestres* are quartzitic, but ISP is one of the three full protection parks of Minas Gerais also harboring ferruginous *campos rupestres*. Thus, this conservation unit in this region plays an important role in protecting biodiversity.

Little is still known about Itacolomi State Park flora, whose domain is an ecotone region between the Atlantic Forest and the *Cerrado*. There were found only a preliminary checklist of *campos rupestres* (Peron 1989), specific family studies about Orchidaceae (Alves 1990, Batista et al. 2004), Leguminosae (Dutra et al. 2005, 2008a, 2008b, 2009, 2014, Lima et al. 2007, 2010), Bromeliaceae (Coser et al. 2010), Myrtaceae (Bünger et al. 2012) and Asteraceae (Barnadesieae e Mutiseae) (Almeida et al. 2014). And also, few floristic studies have been recently published on small patches of *campos rupestres* (Gastauer et al. 2012) and forests (Pedreira & Sousa 2011) of this conservation unit. The aims of this study are to present a brief description of the vegetation of Itacolomi State Park (ISP) and to present a checklist of the phanerogamic flora.

Material and Methods

This survey was carried out in Itacolomi State Park (ISP), situated in the municipalities of Ouro Preto (World Patrimony by Unesco) and Mariana (Brazilian Historic Patrimony), Minas Gerais State, (43º32’30” to 43º22’30”W and 20º22’30” to 20º30’00”S). This Brazilian Conservation Unit was created in 1967 by Minas Gerais State law nº 4465 (Figure 1).

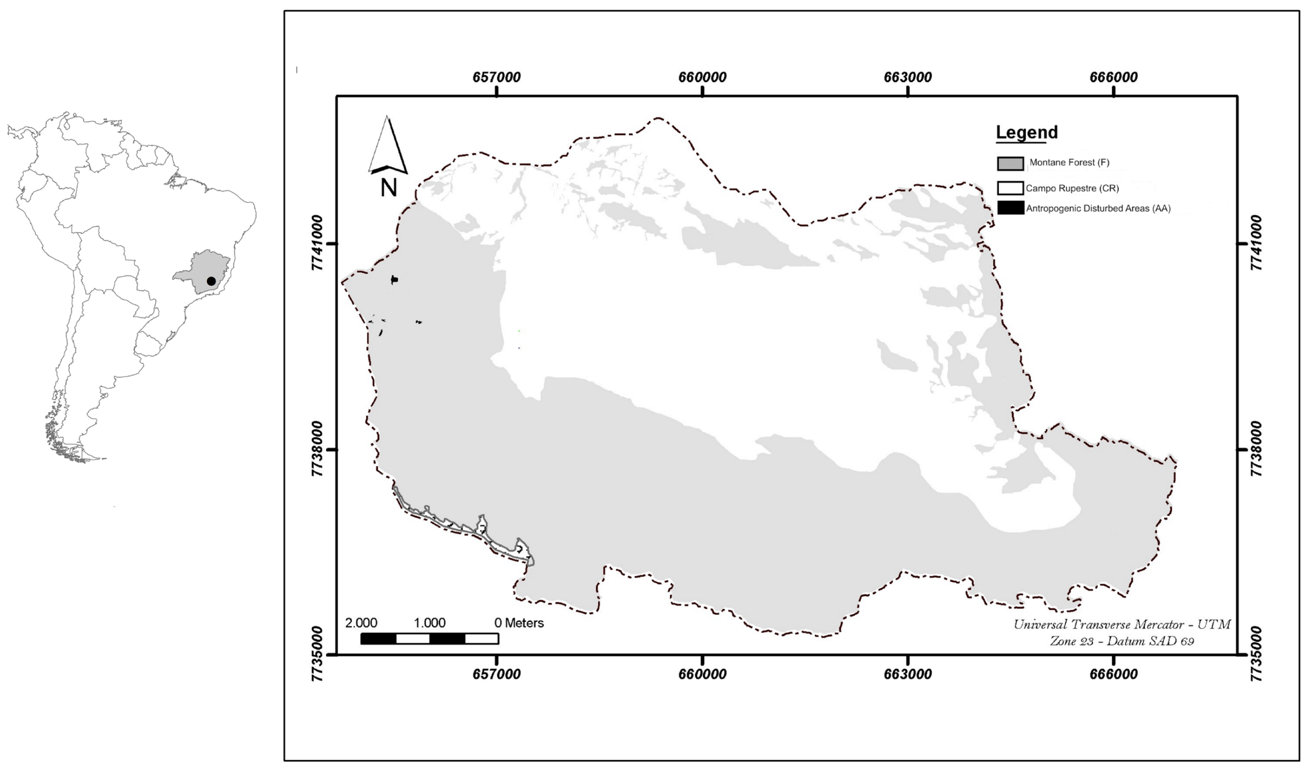

Figure 1. Vegetation map and location of the Itacolomi State Park (ISP), Ouro Preto and Mariana, Minas Gerais, Brazil. F = Montane Forest, CR = Campos rupestres, AA = Anthropogenic disturbed areas.

According to Peron (1989) the vegetation at ISP is formed mainly by *campos rupestres* permeated by riparian or semideciduous forest fragments.

The climate, according Köppen classification is Cwb, i. e. mesotermic, with a warm and rainy season from September to April with a dry season from May to August (Álvares et al. 2013). The annual mean rainfall is 1,250 mm and the annual mean temperature is about 20°C (varying from 4 to 33ºC). Fog is frequent, especially during the dry season.

The relief is characterized by steep slopes where the altitudes varies from 700 to 1772 m. The Park covers the greatest part of Itacolomi Hill, at the southern of Espinhaço Range, with around 7,500 ha (Castañeda 1993). The geology of ISP comprises clastic metasedimentary rocks from Rio das Velhas and Minas Supergroups, Sabará and Itacolomi Groups. The quartzite of Itacolomi Group occurs in the greatest proportion of the Park area. There are also intrusions of metabasic rocks and a superficial iron crusts known as *canga* (Castañeda 1993). According to the same author, the soils at Itacolomi vary from sandy shallow soils, mostly associated to quartzite, occurring in *campos rupestres* to deeper ones, mainly Latossols (Oxisols), at forest patches at lower altitudes.

This survey was carried out from October/1992 to July/2006, when monthly field trips were taken, all over the Park. The collected species were herborized and deposited in the Professor José Badini Herbarium (OUPR) at Federal University of Ouro Preto. The species identification was made by comparison with previously identified material at RB, BHCB, VIC and OUPR herbaria, by using specialized literature and with the help of specialists.

Aiming to supplement the checklist, records from SpeciesLink (CRIA 2016) and JABOT (JBRJ 2016) databank were included. Materials determined only at the family level were not considered and the ones identified at generic level were included only when deposited in OUPR and assumed they were different from the species already listed. Records included in the checklist appear cited with acronyms according to Thiers (2016).

The circumscription of families followed the Angiosperm Phylogeny Group - APG III (Chase & Reveal 2009). The species names were checked with the database of the list of species of the Brazilian Flora 2020 Project (2016) and The Plant List (2013). Each species was described by its habit (Rizzini 1997) and habitat. The forest vegetation was classified according to Veloso et al. (1991) and the grasslands areas followed Ferri (1980), Eiten (1983) and Giulietti et al. (1987). The major vegetation types are Montane Forest (F), *Campos Rupestres* (CR) and Anthropogenic Disturbed Areas (AA). In order to recognize the threatened species, it was consulted the IUCN Red List (2015), as well as the Brazilian (MMA 2014) and Minas Gerais (COPAM 1997) Red Lists.

The vegetation map was made using an IKONOS image, which was provided by the State Forest Institute of Minas Gerais (IEF). The vegetation groups described were identified in the field campaigns and then designed in Geographic Information System (GIS) environment with Arcview 9.2 software (Environmental Systems Research Institute, 2007).

Results and Discussion

A total of 1614 species, one subspecies and eight varieties, belonging to 569 genera and 122 families were identified (Table 1). The species richness in ISP is comparable with other mountainous areas along the Espinhaço Range, e.g. Pico das Almas: 930 species (Stannard et al. 1995), Ibitipoca: 1080 species (Forzza et al. 2013), Grão Mogol: 1073 species (Pirani et al. 2003), Serra de São José: 1087 species (Alves & Kolbek 2009) and Serra do Cipó: 1530 species (Giulietti et al. 1987). These results highlight how diverse is the ISP flora as well as the importance of focusing on further taxonomic studies in this park.

Table 1. Checklist of phanerogamic species of Itacolomi State Park, Ouro Preto/Mariana, Minas Gerais, Brazil and their respective habit, habitat and voucher (Herbarium acronym + number). Habitat: F = Montane forest, CR = Campos rupestres, AA = Anthropogenic disturbed areas. After the species name, in red, the categories of threatened species and references: CR = Critically endangered, DD = Data deficient, EN = Endangered, EX = Probably Extinct, LC = Least concern, NT = Near threatened, VU = Vulnerable, * COPAM (1997), ** MMA (2014), *** IUCN (2015).

Family / Species	Habit	Habitat	Voucher
Acanthaceae			
Aphelandra prismatica (Vell.) Hiern	Subshrub	F	OUPR 9064
Aphelandra sp	Subshrub	F	OUPR 1296
Clistax brasiliensis Mart.	Subshrub	F	BHCB 41493
Justicia monticola (Nees) Profice	Liana	CR	OUPR 102
Justicia sp1	Subshrub	F	OUPR 6780
Justicia sp2	Subshrub	F	OUPR 6825
Justicia sp3	Subshrub	F	OUPR 1403
Justicia sp4	Subshrub	F	OUPR 1991
Justicia sp5	Subshrub	F	OUPR 19286
Mendoncia puberula Mart.	Liana	CR	OUPR 045
Mendoncia velloziana Mart.	Liana	F	OUPR 1273
Ruellia geminiflora Kunth	Subshrub	CR	OUPR 061
Ruellia jussieuoides Schltdl. & Cham.	Subshrub	F	OUPR 7352
Ruellia macrantha (Mart. ex Ness) Lindau	Subshrub	CR,F	OUPR 1996
Ruellia neesiana (Mart. ex Ness) Lindau	Subshrub	CR	NY 1059877
Staurogyne minarum (Nees) Kuntze EN*	Subshrub	F	OUPR 10002
Staurogyne vauthieriana (Nees) Kuntze EN*	Subshrub	F	HRCB 42225
Staurogyne sp	Subshrub	F	OUPR 7411
Alismataceae			
Echinodorus grandiflorus (Cham. & Schltr.) Micheli	Herb	AA	NY 51987
Alstroemeriaceae			
Alstroemeria foliosa Mart. ex Schult. & Schult.f.	Herb	CR	OUPR 617
Alstroemeria haemantha Ruiz & Pav.	Herb	F	OUPR 1308

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Bomarea edulis (Tussac) Herb.	Liana	F	OUPR 577
Amaranthaceae			
Alternanthera brasiliana (L.) Kuntze	Subshrub	F	OUPR 6800
Alternanthera brasiliana var. *moquini* Uline & W.L.Bray	Subshrub	CR,F	RB 170197
Amaranthus spinosus L.	Subshrub	CR	RB 244325
Amaranthus viridis L.	Subshrub	AA	NY 837913
Dysphania ambrosioides (L.) Mosyakin & Clemants	Subshrub	CR	OUPR 2743
Amaryllidaceae			
Hippeastrum correiense (Bury) Worsley	Herb	CR	RB 112160
Hippeastrum damazianum Beauverd	Herb	CR	OUPR 1404
Hippeastrum organense Herb.	Herb	CR	OUPR 7392
Anacardiaceae			
Schinus terebinthifolius Raddi	Shrub	CR,F	OUPR 1201
Tapirira guianensis Aubl.	Tree	F	OUPR 9059
Annonaceae			
Annona emarginata (Schltdl.) H.Rainer	Tree	F	OUPR 19153
Guatteria australis A.St.-Hil.	Shrub	F	OUPR 20217
Guatteria pohliana Schltdl. VU*	Shrub	F	OUPR 5270
Guatteria sellowiana Schltdl. VU*	Tree	F	OUPR 1202
Guatteria villosissima A.St.-Hil. VU*	Tree	F	OUPR 1505
Xylopia brasiliensis Spreng.	Tree	F	OUPR 1398
Apiaceae			
Centella asiatica (L.) Urb.	Herb	CR	OUPR 13609
Ciclospermum leptophyllum (Pers.) Britton P.Wilson	Subshrub	AA	NY 877641
Eryngium eurycephalum Malme	Herb	CR	NY 877731
Eryngium paniculatum Cav. & Dombey ex F.Delaroche	Herb	CR	OUPR 1352
Apocynaceae			
Asclepias curassavica L.	Subshrub	CR	OUPR 19409
Aspidosperma spruceanum Benth. ex Müll.Arg.	Tree	F	OUPR 1468
Blepharodon ampliflorum E.Fourn.	Liana	CR	MO 1246283
Blepharodon pictum (Vahl) W.D.Stevens	Liana	CR	MO 1934634
Condylocarpon intermedium subsp. *laxum* (Müll.Arg.) Fallen	Liana	F	OUPR 366
Condylocarpon isthmicum (Vell.) A. DC.	Liana	CR	OUPR 17722
Ditassa aequicymosa E. Fourn. VU*	Liana	CR	RB 361320
Ditassa laevis Mart. VU*, EN**	Liana	CR	OUPR 833
Ditassa longisepala (Hua) Fontella & E. A. Schwarz VU*, EN**	Liana	CR	OUPR 739
Ditassa mucronata Mart.	Liana	F	OUPR 1379
Ditassa nitida Decne.	Liana	CR	BHCB 110
Ditassa tomentosa (Decne.) Fontella	Liana	CR	OUPR 743
Forsteronia velloziana (A.DC.) Woodson	Liana	F	OUPR 6804
Gonioanthela hilariana (E. Fourn.) Malme	Liana	CR	OUPR 717
Mandevilla atroviolacea (Stadelm.) Woodson	Liana	F	RB 277734
Mandevilla emarginata (Vell.) C.Ezcurra	Shrub	CR	OUPR 1272
Mandevilla immaculata Woodson	Liana	CR	SP 139907
Mandevilla martiana (Stadelm.) Woodson	Liana	CR,F	OUPR 400
Mandevilla sellowii (Müll.Arg.) Woodson	Liana	F	NY 1183021
Mandevilla tenuifolia (J.C.Mikan) Woodson	Subshrub	CR	OUPR 423
Mandevilla sp1	Shrub	CR	RB 277638
Mandevilla sp2	Subshrub	CR	OUPR 8261
Minaria decussata (Mart.) T.U.P.Konno & Rapini	Liana	CR	OUPR 8743
Orthosia scoparia (Nutt.) Liede & Meve	Liana	F	OUPR 6797
Oxypetalum appendiculatum Mart.	Liana	CR	OUPR 883
Oxypetalum foliosum Mart.	Subshrub	CR	OUPR 1488
Oxypetalum glabrum (Decne.) Malme	Liana	CR	BHCB 114
Oxypetalum insigne (Decne.) Malme	Liana	CR	OUPR 799
Oxypetalum minarum E. Fourn.	Liana	CR	OUPR 943
Oxypetalum strictum Mart.	Liana	CR	OUPR 7228
Peplonia asteria (Vell.) Fontella & E.A.Schwarz	Liana	F	OUPR 717

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Stipecoma peltigera (Stadelm.) Müll. Arg.	Liana	F	OUPR 20151
Aquifoliaceae			
Ilex chamaedryfolia Reissek	Shrub	CR	OUPR 478
Ilex conocarpa Reissek	Shrub	CR,F	OUPR 13606
Ilex euryaeformis Reissek	Shrub	CR	OUPR 4639
Ilex grandis Reissek	Shrub	F	OUPR 1358
Ilex loranthoides Mart. ex Reissek VU[*,**]	Shrub	CR	OUPR 1357
Ilex subcordata Reissek	Shrub	CR	OUPR 446
Ilex theezans Mart. ex Reissek	Tree	F	OUPR 19183
Araceae			
Anthurium minarum Sakur. & Mayo	Herb	CR	OUPR 1467
Anthurium scandens (Aubl.) Engl.	Herb	CR,F	OUPR 1486
Philodendron propinquum Schott	Herb	F	OUPR 24872
Araliaceae			
Dendropanax cuneatus (DC.) Decne & Planch	Tree	F	OUPR 565
Hydrocotyle quinqueloba Ruiz & Pav.	Herb	CR	OUPR 1504
Hydrocotyle verticillata Thunb.	Herb	CR	OUPR 1504
Schefflera calva (Cham.) Frodin & Fiaschi	Tree	F	BHCB 8276
Schefflera macrocarpa (Cham. & Schltdl.) Frodin	Tree	CR,F	OUPR 534
Schefflera vinosa (Cham. & Schltdl.) Frodin & Fiaschi	Shrub	F	OUPR 1319
Schefflera sp	Shrub	CR	RB 112206
Araucariaceae			
Araucaria angustifolia (Bertol.) Kuntze EN[*,**]	Tree	F	OUPR 23543
Arecaceae			
Geonoma brevispatha Barb.Rodr.	Palm	F	BHCB 42284
Geonoma schottiana Mart.	Palm	F	OUPR 6955
Aristolochiaceae			
Aristolochia smilacina (Klotzsch) Duch.	Liana	CR	OUPR 687
Asteraceae			
Acanthospermum australe (Loefl.) Kuntze	Subshrub	AA	JB 490409
Achyrocline alata (Kunth) DC.	Subshrub	CR	OUPR 1547
Achyrocline albicans Griseb.	Subshrub	CR	OUPR 1553
Achyrocline satureioides (Lam.) DC.	Subshrub	CR	OUPR 12807
Ageratum conyzoides L.	Subshrub	CR	OUPR 1578
Ageratum fastigiatum (Gardner) R.M.King & H.Rob.	Subshrub	CR	RB 486721
Ageratum myriadenium (Sch.Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	BHCB 41506
Apopyros corymbosus (Hook. & Arn.) G.L.Nesom	Subshrub	CR	NY 787836
Aspilia caudata J.U.Santos CR[*]	Shrub	CR	VIC 26515
Aspilia pohlii (Sch.Bip. ex Baker) Baker DD[**]	Subshrub	CR	VIC 8312
Austrocritonia angulicaulis (Sch. Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	OUPR 18551
Austrocritonia velutina (Gardner) R.M.King & H.Rob.	Subshrub	F	BHCB 161975
Austroeupatorium neglectum (B.L.Rob.) R.M.King & H.Rob.	Shrub	CR	OUPR 14853
Ayapana amygdalina (Lam.) R.M.King & H.Rob.	Subshrub	CR	OUPR 16892
Baccharis aphylla (Vell.) DC.	Subshrub	CR	OUPR 2320
Baccharis articulata (Lam.) Pers.	Subshrub	CR	VIC 22077
Baccharis brevifolia DC.	Subshrub	CR	RB 275538
Baccharis calvescens DC.	Shrub	CR	RB 486583
Baccharis cognata DC.	Subshrub	CR	RB 486604
Baccharis crispa Spreng.	Subshrub	CR	RB 486597
Baccharis dentata (Vell.) G.M.Barroso	Subshrub	CR	HUFU 42320
Baccharis dracunculifolia DC.	Subshrub	CR	OUPR 1287
Baccharis genistelloides (Lam.) Pers.	Subshrub	CR	OUPR 12791
Baccharis glutinosa Pers.	Subshrub	CR	VIC 22069
Baccharis gnidiifolia Kunth	Shrub	CR	OUPR 4937
Baccharis helichrysoides DC.	Shrub	CR	OUPR 1288
Baccharis hirta DC.	Subshrub	CR	OUPR 4852
Baccharis illinita DC.	Subshrub	CR	VIC22071
Baccharis junciformis DC.	Subshrub	CR	OUPR 4928

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Baccharis lateralis Baker	Shrub	CR	RB 157380
Baccharis latifolia (Ruiz & Pav.) Pers.	Subshrub	CR	OUPR 3456
Baccharis ligustrina DC.	Shrub	CR	HUFU 42319
Baccharis linearifolia (Lam.) Pers.	Shrub	CR	VIC 22176
Baccharis lychnophora Gardner VU*, EN**	Shrub	CR	RB 275530
Baccharis microcephala (Less.) DC.	Subshrub	CR	VIC 22063
Baccharis montana DC.	Subshrub	CR	OUPR 1747
Baccharis oblongifolia (Ruiz & Pav.) Pers	Subshrub	CR	RB 486562
Baccharis platypoda DC.	Shrub	CR	OUPR 9054
Baccharis pseudomyriocephala Malag.	Shrub	CR	OUPR 12806
Baccharis punctulata DC.	Shrub	CR	OUPR 3453
Baccharis reticularia DC.	Shrub	CR	OUPR 4865
Baccharis retusa DC.	Subshrub	CR	RB 486582
Baccharis rufidula (Spreng.) Joch.Müll.	Shrub	CR	RB 277732
Baccharis sagittalis (Less.) DC.	Subshrub	CR	OUPR 4928
Baccharis semiserrata DC.	Shrub	CR	RB 486706
Baccharis serrulata (Lam.) Pers.	Subshrub	CR	OUPR 23270
Baccharis sessiliflora Vahl	Shrub	CR	OUPR 2664
Baccharis subdentata DC.	Shrub	CR	RB 486595
Baccharis tarchonanthoides DC.	Subshrub	CR	OUPR 12790
Baccharis tridentata Vahl	Subshrub	CR	RB 486741
Baccharis trinervis Pers.	Liana	CR	RB 486620
Baccharis vulneraria Baker	Shrub	CR	OUPR 4994
Baccharis sp1	Shrub	CR	OUPR 9053
Baccharis sp2	Shrub	CR	OUPR 15602
Barrosoa organensis (Gardner) R.M.King & H.Rob.	Shrub	CR	RB 486774
Bartlettina hemisphaerica (DC.) R.M.King & H.Rob.	Shrub	CR	OUPR 14502
Bidens pilosa L.	Subshrub	CR	OUPR 13594
Bidens rubifolia Kunth	Liana	CR,F	OUPR 1354
Bidens segetum Mart. ex Colla	Liana	F	VIC 26510
Calea clausseniana Baker	Subshrub	CR	VIC 26502
Calea clematidea Baker	Subshrub	CR	OUPR 22365
Calea fruticosa (Gardner) Urbatsch, Zlotsky & Pruski	Shrub	CR	OUPR 1286
Calea lemmatioides Sch.Bip. ex Baker	Subshrub	CR	OUPR 1338
Calea rotundifolia (Less.) Baker	Subshrub	CR	RB 489982
Campovassouria cruciata (Vell.) R.M.King & H.Rob.	Shrub	CR	OUPR 16909
Centratherum punctatum Cass.	Subshrub	CR	OUPR 20305
Chaptalia graminifolia (Dusén) Cabrera	Subshrub	CR	VIC 21840
Chaptalia integerrima (Vell.) Burkart	Subshrub	CR	RB 324851
Chaptalia martii (Baker) Zardini EN*	Subshrub	CR	OUPR 7038
Chaptalia nutans (L.) Pol.	Subshrub	CR	OUPR 13583
Chaptalia runcinata Kunth	Subshrub	CR	NY 362359
Chevreulia acuminata Less.	Subshrub	CR	RB 490161
Chionolaena capitata (Baker) S.E.Freire	Subshrub	CR	RB 1468
Chionolaena lychnophorioides Sch.Bip. VU*	Subshrub	CR	RB 324850
Chromolaena adenolepis (Sch.Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	VIC 26568
Chromolaena barbacensis (Hieron.) R.M.King & H.Rob.	Subshrub	CR	RB 486723
Chromolaena cylindrocephala (Sch.Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	RB 275393
Chromolaena decumbens Gardner	Subshrub	CR	OUPR 1341
Chromolaena laevigata (Lam.) R.M.King & H.Rob.	Shrub	CR	OUPR 18318
Chromolaena latisquamulosa (Hieron.) R.M.King & H.Rob	Subshrub	CR	RB 489213
Chromolaena maximilianii (Schrad. ex DC.) R.M.King & H.Rob.	Subshrub	CR	OUPR 18517
Chromolaena multiflosculosa (DC.) R.M.King & H.Rob.	Subshrub	CR	OUPR 18843
Chromolaena odorata (L.) R.M.King & H.Rob.	Shrub	AA,CR	OUPR 14460
Chromolaena pedalis (Sch. Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	OUPR 1343
Chromolaena perforata (Sch.Bip. ex Baker) R.M.King & H.Rob.	Subshrub	CR	RB 486781
Chromolaena squalida (DC.) R.M.King & H.Rob.	Subshrub	CR	OUPR 16856
Chromolaena squarrulosa (Hook. & Arn.) R.M.King & H.Rob.	Subshrub	CR	OUPR 18567

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Chromolaena stachyophylla (Spreng.) R.M.King & H.Rob.	Subshrub	CR	OUPR 15603
Chromolaena vindex (DC.) R.M.King & H.Rob.	Subshrub	CR	RB 486779
Chrysolaena cognata (Less.) Dematt.	Subshrub	CR	OUPR 19959
Chrysolaena simplex (Less.) Dematt.	Subshrub	CR	OUPR 3776
Chrysolaena obovata (Less.) Dematt.	Subshrub	CR	HUFU 54022
Chrysolaena platensis (Spreng.) H.Rob.	Subshrub	CR	OUPR 20752
Chrysolaena propringua (Hieron.) H.Rob.	Subshrub	CR	RB 158177
Chrysolaena verbascifolia (Less.) H.Rob.	Subshrub	F	OUPR 2939
Clibadium armani (Balb.) Sch. Bip. ex O.E. Schulz	Subshrub	CR	OUPR 1458
Conyza bonariensis (L.) Cronquist	Subshrub	CR	RB 486589
Conyza canadensis (L.) Cronquist	Subshrub	AA	VIC 22160
Conyza monorchis (Griseb.) Cabrera	Subshrub	CR	RB 162919
Conyza primulifolia (Lam.) Cuatrec. & Lourteig	Subshrub	F	RB 486622
Conyza sumatrensis (Retz.) E.Walker	Subshrub	F	RB 486608
Cyrtocymura scorpioides (Lam.) H.Rob.	Subshrub	CR	OUPR 13602
Dasyphyllum brasiliense (Spreng.) Cabrera	Shrub	CR	RB 468820
Dasyphyllum candolleanum (Gardner) Cabrera	Shrub	CR	VIC 21807
Dasyphyllum flagellare (Casar.) Cabrera.	Shrub	CR	OUPR 1401
Dasyphyllum fodinarum (Gardner) Cabrera	Shrub	CR	OUPR 5152
Dasyphyllum sprengelianum var. *inerme* (Gardner) Cabrera	Shrub	CR	VIC 21799
Dasyphyllum sprengelianum var. *sprengelianum* (Gardner) Cabrera	Shrub	CR	VIC 21796
Dendrophorbium pellucidinerve (Sch.Bip. ex Baker) C.Jeffrey	Shrub	CR	OUPR 15109
Echinocoryne holosericea (Mart. ex DC.) H.Rob.	Subshrub	CR	OUPR 20145
Echinocoryne schwenkiifolia (Mart. ex DC.) H.Rob.	Subshrub	CR	VIC 21974
Eclipta prostrata (L.) L.	Subshrub	AA,CR	OUPR 14333
Elephantopus biflorus (Less.) Sch.Bip.	Subshrub	CR	OUPR 14344
Elephantopus mollis Kunth	Herb	AA,CR	OUPR 14353
Emilia fosbergii Nicolson	Subshrub	CR	VIC 22225
Emilia sonchifolia (L.) DC. ex Wight	Subshrub	AA,CR	OUPR 2166
Erechtites hieracifolius (L.) Raf. ex DC.	Subshrub	CR	OUPR 14372
Erechtites valerianifolius (Wolf) DC	Subshrub	CR	OUPR 14380
Eremanthus crotonoides (DC.) Sch.Bip.	Shrub	CR	OUPR 19248
Eremanthus erythropappus (DC.) MacLeish	Tree	CR,F	OUPR 1385
Eremanthus glomerulatus Less.	Tree	CR	VIC 21891
Eremanthus incanus (Less.) Less.	Shrub	CR	OUPR 16943
Fleischmannia laxa (Gardner) R.M. King & H. Rob.	Subshrub	CR	OUPR 14028
Galatella linosyris (L.) Rchb.f.	Subshrub	CR	OUPR 2080
Galinsoga parviflora Cav.	Subshrub	AA	VIC 26498
Gamochaeta americana (Mill.) Wedd.	Subshrub	CR	RB 490033
Gamochaeta purpurea (L.) Cabrera	Subshrub	CR	RB 490162
Gamochaeta simplicicaulis (Willd. ex Spreng.) Cabrera	Subshrub	CR	RB 163267
Gamochaeta sp	Subshrub	CR	RB 190526
Gnaphalium cheiranthifolium Bertero ex Lam.	Subshrub	CR	OUPR 14961
Grazielia coriacea (Scheele) R.M.King & H.Rob.	Subshrub	CR	OUPR 14457
Grazielia gaudichaudeana (DC.) R.M.King & H.Rob.	Shrub	CR	RB 163258
Grazielia intermedia (DC.) R.M.King & H.Rob.	Shrub	CR	RB 486734
Grazielia serrata (Spreng.) R.M.King & H.Rob.	Subshrub	CR	OUPR 18489
Guayania yaviana (Lasser & Maguire) R.M.King & H.Rob.	Shrub	CR	OUPR 18551
Heterocondylus alatus (Vell.) R.M.King & H.Rob.	Shrub	AA,CR	RB 486729
Heterocondylus amphidictyus (DC.) R.M.King & H.Rob.	Subshrub	CR	OUPR 1337
Heterocondylus pumilus (Gardner) R.M.King & H.Rob.	Subshrub	CR	RB 277730
Hololepis pedunculata (DC. ex Pers.) DC. VU[*]	Shrub	CR	OUPR 1389
Hoehnephytum trixoides (Gardner) Cabrera VU[*]	Subshrub	CR	RB 324847
Hypochaeris chillensis (Kunth) Hieron.	Herb	AA,CR	OUPR 14940
Hypochaeris gardneri Baker	Herb	CR	VIC 21875
Inulopsis scaposa (DC.) O.Hoffm. EN[*]	Herb	CR	OUPR 8353
Jaegeria hirta (Lag.) Less.	Herb	AA,CR	VIC 26495
Koanophyllon adamantium (Gardner) R.M. King & H. Rob. EN[*]	Subshrub	CR	OUPR 14308

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Koanophyllon consanguineum (DC.) R.M.King & H.Rob.	Subshrub	CR	OUPR 14464
Koanophyllon thysanolepis (B.L.Rob.) R.M.King & H.Rob.	Shrub	CR	RB 163210
Lepidaploa araripensis (Gardner) H.Rob.	Liana	CR	OUPR 20101
Lepidaploa argyrotricha (Sch.Bip. ex Baker) H.Rob.	Subshrub	CR	HUFU 54025
Lepidaploa cotoneaster (Willd. ex Spreng.) H.Rob.	Liana	F	OUPR 19247
Lepidaploa helophila (Mart. ex DC.) H.Rob.	Shrub	CR	VIC 21934
Lepidaploa lilacina (Mart. ex DC.) H.Rob. VU* (=*Vernonia adamantium* Gardner)	Subshrub	CR	OUPR 19432
Lepidaploa muricata (DC.) H.Rob.	Subshrub	CR	VIC 21917
Lepidaploa persericea (H.Rob.) H.Rob.	Subshrub	CR	VIC 21916
Lepidaploa remotiflora (Rich.) H.Rob.	Shrub	CR	OUPR 24656
Lepidaploa rufogrisea (A. St.-Hil.) H.Rob.	Subshrub	CR,F	RB 275396
Lepidaploa salzmannii (DC.) H.Rob.	Subshrub	CR	OUPR 2920
Lepidaploa sororia (DC.) H.Rob.	Subshrub	CR	RB 56519
Lepidaploa vauthieriana (DC.) H.Rob.	Subshrub	CR	OUPR 14559
Leptostelma maximum D.Don	Subshrub	CR	OUPR 1336
Lessingianthus brevipetiolatus (Sch.Bip. ex Baker) H.Rob.	Shrub	CR,F	HUFU 54037
Lessingianthus linearifolius (Less.) H.Rob.	Subshrub	CR	OUPR 4131
Lessingianthus obscurus (Less.) H.Rob.	Shrub	CR	OUPR 20146
Lessingianthus psilophyllus (DC.) H.Rob.	Subshrub	CR	RB 324843
Lessingianthus rubricaulis (Humb. & Bonpl.) H.Rob.	Subshrub	CR	HUFU 61540
Lessingianthus syncephalus (Sch.Bip. ex Baker) H.Rob.	Subshrub	CR	OUPR 1339
Lessingianthus tomentellus (Mart. *ex* DC.) H.Rob.	Subshrub	CR	OUPR 1335
Lessingianthus vepretorum (Mart. *ex* DC.) H.Rob.	Subshrub	CR	RB 490166
Lessingianthus virgulatus (Mart. ex DC.) H.Rob.	Subshrub	CR	OUPR 2932
Lucilia linearifolia Baker	Subshrub	CR	VIC 22005
Lucilia lycopodioides (Less.) S.E.Freire	Subshrub	CR	OUPR 1507
Lychnophora ericoides Mart. VU*, NT**	Shrub	CR	OUPR 13498
Lychnophora passerina (Mart. ex DC.) Gardner VU*	Shrub	CR	BHCB 318
Lychnophora pinaster Mart. VU*,NT**	Shrub	CR	OUPR 9057
Lychnophora reticulata Gardner EN**	Shrub	CR	HUFU 54028
Melampodium divaricatum (Rich. ex Pers.) DC	Subshrub	CR	VIC 26494
Mikania acuminata DC.	Liana	F	BHCB 54647
Mikania argyreiae DC. VU*,**	Liana	CR	OUPR 24668
Mikania badiniana G.S.S. Almeida & Carvalho-Okano	Subshrub	CR	VIC 29114
Mikania banisteriae DC.	Liana	CR	OUPR 5941
Mikania candolleana Gardner	Liana	CR	OUPR 24669
Mikania capricorni B.L. Rob.	Liana	F	OUPR 1332
Mikania clematidifolia Dusén VU**	Shrub	F	VIC 27606
Mikania duckei G.M. Barroso	Liana	CR	OUPR 14646
Mikania glauca Mart. ex Baker EN*,**	Subshrub	CR	BHCB 41476
Mikania hemisphaerica Sch.Bip. ex Baker	Liana	CR	HUFU 39918
Mikania hirsutissima DC.	Liana	CR	RB 98143
Mikania lasiandrae DC.	Liana	CR,F	BHCB 41501
Mikania lindbergii Baker	Liana	CR	OUPR 14706
Mikania microcephala DC.	Liana	CR	VIC 28777
Mikania microdonta DC.	Liana	CR	RB 324845
Mikania microphylla Sch.Bip.	Subshrub	CR	BHCB 56217
Mikania microptera DC.	Liana	F	OUPR 15006
Mikania nummularia DC.	Subshrub	CR	OUPR 16755
Mikania oblongifolia DC.	Subshrub	CR	OUPR 14758
Mikania obtusata DC.	Subshrub	CR	RB 162942
Mikania officinalis Mart.	Subshrub	CR	VIC 28764
Mikania parviflora (Aubl.) H.Karst.	Liana	F	OUPR 14618
Mikania parvifolia Baker	Subshrub	CR	BHCB 50618
Mikania phaeoclados Mart.	Liana	CR	VIC 28754
Mikania premnifolia Gardner VU*,EN**	Subshrub	CR	OUPR 14768
Mikania psilostachya DC.	Liana	F	VIC 8250

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Mikania ramosissima Gardner	Shrub	CR	OUPR 15002
Mikania reticulata Gardner	Shrub	CR	BHCB 161990
Mikania scandens (L.) Willd.	Liana	F	OUPR 15006
Mikania schenckii Hieron.	Liana	CR	VIC 28752
Mikania selloi Spreng.	Liana	F	OUPR 15015
Mikania sericea Hook. & Arn.	Liana	F	VIC 28750
Mikania sessilifolia DC.	Subshrub	CR	BHCB 54693
Mikania subverticillata Baker	Subshrub	CR	OUPR 14809
Mikania ternata (Vell.) B.L.Rob.	Liana	F	BHCB 8210
Mikania testudinaria DC.	Liana	F	BHCB 152825
Mikania trinervis Hook. & Arn.	Liana	F	BHCB 54658
Mikania vauthieriana Baker	Liana	F	BHCB 152826
Mikania vismiifolia DC.	Liana	CR	OUPR 15046
Mikania warmingii Sch.Bip. ex Baker EN*	Shrub	CR	VIC 29120
Mikania sp1	Shrub	CR	OUPR 1879
Mikania sp2	Liana	F	OUPR 16754
Mikania sp3	Subshrub	CR	OUPR 7281
Mikania sp4	Subshrub	CR	OUPR 15604
Mikania sp5	Subshrub	CR	OUPR 16600
Moquinia racemosa (Spreng.) DC.	Shrub	CR	RB 324853
Moquiniastrum densicephalum (Cabrera) G.Sancho	Subshrub	CR	HUFU 54032
Moquiniastrum floribundum (Cabrera) G.Sancho	Shrub	CR	RB 162938
Moquiniastrum paniculatum (Less.) G.Sancho	Shrub	CR	OUPR 14886
Moquiniastrum polymorphum (Less.) G.Sancho	Shrub	F	HUFU 54040
Moquiniastrum pulchrum (Cabrera) G.Sancho	Shrub	CR	VIC 21818
Mutisia campanulata Less.	Liana	CR	OUPR 1208
Mutisia speciosa Aiton ex Hook.	Shrub	CR	VIC 21817
Ophryosporus freyreysii (Thunb.) Baker	Subshrub	CR	RB 145118
Orthopappus angustifolius (Sw.) Gleason	Subshrub	CR	HUFU 54018
Pentacalia desiderabilis (Vell.) Cuatrec.	Liana	F	OUPR 16117
Piptocarpha axillaris (Less.) Baker	Tree	F	HUFU 54024
Piptocarpha macropoda (DC.) Baker	Tree	F	OUPR 19150
Piptocarpha oblonga (Gardner) Baker	Shrub	F	OUPR 16027
Piptocarpha regnellii (Sch.Bip.) Cabrera	Tree	F	OUPR 16039
Piptocarpha tomentosa Baker	Tree	F	OUPR 5771
Piptocarpha sp	Tree	F	OUPR 1206
Piptolepis ericoides Sch. Bip. VU*	Subshrub	CR,F	OUPR 6815
Piptolepis sp	Subshrub	CR	OUPR 1457
Pluchea oblongifolia DC.	Subshrub	CR	RB 163287
Pluchea sagittalis (Lam.) Cabrera	Subshrub	AA,CR	OUPR 16061
Porophyllum ruderale (Jacq.) Cass.	Subshrub	AA,CR	VIC 26523
Praxelis kleinioides (Kunth) Sch. Bip.	Subshrub	CR	RB 486724
Praxelis pauciflora (Kunth) R.M.King & H.Rob.	Subshrub	CR	VIC 27577
Pseudobrickellia brasiliensis (Spreng.) R.M.King & H.Rob.	Subshrub	CR	OUPR 5095
Pseudognaphalium cheiranthifolium (Lam.) Hilliard & Burtt	Subshrub	CR	RB 490164
Pterocaulon alopecuroides (Lam.) DC.	Subshrub	AA,CR	OUPR 15097
Pterocaulon balansae Chodat	Subshrub	CR	VIC 22023
Pterocaulon rugosum (Vahl) Malme	Subshrub	CR	VIC 22021
Pterocaulon sp	Subshrub	CR	OUPR 23275
Raulinoreitzia crenulata (Spreng.) R.M.King & H.Rob.	Shrub	CR	OUPR 14447
Richterago campestris Roque & J.N. Nakaj. EN*	Herb	CR	VIC 21866
Richterago discoidea (Less.) Kuntze	Subshrub	CR	OUPR 14903
Richterago hatschbachii (Zardini) Roque EN*	Herb	CR	OUPR 23296
Richterago petiolata Roque & J.N.Nakaj. EN*	Herb	CR	VIC 21862
Richterago polymorpha (Less.) Roque	Herb	CR	HUFU 54033
Richterago radiata (Vell.) Roque	Herb	CR	OUPR 1453
Richterago sp	Herb	CR	OUPR 3143
Senecio adamantinus Bong.	Subshrub	CR	OUPR 20458

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Senecio brasiliensis (Spreng.) Less.	Shrub	CR	OUPR 21503
Senecio colpodes Bong.	Subshrub	CR	RB 490410
Senecio pohlii Sch.Bip. ex Baker VU*	Subshrub	CR	BHCB118650
Sonchus asper (L.) Hill	Herb	AA	VIC 21873
Sonchus oleraceus L.	Herb	AA	VIC 21871
Sphagneticola trilobata (L.) Pruski	Subshrub	CR	OUPR 21504
Stenocephalum megapotamicum (Spreng.) Sch.Bip.	Subshrub	CR	RB 56531
Stenocline chionaea (ex de Candolle) DC.	Shrub	CR	OUPR 16188
Stenophalium chionaeum (DC.) Anderb.	Subshrub	CR	RB 487378
Stenophalium gardneri (Baker) D.J.N.Hind	Subshrub	CR	BHCB125649
Stevia alexii G.S.S.Almeida & Carvalho-Okano	Subshrub	CR	VIC 29113
Stevia camporum Baker	Subshrub	CR	VIC 29116
Stevia claussenii Sch.Bip. ex Baker	Subshrub	CR	OUPR 1374
Stevia hilarii B.L.Rob. VU*,CR**	Subshrub	CR	VIC 29111
Stevia myriadenia Sch.Bip. ex Baker	Subshrub	CR	VIC 29110
Stevia resinosa Gardner	Subshrub	CR	RB 98089
Stevia urticaefolia Thunb.	Subshrub	CR	OUPR 24672
Symphyopappus angustifolius Cabrera	Shrub	CR	RB 110427
Symphyopappus compressus (Gardner) B.L.Rob.	Shrub	CR	OUPR 1481
Symphyopappus cuneatus (DC.) Sch.Bip. ex Baker	Shrub	CR	BHCB 137583
Symphyopappus itatiayensis (Hieron.) R.M.King & H.Rob.	Shrub	CR	VIC 28810
Symphyopappus reticulatus Baker	Shrub	CR	OUPR 1480
Symphyotrichum regnellii (Baker) G.L.Nesom	Subshrub	CR	VIC 22231
Symphyotrichum squamatum (Spreng.) G.L.Nesom	Subshrub	CR	RB 486586
Tagetes minuta L.	Subshrub	CR	OUPR 12812
Trichogonia eupatorioides (Gardner) R.M.King & H.Rob. EX*	Subshrub	CR	OUPR 17246
Trichogonia hirtiflora (DC.) Sch. Bip. ex Baker	Shrub	CR	OUPR 17207
Trichogonia salviifolia Gardner	Subshrub	CR	OUPR 1344
Trichogonia villosa (Spreng.) Sch.Bip. ex Baker	Subshrub	CR	OUPR 1345
Trixis antimenorrhoea (Schrank) Kuntze	Shrub	CR	OUPR 18367
Trixis glaziovii Baker VU**	Subshrub	CR	HUFU 54041
Trixis lessingii DC.	Subshrub	CR	OUPR 7386
Trixis nobilis (Vell.) Katinas	Subshrub	CR	OUPR 1456
Trixis verbascifolia (Gardner) Blake	Subshrub	CR	OUPR 15255
Trixis sp	Subshrub	CR	OUPR 1455
Verbesina glabrata Hook & Arn.	Shrub	CR	OUPR 1451
Verbesina luetzelburgii Mattf.	Shrub	CR	VIC 26488
Vernonanthura discolor (Spreng.) H.Rob.	Tree	F	OUPR 7430
Vernonanthura lindbergii (Baker) H. Rob.	Subshrub	CR	OUPR 20099
Vernonanthura montevidensis (Spreng.) H.Rob.	Subshrub	CR	OUPR 19249
Vernonanthura mucronulata (Less.) H.Rob.	Subshrub	CR	VIC 21919
Vernonanthura phosphorica (Vell.) H.Rob.	Shrub	CR	BHCB41489
Vernonanthura viscidula (Less.) H.Rob.	Shrub	CR	OUPR 2932
Vernonanthura westiniana (Less.) H.Rob.	Shrub	CR	OUPR 2948
Vittetia orbiculata (DC.) R.M.King & H.Rob.	Subshrub	CR	BHCB118661
Willoughbya acuminata (DC.) Kuntze	Liana	F	OUPR 14619
Willoughbya candolleana (Gardner) Kuntze	Liana	CR	OUPR 14608
Willoughbya estrellensis (Baker) Kuntze	Liana	CR	OUPR 14644
Willoughbya glauca (Mart.) Kuntze	Subshrub	CR	OUPR 1333
Willoughbya lasiandra (DC.) Kuntze	Liana	CR	OUPR 1334
Willoughbya leiolaena DC.	Subshrub	CR	RB 362360
Willoughbya microdonta (DC.) Kuntze	Liana	CR	RB 324845
Willoughbya oblongifolia (DC.) Kuntze	Shrub	CR	RB 163216
Willoughbya obtusata (DC.) Kuntze	Subshrub	CR	OUPR 15610
Willoughbya ramosissima (Gardner) Kuntze	Liana	F	OUPR 3142
Willoughbya selloi (Spreng.) Kuntze	Liana	F	OUPR 15015
Willoughbya sessilifolia (DC.) Kuntze	Shrub	CR	OUPR 14793
Willoughbya subverticillata (Sch. Bip. ex Baker) Kuntze	Subshrub	CR	OUPR 14809

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Willoughbya vismiifolia (DC.) Kuntze	Liana	CR	OUPR 15046
Balanophoraceae			
Langsdorffia hypogaea Mart.	Herb	F	OUPR 987
Balsaminaceae			
Impatiens balsamina L.	Subshrub	AA	OUPR 19303
Begoniaceae			
Begonia cucullata Willd.	Subshrub	CR	NY 877944
Begonia rufa Thunb.	Subshrub	CR	OUPR 9090
Begonia valdensium A.DC.	Subshrub	CR	RB 47524
Bignoniaceae			
Adenocalymma bracteatum (Cham.) DC.	Liana	F	VIC 30460
Adenocalymma cymbalum (Cham.) Bureau & K.Schum.	Liana	F	VIC 30459
Adenocalymma magnoalatum Scud. EN*, CR**	Liana	F	VIC 30454
Adenocalymma marginatum (Cham.) DC.	Liana	F	VIC 30451
Adenocalymma subsessilifolium DC.	Liana	F	VIC 30448
Adenocalymma ternatum (Vell.) Mello ex Bureau & K.Schum.	Shrub	F	OUPR 1429
Adenocalymma sp	Liana	CR	OUPR 1141
Amphilophium crucigerum (L.) L.G.Lohmann	Liana	F	VIC 29400
Amphilophium elongatum (Vahl) L.G.Lohmann	Liana	F	VIC 30376
Amphilophium paniculatum (L.) Kunth	Liana	F	VIC 30447
Anemopaegma arvense (Vell.) Stellfeld ex de Souza EN**	Subshrub	CR	VIC 30445
Anemopaegma chamberlaynii (Sims) Bureau & K. Schum.	Liana	F	OUPR 1034
Anemopaegma floridum Mart. ex DC.	Liana	F	VIC 30442
Anemopaegma prostratum DC.	Liana	CR	OUPR 7513
Anemopaegma setilobum A.H.Gentry	Shrub	CR	VIC 30424
Bignonia binata Thunb.	Liana	F	VIC 21770
Bignonia costata (Bureau & K.Schum.) L.G.Lohmann	Liana	F	VIC 30384
Bignonia prieurii DC.	Liana	F	VIC 29404
Callichlamys latifolia (Rich.) K.Schum.	Liana	F	VIC 30393
Cuspidaria floribunda (DC.) A.H.Gentry	Liana	F	VIC 30380
Cybistax antisyphilitica (Mart.) Mart.	Tree	F	VIC 30378
Dolichandra quadrivalvis (Jacq.) L.G.Lohmann	Liana	F	VIC 29405
Dolichandra unguiculata (Vell.) L.G.Lohmann	Liana	F	VIC 29402
Dolichandra unguis-cati (L.) L.G.Lohmann	Liana	F	VIC 29411
Fridericia candicans (Rich.) L.G.Lohmann	Liana	F	VIC 30417
Fridericia florida (DC.) L.G.Lohmann	Liana	F	VIC 30415
Fridericia formosa (Bureau) L.G.Lohmann	Liana	F	VIC 30410
Fridericia platyphylla (Cham.) L.G.Lohmann	SubShrub	CR	VIC 30418
Fridericia pubescens (L.) L.G.Lohmann	Liana	F	VIC 30408
Fridericia rego (Vell.) L.G.Lohmann	Liana	F	VIC 30401
Fridericia samydoides (Cham.) L.G.Lohmann	Liana	CR,F	OUPR 1297
Fridericia speciosa Mart.	Liana	CR	OUPR 1329
Fridericia triplinervia (Mart. ex DC.) L.G.Lohmann	Liana	F	VIC 30398
Fridericia tynanthoides (A.H.Gentry) L.G.Lohmann	Liana	F	VIC 30395
Handroanthus albus (Cham.) Mattos VU*	Tree	F	OUPR 19280
Handroanthus bureavii (Sandwith) S.Grose	Tree	CR,F	VIC 29442
Handroanthus chrysotrichus (Mart. ex DC.) Mattos	Tree	F	VIC 29435
Handroanthus vellosoi (Toledo) Mattos	Tree	F	VIC 29431
Jacaranda macrantha Cham.	Tree	F	VIC 29248
Jacaranda mimosifolia D.Don	Tree	F	OUPR 8202
Jacaranda montana Morawetz	Tree	F	VIC 29427
Jacaranda puberula Cham.	Tree	F	VIC 29425
Lundia cordata (Vell.) DC.	Liana	F	VIC 29424
Lundia corymbifera (Vahl) Sandwith	Liana	F	VIC 29416
Manaosella cordifolia (DC.) A.H.Gentry	Liana	F	VIC 29409
Pleonotoma stichadenia K.Schum.	Liana	F	VIC 29399
Pleonotoma tetraquetra (Cham.) Bureau	Liana	F	VIC 29398
Pyrostegia venusta (Ker Gawl.) Miers	Liana	CR,F	OUPR 1134

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Sparattosperma leucanthum (Vell.) K. Schum.	Tree	F	OUPR 20259
Stizophyllum aff. *inaequilaterum* Bureau & K.Schum.	Liana	F	VIC 29392
Stizophyllum perforatum (Cham.) Miers	Liana	F	VIC 29391
Stizophyllum riparium (Kunth) Sandwith	Liana	F	VIC 29280
Tabebuia heterophylla (DC.) Britton	Liana	F	VIC 29390
Tabebuia nodosa (Griseb.) Griseb.	Tree	F	OUPR 20259
Tecoma stans (L.) Juss. ex Kunth	Shrub	AA	OUPR 19374
Tynanthus fasciculatus (Vell.) Miers	Liana	F	VIC 29388
Xylophragma myrianthum (Cham. ex Steud.) Sprague	Liana	F	VIC 29386
Zeyheria montana Mart.	Shrub	CR	VIC 29384
Bixaceae			
Bixa orellana L.	Shrub	F	OUPR 1361
Boraginaceae			
Cordia curassavica (Jacq.) Roem. & Schult.	Subshrub	CR	OUPR 3211
Cordia sellowiana Cham.	Tree	CR,F	OUPR 4812
Heliotropium arborescens L.	Shrub	AA	OUPR 1203
Bromeliaceae			
Acanthostachys strobilacea (Schult. & Schult.f.) Klotzsch	Epiphyte	CR	VIC 37212
Aechmea bromeliifolia (Rudge) Baker var. *bromeliifolia*	Herb	CR	VIC 21067
Aechmea lamarchei Mez	Epiphyte	F	VIC 21069
Aechmea nudicaulis var. *aureorosea* (Antoine) L.B.Sm.	Herb	CR	VIC 21070
Aechmea sp	Herb	CR	OUPR 13880
Billbergia elegans Mart. ex Schult. & Schult.f.	Herb	CR,F	OUPR 20235
Billbergia minarum L.B.Sm.	Herb	CR	RB 258593
Billbergia vittata Brongn.	Herb	CR	VIC 21090
Cryptanthus schwackeanus Mez.	Herb	CR	VIC 21098
Dyckia bracteata (Wittm.) Mez	Herb	CR	RB 112248
Dyckia cinerea Mez	Herb	CR	VIC 27818
Dyckia saxatilis Mez	Herb	CR	VIC 27806
Dyckia schwackeana Mez	Herb	CR	RB 275535
Dyckia trichostachya Baker	Herb	CR	RB 275531
Neoregelia mucugensis Leme	Herb	CR	VIC 21104
Nidularium marigoi Leme	Herb	CR	VIC 21107
Pitcairnia flammea Lindl. var. *flammea*	Herb	CR	VIC 27803
Pseudananas sagenarius (Arruda) Camargo	Herb	CR	VIC 21108
Racinaea aerisincola (Mez) M.A.Spencer & L.B.Sm.	Epiphyte	CR	VIC 23656
Tillandsia pohliana Mez	Epiphyte	F	OUPR 14301
Tillandsia polystachia (L.) L.	Epiphyte	CR	VIC (T.S. Coser *et al.* 24)
Tillandsia stricta Sol.	Epiphyte	F	VIC 27798
Vriesea bituminosa Wawra	Herb	CR	VIC 23657
Vriesea clausseniana (Baker) Mez VU*	Herb	CR	VIC 27793
Vriesea hoehneana L.B.Sm.	Herb	CR	VIC 27785
Vriesea minarum L.B.Sm. VU*, EN**	Herb	CR	VIC 36491
Vriesea minor (L.B.Sm.) Leme	Herb	CR	RB 112301
Vriesea regnellii Mez.	Herb	CR	VIC 26453
Vriesea sp1	Herb	CR	VIC 26457
Vriesea sp2	Herb	CR	VIC 26458
Burmanniaceae			
Burmannia damazii Beauverd EN*	Herb	CR	OUPR 13042
Burmannia sp	Herb	CR	OUPR 13471
Burseraceae			
Protium brasiliense (Spreng.) Engl.	Tree	F	OUPR 2452
Cactaceae			
Hatiora salicornioides (Haw.) Britton & Rose	Epiphyte	CR,F	OUPR 7431
Rhipsalis floccosa subsp. *pulvinigera* (Lindb.) Barthlott & N.P.Taylor	Herb	CR	OUPR 20568
Campanulaceae			
Centropogon cornutus (L.) Druce	Subshrub	CR	OUPR 1204
Lobelia camporum Pohl.	Subshrub	CR	OUPR 13425

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Lobelia thapsoidea Schott	Subshrub	CR	RB 97999
Siphocampylus nitidus Pohl.	Subshrub	CR	OUPR 12800
Siphocampylus verticillatus (Cham.) G. Don	Subshrub	F	OUPR 1264
Siphocampylus westinianus (Thunb.) Pohl	Subshrub	CR	BHCB 8249
Siphocampylus sp1	Subshrub	CR	OUPR 5058
Siphocampylus sp2	Subshrub	F	OUPR 6825
Siphocampylus sp3	Subshrub	F	OUPR 6826
Cannabaceae			
Trema micrantha (L.) Blume	Tree	F	OUPR 20241
Caprifoliaceae			
Lonicera japonica Thunb.	Shrub	AA	OUPR 19304
Valeriana organensis Gardner CR**	Liana	F	OUPR 1798
Valeriana scandens L.	Liana	F	OUPR 24838
Celastraceae			
Maytenus longifolia Reissek ex Loes	Shrub	F	RB 398994
Maytenus salicifolia Reissek	Subshrub	F	OUPR 18916
Peritassa campestris (Cambess.) A.C.Sm.	Subshrub	F	BHCB 41525
Chloranthaceae			
Hedyosmum brasiliense Mart. ex Miq.	Shrub	CR,F	OUPR 1441
Chrysobalanaceae			
Couepia grandiflora (Mart. & Zucc.) Benth.	Shrub	F	OUPR 1431
Hirtella floribunda Cham. & Schltdl.	Liana	CR	OUPR 20274
Licania humilis Cham. & Schltdl.	Shrub	F	OUPR 22222
Clethraceae			
Clethra scabra Pers.	Tree	CR,F	OUPR 1275
Clusiaceae			
Clusia arrudea Planch. & Triana	Shrub	CR	NY 1022677
Clusia fluminensis Planch. & Triana	Shrub	CR	OUPR 1360
Clusia spathulifolia Engl.	Shrub	CR,F	OUPR 20150
Commelinaceae			
Commelina diffusa Burm.f.	Herb	CR	MO 3020454
Commelina sp	Herb	CR	OUPR 17721
Dichorisandra pubescens Mart. ex Schult & Schult.f.	Herb	CR,F	OUPR 27241
Dichorisandra thyrsiflora J.C.Mikan	Subshrub	CR	OUPR 22794
Dichorisandra sp	Herb	CR	OUPR 16390
Gibasis geniculate (Jacq.) Rohweder	Herb	CR	OUPR 1414
Tripogandra diuretica (Mart.) Handlos	Herb	CR	OUPR 9063
Tripogandra serrulata (Vahl) Handlos	Herb	F	OUPR 1413
Convolvulaceae			
Ipomoea cairica (L.) Sweet	Liana	CR	OUPR 19301
Ipomoea delphinioides Choisy	Liana	CR,F	OUPR 1210
Ipomoea indica (Burm.f.) Merr.	Liana	AA,F	BHCB 41509
Ipomoea purpurea (L.) Roth	Liana	CR,F	OUPR 1370
Jacquemontia densiflora (Meisn.) Hallier f.	Liana	F	OUPR 8250
Jacquemontia ferruginea Choisy	Liana	F	HRCB 41557
Jacquemontia prostrata Choisy	Liana	CR	OUPR 3022
Jacquemontia rufa (Choisy) Hallier f.	Liana	CR	BHCB 41512
Merremia contorquens (Choisy) Hallier f.	Liana	F	BHCB 41520
Merremia macrocalyx (Ruiz & Pav.) O'Donell	Liana	CR,F	OUPR 1406
Merremia repens D.F.Austin EN**	Liana	F	NY 1014441
Merremia sp	Liana	F	OUPR 1465
Turbina corymbosa (L.) Raf.	Liana	F	OUPR 7746
Cucurbitaceae			
Cayaponia sp	Liana	CR	OUPR 5378
Melothrianthus smilacifolius (Cogn.) M.Crov.	Liana	F	OUPR 9973
Cunoniaceae			
Lamanonia cuneata (Cambess.) Kuntze	Tree	F	OUPR 1397
Lamanonia ternata Vell.	Tree	F	OUPR 4807

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Weinmannia paulliniifolia Pohl ex Ser.	Subshrub	CR	BHCB 460
Weinmannia pinnata L.	Subshrub	CR	OUPR 1396
Cyperaceae			
Ascolepis brasiliensis (Kunth) Benth. ex C.B.Clarke	Herb	F	OUPR 1411
Bulbostylis capillaris (L.) C.B.Clarke	Herb	CR,F	OUPR 12582
Bulbostylis consanguinea (Kunth) C.B.Clarke	Herb	CR	NY 916633
Bulbostylis hirtella (Schrad.) Urb.	Herb	CR	NY 916717
Bulbostylis junciformis (Kunth) C.B.Clarke	Herb	CR	OUPR 1502
Bulbostylis juncoides (Vahl) Kük.	Herb	CR	NY 916717
Bulbostylis paradoxa (Spreng.) Lindm.	Herb	CR	OUPR 12505
Bulbostylis sphaerocephala (Boeckeler) C.B.Clarke	Herb	CR	MO 2941369
Bulbostylis vestita (Kunth) C.B.Clarke	Herb	CR	NY918765
Cryptangium claussenii C.B.Clarke	Herb	CR	OUPR 13058
Cryptangium junciforme (Kunth) Boeckeler	Herb	CR	OUPR 13071
Cryptangium minarum (Nees) Boeckeler	Herb	CR	OUPR 13072
Cyperus aggregatus (Willd.) Endl.	Herb	CR	NY 918974
Cyperus gardneri Nees	Herb	CR	NY 919012
Cyperus haspan L.	Herb	CR	NY 919056
Cyperus odoratus L.	Herb	CR	OUPR 15239
Cyperus pohlii (Nees) Steud.	Herb	CR	OUPR 15945
Cyperus rotundus L.	Herb	CR	OUPR 15240
Cyperus simplex Kunth	Herb	CR	BHCB 483
Cyperus sp	Herb	CR	OUPR 17495
Eleocharis debilis Kunth	Herb	CR	NY 925594
Eleocharis filiculmis Kunth	Herb	CR	NY 925630
Eleocharis sellowiana Kunth	Herb	CR	NY 925702
Fimbristylis complanata (Retz.) Link	Herb	CR	NY 925764
Fimbristylis dichotoma (L.) Vahl	Herb	CR	UB 163367
Kyllinga brevifolia Rottb.	Herb	CR	NY 926309
Lagenocarpus rigidus Nees	Herb	CR	OUPR 7294
Lipocarpha micrantha (Vahl) G.C.Tucker	Herb	CR	OUPR 1502
Pycreus lanceolatus (Poir.) C.B.Clarke	Herb	CR	NY 1112699
Rhynchospora albobracteata A.C.Araújo	Herb	CR	OUPR 15953
Rhynchospora brasiliensis Boeckeler	Herb	CR	OUPR 17499
Rhynchospora ciliata Kük.	Herb	CR	OUPR 12463
Rhynchospora consanguinea (Kunth) Boeckeler	Herb	CR	RB 287166
Rhynchospora corymbosa (L.) Britton	Herb	CR	OUPR 16609
Rhynchospora cryptantha C.B.Clarke	Herb	CR	OUPR 13059
Rhynchospora emaciata (Nees) Boeckeler	Herb	CR	NY 938535
Rhynchospora polyphylla (Vahl) Vahl	Herb	CR	OUPR 15953
Rhynchospora rugosa subsp. *brownii* (Roem. & Schult.) T.Koyama	Herb	CR	OUPR 17703
Rhynchospora speciosa (Kunth) Boeckeler	Herb	CR	OUPR 17736
Rhynchospora tenuis Link	Herb	CR	OUPR 15963
Rhynchospora sp1	Herb	CR	OUPR 10019
Rhynchospora sp2	Herb	CR	OUPR 17736
Rhynchospora sp3	Herb	CR	OUPR 11503
Rhynchospora sp4	Herb	CR	OUPR 23279
Scleria sp	Herb	CR	OUPR 22769
Dilleniaceae			
Davilla angustifolia A. St.-Hil.	Liana	CR	OUPR 1331
Davilla rugosa Poir	Liana	F	OUPR 1318
Dioscoreaceae			
Dioscorea debilis Uline ex R.Knuth	Liana	CR	BHCB 8232
Dioscorea demourae Uline ex R.Knuth	Liana	F	OUPR 1824
Dioscorea grisebachii Kunth	Liana	F	RB 324861
Dioscorea monadelpha (Kunth) Griseb.	Liana	CR	OUPR 1824
Dioscorea ovata Vell.	Liana	CR	OUPR 13382A
Dioscorea piperifolia Humb. & Bonpl. ex Willd.	Liana	CR	OUPR 13382B

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Dioscorea schwakei Uline ex Knuth	Liana	CR	RB 61004
Droseraceae			
Drosera communis A.St.-Hil.	Herb	CR	OUPR 9071
Drosera latifolia (Eichler) Gonella & Rivadavia	Herb	CR	NY 918650
Drosera montana A.St.-Hil.	Herb	CR	OUPR 12788
Drosera villosa A.St.-Hil.	Herb	CR	RB 116710
Ebenaceae			
Diospyros kaki Thunb.	Tree	AA	OUPR 22827
Diospyros sp	Shrub	F	OUPR 9067
Elaeocarpaceae			
Sloanea hirsuta (Schott) Planch. ex Benth.	Tree	F	OUPR 18921
Ericaceae			
Agarista coriifolia var. *bradei* (Sleumer) Judd	Subshrub	CR	RB 182938
Agarista oleifolia (Cham.) G.Don	Shrub	F	OUPR 6807
Agarista pulchella Cham. ex G.Don	Tree	F	OUPR 19288
Agarista pulchella var. *cordifolia* (Meisn.) Judd	Shrub	CR	OUPR 4581
Gaultheria eriophylla (Pers.) Sleumer ex Burtt	Subshrub	CR	OUPR 12793
Gaylussacia chamissonis Meisn.	Shrub	CR	OUPR 8247
Gaylussacia decipiens Cham.	Subshrub	CR	OUPR 13626
Gaylussacia densa Cham.	Subshrub	CR	OUPR 6026
Gaylussacia gardneri Meisn.	Shrub	CR	NY 943288
Gaylussacia incana Cham.	Subshrub	CR	OUPR 6025
Gaylussacia pallida Cham.	Subshrub	CR	RB 139389
Gaylussacia pinifolia Cham. & Schltdl.	Shrub	CR	OUPR 4183
Gaylussacia pseudogaultheria Cham. & Schltd	Subshrub	CR	BHCB 162557
Gaylussacia reticulata Mart. ex Meisn.	Shrub	CR,F	OUPR 1294
Gaylussacia rugosa Cham. & Schltdl.	Shrub	CR	OUPR 6817
Gaylussacia salicifolia Sleumer	Shrub	CR	OUPR 12789
Leucothoe ambigua Mart.	Shrub	CR	OUPR 7286
Leucothoe laxiflora Meiss.	Subshrub	CR	OUPR 1384
Rhododendron simsii Planch.	Subshrub	AA	OUPR 21502
Eriocaulaceae			
Actinocephalus bongardii (A.St.-Hil.) Sano	Herb	CR	BHCB 134051
Comanthera nivea (Bong.) L.R.Parra & Giul.	Herb	CR	R 3711
Eriocaulon ligulatum (Vell.) L.B.Sm.	Herb	CR	OUPR 6981
Leiothrix flavescens (Bong.) Ruhland	Herb	CR	OUPR 13145
Leiothrix vivipara (Bong.) Ruhland	Herb	CR	OUPR 5741
Paepalanthus aequalis (Vell.) J.F.Macbr.	Herb	CR	OUPR 13184
Paepalanthus ciliolatus Ruhland	Herb	CR	OUPR 4569
Paepalanthus conduplicatus Körn.	Herb	CR	OUPR 13193
Paepalanthus elongatus (Bong.) Körn.	Herb	CR	OUPR 12561
Paepalanthus exiguus (Bong.) Körn.	Herb	CR	OUPR 12572
Paepalanthus flaccidus (Bong.) Kunth	Herb	CR	NY 897855
Paepalanthus freyreissii (Thunb.) Körn.	Herb	CR	OUPR 12589
Paepalanthus mollis Kunth	Herb	CR	B 100243890
Paepalanthus planifolius (Bong.) Körn.	Herb	CR	OUPR 1212
Paepalanthus plantagineus (Bong.) Körn.	Herb	CR	OUPR 13286
Syngonanthus caulescens (Poir.) Ruhland	Herb	CR	OUPR 13348
Erythroxylaceae			
Erythroxylum campestre A.St.-Hil.	Shrub	CR	OUPR 6038
Erythroxylum gonocladum (Mart.) O.E.Schulz	Subshrub	F	OUPR 10776
Erythroxylum microphyllum A.St.-Hil.	Subshrub	CR	BHCB 574
Erythroxylum sp1	Shrub	CR	RB 324862
Erythroxylum sp2	Shrub	CR	RB 324863
Escalloniaceae			
Escallonia hispida (Vell.) Sleumer	Shrub	CR	OUPR 1255
Euphorbiaceae			
Alchornea glandulosa subsp. *iricurana* (Casar.) Secco	Shrub	F	OUPR 7429

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Alchornea triplinervia (Spreng.) Müll. Arg.	Tree	CR,F	OUPR 9061
Croton argyroglossus Baill.	Shrub	F	OUPR 9058
Croton ceanothifolius Baill.	Subshrub	CR	NY 870391
Croton celtidifolius Baill.	Tree	F	OUPR 1242
Croton fuscescens Spreng.	Subshrub	CR,F	OUPR 20289
Croton lundianus (Didr.) Müll. Arg.	Subshrub	CR	OUPR 13619
Croton salutaris Casar.	Tree	F	OUPR 1281
Croton vulnerarius Baill.	Tree	CR,F	OUPR 6092
Euphorbia chrysophylla (Klotzsch & Garcke) Boiss.	Subshrub	CR	BHCB 602
Euphorbia hyssopifolia L.	Subshrub	CR	OUPR 1408
Euphorbia portulacoides subsp. *collina* (Phil.) Croizat	Subshrub	CR	BHCB 602
Manihot pilosa Pohl	Shrub	F	OUPR 18256
Microstachys daphnoides (Mart.) Müll. Arg.	Shrub	CR	OUPR 6930
Microstachys hispida (Mart.) Govaerts	Subshrub	CR	RB 277642
Sapium glandulosum (L.) Morong	Shrub	F	OUPR 1213
Sapium sellowianum (Müll.Arg.) Klotzsch ex Baill.	Tree	F	NY 528680
Fabaceae			
Abarema brachystachya (DC.) Barneby & J.W.Grimes	Tree	CR	OUPR 21252
Abarema langsdorffii (Benth.) Barneby & J.W.Grimes	Tree	CR	OUPR 18931
Aeschynomene elegans Schltdl. & Cham	Subshrub	CR	OUPR 13590
Anadenanthera colubrina (Vell.) Brenan	Tree	F	OUPR 20242
Anadenanthera peregrina (L.) Speg.	Tree	F	OUPR 21354
Ancistrotropis peduncularis (Kunth) A. Delgado	Liana	CR	OUPR 13587
Andira fraxinifolia Benth.	Tree	F	VIC 20033
Andira humilis Mart. ex Benth.	Shrub	CR	OUPR 21201
Andira surinamensis (Bondt) Splitg. ex Amshoff	Tree	F	RB 411625
Bauhinia forficata Link	Tree	F	OUPR 15889
Bauhinia fusconervis (Bong.) Steud.	Tree	F	RB 98287
Bauhinia longifolia (Bong.) Steud.	Tree	F	VIC 20875
Bauhinia ungulata var. *cuiabensis* (Bong.) Vaz	Shrub	F	OUPR 20590
Bauhinia sp	Shrub	F	OUPR 7403
Bionia bella Mart. ex Benth.	Liana	F	OUPR 1306
Bowdichia virgilioides Kunth	Tree	F	OUPR 20575
Cajanus cajan (L.) Huth	Shrub	AA	OUPR 12125
Calliandra parvifolia (Hook. & Arn.) Speg.	Shrub	F	OUPR 19479
Calliandra surinamensis Benth.	Shrub	F	OUPR 7349
Calopogonium mucunoides Desv.	Subshrub	CR	OUPR 12131
Camptosema scarlatinum var. *pohlianum* (Benth.) Burkart	Liana	CR	OUPR 13608
Cassia ferruginea (Schrad.) Schrad ex DC.	Tree	F	OUPR 20585
Centrosema coriaceum Benth.	Liana	CR	OUPR 1407
Centrosema virginianum (L.) Benth.	Liana	CR	OUPR 12134
Chaetocalyx longiflora Benth. ex A.Gray	Liana	F	VIC 21000
Chamaecrista andromedea (Mart. ex Benth.) H.S.Irwin & Barneby	Shrub	CR	OUPR 3489
Chamaecrista calycioide s(DC. ex Collad.) Greene	Subshrub	CR	VIC 28986
Chamaecrista dentata (Vogel) H.S.Irwin & Barneby EN*	Shrub	CR	OUPR 1220
Chamaecrista desvauxii (Collad.) Killip	Subshrub	CR	OUPR 22195
Chamaecrista flexuosa (L.) Greene var. *flexuosa*	Shrub	CR	OUPR 15934
Chamaecrista hedysaroides (Vogel) H.S.Irwin & Barneby	Shrub	CR,F	OUPR 19290
Chamaecrista langsdorffii (Kunth ex Vogel) Britton ex Pittier	Shrub	CR	OUPR 1423
Chamaecrista mucronata (Spreng.) H.S.Irwin & Barneby.	Shrub	CR	OUPR 1349
Chamaecrista multipennis (H.S.Irwin & Barneby) H.S.Irwin & Barneby	Shrub	CR	OUPR 18612
Chamaecrista nictitans (L.) Moench	Subshrub	CR	OUPR 16603
Chamaecrista ochnacea (Vogel) H.S.Irwin & Barneby	Shrub	CR	OUPR 20197
Chamaecrista rotundata var. *grandistipula* (Vogel) H.S.Irwin & Barneby	Shrub	CR	OUPR 18982
Chamaecrista rotundata var. *rotundata* (Vogel) H.S.Irwin & Barneby	Shrub	CR	OUPR 18982
Chamaecrista rotundifolia var. *rotundifolia* (Pers.) Greene	Herb	CR	OUPR 18980
Chamaecrista trichopoda (Benth.) Britton & Rose ex Britton & Killip	Subshrub	CR	VIC 28986
Chamaecrista sp	Shrub	CR	OUPR 7400

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Clitoria falcata Lam. var. *falcata*	Liana	F	OUPR 18254
Clitoria rufescens (Benth.) Benth.	Liana	CR	OUPR 1433
Collaea speciosa (Loisel.) DC.	Subshrub	CR	OUPR 17741
Copaifera langsdorffii Desf.	Tree	F	RB 377703
Copaifera reticulata Ducke	Tree	F	OUPR 13761
Crotalaria breviflora DC.	Subshrub	F	OUPR 13501
Crotalaria grandiflora Benth.	Subshrub	CR	OUPR 2337
Crotalaria incana L.	Subshrub	CR	OUPR 14408
Crotalaria lanceolata E.Mey.	Subshrub	CR	OUPR 14414
Crotalaria maypurensis Kunth	Subshrub	CR	NY 983419
Crotalaria micans Link	Subshrub	CR	OUPR 1279
Crotalaria nitens Kunth	Subshrub	CR	OUPR 13813
Crotalaria otoptera Benth.	Subshrub	CR	OUPR 5256
Crotalaria pallida Aiton	Subshrub	CR	FUNED 1275
Crotalaria paulina Schrank	Subshrub	F	OUPR 4817
Crotalaria pilosa Mill.	Subshrub	CR	VIC 24905
Crotalaria stipularia Desv.	Subshrub	CR	VIC 28478
Crotalaria velutina Benth.	Subshrub	CR	VIC 28479
Dalbergia acuta Benth.	Shrub	CR	RB 404382
Dalbergia brasiliensis Vogel	Tree	F	OUPR 21241
Dalbergia foliosa (Benth.) A.M.Carvalho	Shrub	CR	RB 404382
Dalbergia frutescens (Vell.) Britton	Shrub	CR,F	OUPR 9091
Dalbergia nigra (Vell.) Allemão ex Benth. VU*	Tree	CR,F	OUPR 8254
Dalbergia villosa (Benth.) Benth.var. *villosa*	Tree	CR,F	OUPR 12140
Dalbergia violacea (Jacq.) Hoffmanns.	Tree	F	OUPR 6377
Dalbergia sp	Tree	F	VIC 21402
Desmodium adscendens (Sw.) DC.	Subshrub	CR,F	OUPR 12127
Desmodium affine Schltdl.	Subshrub	CR	OUPR 12129
Desmodium barbatum (L.) Benth.	Subshrub	CR	OUPR 14418
Desmodium incanum DC.	Subshrub	CR	OUPR 14402
Desmodium subsericeum Malme	Subshrub	CR	ICN 138601
Desmodium uncinatum (Jacq.) DC.	Subshrub	CR,F	OUPR 12130
Dioclea violacea Mart. ex Benth.	Liana	F	VIC 21026
Eriosema crinitum var. *discolor* Fortunato	Subshrub	CR	OUPR 19766
Erythrina speciosa Andrews	Tree	CR	OUPR 13711
Indigofera suffruticosa Mill.	Subshrub	CR	OUPR 12124
Inga barbata Benth.	Tree	CR	OUPR 18930
Inga cylindrica (Vell.) Mart.	Tree	F	OUPR 21340
Inga edulis Mart.	Tree	F	VIC 20961
Inga ingoides (Rich.) Willd.	Tree	F	OUPR 21339
Inga marginata Willd.	Tree	F	OUPR 20583
Inga schinifolia Benth.	Tree	F	VIC 20963
Inga sessilis (Vell.) Mart.	Tree	F	OUPR 1439
Inga vera subsp. *affinis* (DC.) T.D.Penn.	Tree	F	OUPR 20581
Inga vulpina Mart. ex Benth.	Tree	CR,F	OUPR 9121
Inga sp1	Tree	F	OUPR 7401
Inga sp2	Tree	F	OUPR 9060
Leptospron adenanthum (G. Mey.) A.Delgado	Liana	CR	RB 255436
Machaerium aculeatum Raddi	Tree	CR,F	OUPR 4234
Machaerium brasiliense Vogel LC***	Shrub	CR	OUPR 13611
Machaerium hirtum (Vell.) Stellfeld	Tree	F	OUPR 8259
Machaerium nyctitans (Vell.) Benth.	Tree	F	OUPR 20070
Machaerium oblongifolium Vogel	Liana	F	OUPR 13715
Machaerium reticulatum (Poir.) Pers.	Tree	F	OUPR 13712
Machaerium villosum Vogel VU***	Tree	F	OUPR 20071
Machaerium sp	Shrub	F	OUPR 8259
Macroptilium sabaraense (Hoehne) V.P.Barbosa	Liana	CR	VIC 29114
Melanoxylon brauna Schott VU*, **	Tree	F	OUPR 7405

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Mimosa aurivillus Mart. var. *aurivillus*	Shrub	CR	OUPR 19029
Mimosa aurivillus var. *calothamnos* (Benth.) Barneby	Shrub	CR	OUPR 1223
Mimosa bimucronata (DC.) Kuntze var. *bimucronata*	Shrub	F	OUPR 19477
Mimosa calodendron Mart. ex Benth.	Shrub	CR	OUPR 24799
Mimosa debilis Humb. & Bonpl. ex Willd. var. *debilis*	Subshrub	CR	OUPR 12803
Mimosa diplotricha C.Wright ex Sauvalle var. *diplotricha*	Liana	CR	VIC 30737
Mimosa dolens Vell. var. *dolens*	Subshrub	CR	OUPR 18927
Mimosa montis-carasae Barneby EN[*, **]	Shrub	CR	OUPR 1452
Mimosa ourobrancoensis Burkart	Shrub	CR	OUPR 6385
Mimosa peduncularis Bong. ex Benth.	Shrub	CR	CEN 80702
Mimosa pigra L.	Shrub	CR	VIC 28302
Mimosa pudica var. *hispida* Brenan	Subshrub	CR	VIC 30736
Mimosa scabrella Benth.	Tree	CR,F	OUPR 12408
Mimosa sensitiva var. *malitiosa* (Mart.) Barneby	Liana	CR	VIC 30738
Ormosia friburgensis Taub. ex Glaz.	Tree	F	VIC 21045
Ormosia ruddiana Yakovlev	Tree	F	RB 419029
Parapiptadenia rigida (Benth.) Brenan	Tree	F	OUPR 1328
Peltophorum dubium (Spreng.) Taub.	Tree	F	NY 1093141
Periandra mediterranea (Vell.) Taub.	Shrub	CR	OUPR 14407
Piptadenia adiantoides (Spreng.) J.F.Macbr.	Shrub	F	OUPR 868
Piptadenia gonoacantha (Mart.) J.F.Macbr.	Tree	F	OUPR 20287
Piptadenia micracantha Benth.	Liana	F	OUPR 1450
Platypodium elegans Vogel	Tree	F	VIC 21048
Poiretia punctata (Willd.) Desv.	Liana	F	OUPR 19092
Pseudopiptadenia contorta (DC.) G.P. Lewis & M.P. Lima	Tree	F	OUPR 20582
Pterocarpus rohrii Vahl	Tree	F	OUPR 15587
Rhynchosia minima (L.) DC.	Liana	CR	OUPR 19110
Rhynchosia reticulata (Sw.) DC.	Liana	CR	OUPR 19024
Schnella macrostachya Raddi	Liana	CR	VIC 20874
Senegalia martiusiana (Steud.) Seigler & Ebinger	Liana	F	VIC 20930
Senegalia nitidifolia (Speg.) Seigler & Ebinger	Shrub	F	RB 421786
Senegalia riparia (Kunth) Britton & Rose ex Britton & Killip	Liana	F	VIC 20935
Senna bicapsularis (L.) Roxb. var. *bicapsularis*	Shrub	CR	OUPR 18666
Senna macranthera (DC. ex Collad.) H.S.Irwin & Barneby	Tree	F	OUPR 1346
Senna macranthera var. *nervosa* (Vogel) H.S.Irwin & Barneby	Tree	F	OUPR 1347
Senna multijuga var. *lindleyana* (Gardner) H.S.Irwin & Barneby	Tree	F	OUPR 20587
Senna neglecta var. *oligophylla* (Benth.) H.S.Irwin & Barneby	Tree	CR	OUPR 20197
Senna pendula var. *glabrata* (Vogel) H.S.Irwin & Barneby	Shrub	CR,F	OUPR 8258
Senna pneumatica H.S.Irwin & Barneby	Shrub	F	OUPR 20589
Senna reniformis (G.Don) H.S.Irwin & Barneby	Shrub	CR,F	OUPR 1305
Sesbania virgata (Cav.) Pers.	Shrub	CR	OUPR 19022
Spartium junceum L.	Shrub	AA	OUPR 13710
Stryphnodendron adstringens (Mart.) Coville	Tree	F	OUPR 7443
Stryphnodendron polyphyllum Mart.	Tree	F	OUPR 21204
Stylosanthes gracilis Kunth	Subshrub	CR	OUPR 19922
Stylosanthes guianensis var. *guianensis* (Aubl.) Sw.	Subshrub	CR	MO 3210340
Stylosanthes guianensis var. *pauciflora* M.B.Ferreira & Sousa Costa	Subshrub	CR	NY 623171
Stylosanthes macrocephala M.B.Ferreira & Sousa Costa	Subshrub	CR	OUPR 12126
Stylosanthes montevidensis Vogel	Subshrub	CR	OUPR 12126
Stylosanthes scabra Vogel	Subshrub	CR	RB 190484
Stylosanthes viscosa (L.) Sw.	Subshrub	CR	OUPR 13599
Swartzia hilaireana Mansano & Torke	Tree	F	OUPR 21248
Swartzia oblata R.S.Cowan	Shrub	CR	OUPR 19025
Tachigali friburgensis (Harms) L.G.Silva & H.C.Lima	Tree	F	OUPR 20588
Tachigali rugosa (Mart. ex Benth.) Zarucchi & Pipoly	Tree	F	OUPR 7449
Trifolium repens L.	Subshrub	F	OUPR 13754
Zornia curvata Mohlenbr.	Subshrub	CR	OUPR 27805
Zornia hebecarpa Mohlenbr.	Subshrub	CR	OUPR 27798

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Zornia reticulata Sm.	Subshrub	CR	OUPR 8256
Gentianaceae			
Calolisianthus amplissimus (Mart.) Gilg	Subshrub	CR	UPCB 38616
Calolisianthus pedunculatus (Cham. & Schltdl.) Gilg	Subshrub	CR	OUPR 4564
Calolisianthus pendulus (Mart.) Gilg	Subshrub	CR	OUPR 15385
Calolisianthus pulcherrimus (Mart.) Gilg	Subshrub	CR	OUPR 1375
Calolisianthus speciosus (Cham. & Schltdl.) Gilg	Subshrub	CR	VIC 8272
Deianira damazioi E.F.Guimar.	Subshrub	CR	RB 471530
Deianira nervosa Cham. & Schltdl.	Subshrub	CR	OUPR 9094
Helia alpestris (Mart.) Kuntze	Subshrub	CR	OUPR 16387
Irlbachia elegans Mart.	Subshrub	CR	OUPR 20470
Gesneriaceae			
Anetanthus gracilis Hiern VU*	Subshrub	CR	OUPR 13296
Nematanthus dichrus (Spreng.) Wiehler	Subshrub	CR,F	OUPR 1215
Paliavana sericiflora Benth.	Subshrub	CR	OUPR 1350
Sinningia allagophylla (Mart.) Wiehler	Subshrub	CR	OUPR 1271
Sinningia elatior (Kunth) Chautems	Subshrub	CR	OUPR 17707
Sinningia magnifica (Otto & A.Dietr.) Wiehler	Subshrub	CR	OUPR 13427
Sinningia tuberosa (Mart.) H.E.Moore VU*,**	Subshrub	CR	OUPR 16437
Griseliniaceae			
Griselinia ruscifolia (Clos) Taub.	Liana	CR	OUPR 3307
Humiriaceae			
Humiriastrum glaziovii (Urb.) Cuatrec.	Tree	F	RB 277735
Sacoglottis dentata (Casar.) Urb.	Tree	F	OUPR 3563
Hydrangeaceae			
Hydrangea macrophylla (Thunb.) Ser.	Subshrub	AA	OUPR 21501
Hypericaceae			
Hypericum brasiliense Choisy	Subshrub	CR	RB 98245
Hypericum denudatum A.St.-Hil.	Subshrub	CR	RB 148714
Hypericum polyanthemum Klotzsch ex Reichardt	Subshrub	CR,F	OUPR 1876
Hypericum rigidum A.St.-Hil.	Subshrub	CR	BHCB 8278
Vismia brasiliensis Choisy	Shrub	CR	OUPR 12795
Vismia magnoliifolia Cham. & Schltdl.	Shrub	CR,F	OUPR 1216
Vismia micrantha A.St.-Hil.	Shrub	CR	OUPR 12799
Vismia parviflora Cham. & Schltdl.	Shrub	CR,F	OUPR 1284
Hypoxidaceae			
Hypoxis decumbens L.	Herb	CR	OUPR 17505
Iridaceae			
Crocosmia crocosmiiflora (Lemoine ex Morren) N.E.Br.	Herb	CR	OUPR 1348
Cypella caerulea(Ker Gawl.) Seub. ex Hook. f.	Herb	CR	OUPR 15601
Cypella sp	Herb	CR	OUPR 13554
Dietes bicolor Sweet ex Klatt	Herb	CR	OUPR 19307
Neomarica caerulea (Ker Gawl.) Sprague	Herb	CR	OUPR 13546
Neomarica glauca (Seub. ex Klatt) Sprague	Herb	CR	OUPR 13545
Sisyrinchium itabiritense Ravenna	Herb	CR	NY 910565
Sisyrinchium minutiflorum Klatt	Herb	CR	OUPR 11513
Sisyrinchium vaginatum Spreng.	Herb	CR	OUPR 9096
Sisyrinchium sp	Herb	CR	OUPR 23282
Trimezia juncifolia (Klatt) Benth. & Hook.	Herb	CR	OUPR 10774
Trimezia rupestris Ravenna	Herb	CR	MBML 7050
Juncaceae			
Juncus microcephalus Kunth	Herb	CR	OUPR 15950
Juncus tenuis Willd.	Herb	CR	NY 402417
Juncus sp1	Herb	CR	OUPR 22510
Juncus sp2	Herb	CR	OUPR 22511
Lacistemataceae			
Lacistema pubescens Mart.	Shrub	F	OUPR 10769
Lamiaceae			

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Aegiphila integrifolia (Jacq.) Moldenke	Shrub	F	OUPR 7295
Aegiphila obducta Vell.	Shrub	CR	OUPR 12407
Aegiphila verticillata Vell.	Shrub	F	RB 183422
Cantinoa racemulosa (Mart. ex Benth.) Harley & J.F.B.Pastore	Subshrub	CR	NY 500959
Eriope macrostachya Mart. ex Benth.	Shrub	CR	OUPR 1293
Hyptidendron asperrimum (Spreng.) Harley	Tree	CR,F	OUPR 5609
Hyptis homalophylla Pohl ex Benth.	Subshrub	CR	OUPR 16291
Hyptis monticola Mart. ex Benth.	Subshrub	CR	OUPR 17860
Hyptis pusilla (Pohl) Harley & J.F.B.Pastore	Subshrub	CR	UB 172265
Hyptis radicans (Pohl) Harley & J.F.B.Pastore	Subshrub	CR	OUPR 12804
Hyptis rotundifolia Benth.	Subshrub	CR	OUPR 17887
Leonurus japonicus Houtt.	Subshrub	CR	OUPR 4233
Marsypianthes chamaedrys (Vahl) Kuntze	Subshrub	CR	OUPR 7347
Mesosphaerum pectinatum (L.) Kuntze	Subshrub	CR	UEFS 150032
Mesosphaerum sidifolium (L'Hérit.) Harley & J.F.B.Pastore	Subshrub	CR	UB 111148
Oocephalus oppositiflorus (Schrank) Harley & J.F.B.Pastore	Subshrub	CR	OUPR 17830
Vitex polygama Cham.	Tree	CR,F	OUPR 1376
Vitex rufescens A.Juss.	Tree	F	UB 161215
Vitex sellowiana Cham.	Shrub	F	OUPR 6422
Lauraceae			
Cassytha filiformis L.	Liana	F	OUPR 20257
Cinnamomum erythropus (Nees & Mart.) Kosterm. EN[*], [**]	Tree	F	RB 418224
Cinnamomum quadrangulum Kosterm. VU[*],[**]	Shrub	CR	OUPR 22531
Cinnamomum sp.	Tree	F	RB 48737
Nectandra nitidula Nees & Mart.	Tree	CR,F	OUPR 7375
Nectandra oppositifolia Nees & Mart.	Tree	F	OUPR 25200
Ocotea densiflora (Meisn.) Mez	Tree	F	OUPR 18144
Ocotea diospyrifolia (Meisn.) Mez	Tree	F	OUPR 21331
Ocotea dispersa (Nees & Mart.) Mez	Tree	F	OUPR 15550
Ocotea divaricata (Nees) Mez	Shrub	F	OUPR 1489
Ocotea felix Coe-Teix. CR[*],EN[**]	Shrub	F	OUPR 8331
Ocotea hypoglauca (Nees & Mart.) Mez	Shrub	CR,F	OUPR 18154
Ocotea lancifolia (Schott) Mez	Tree	F	RB 324873
Ocotea laxa (Nees) Mez	Tree	F	OUPR 6827
Ocotea nutans (Nees) Mez	Tree	F	RB 48725
Ocotea percoriacea Kosterm. EN[*]	Tree	CR,F	OUPR 18154
Ocotea pomaderroides (Meisn.) Mez EN[*]	Shrub	F	OUPR 19284
Ocotea puberula (Rich.) Nees	Tree	F	OUPR 21697
Ocotea sassafras (Meisn.) Mez.	Tree	F	OUPR 18195
Ocotea semicompleta (Nees & Mart.) Mez	Tree	F	MO 2158206
Ocotea spixiana (Nees) Mez	Tree	F	OUPR 1390
Ocotea tabacifolia (Meisn.) Rohwer EN[**]	Shrub	F	OUPR 18144
Ocotea tristis (Nees & C. Mart.) Mez	Shrub	CR	OUPR 1394
Ocotea vaccinioides (Meisn.) Mez	Shrub	CR	OUPR 18270
Ocotea sp1	Shrub	F	OUPR 9072
Ocotea sp2	Shrub	F	OUPR 6801
Persea americana Mill.	Tree	AA	OUPR 19302
Persea fulva L.E.Kopp	Tree	F	MO 1899923
Persea pedunculosa Meisn. EN[**]	Tree	F	RB 48653
Lecythidaceae			
Cariniana estrellensis (Raddi) Kuntze	Tree	F	OUPR 140
Lentibulariaceae			
Genlisea aurea A.St.-Hil.	Herb	CR	OUPR 3544
Genlisea repens Benj.	Herb	CR	OUPR 22811
Genlisea violacea A.St.-Hil.	Herb	CR	VIC 19479
Utricularia amethystina Salzm. ex A.St.-Hil. & Girard	Herb	CR	OUPR 22812
Loganiaceae			
Spigelia linarioides A.DC.	Subshrub	CR	RB 111041

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Spigelia spartioides Cham.	Subshrub	CR	OUPR 13473
Loranthaceae			
Phthirusa sp	Shrub	F	BHCB 41488
Psittacanthus dichrous Mart.	Shrub	F	OUPR 1371
Struthanthus concinnus (Mart.) Mart.	Shrub	F	OUPR 1224
Struthanthus salicifolius Mart.	Shrub	F	OUPR 6770
Lythraceae			
Cuphea carthagenensis (Jacq.) J.Macbr.	Subshrub	CR	OUPR 12579
Cuphea ingrata Cham. & Schltdl.	Shrub	CR	OUPR 1227
Cuphea thymoides Cham. & Schltdl.	Subshrub	CR	RB 74307
Diplusodon buxifolius (Cham. & Schltdl.) A.DC.	Shrub	CR	OUPR 8336
Diplusodon hirsutus (Cham. & Schltdl.) A.DC.	Shrub	CR	OUPR 1330
Diplusodon microphyllus Pohl	Subshrub	CR	OUPR 6823
Diplusodon virgatus Pohl	Subshrub	CR	OUPR 6824
Lafoensia pacari A.St.-Hil.	Shrub	CR	OUPR 16274
Magnoliaceae			
Magnolia ovata (A.St.-Hil.) Spreng.	Tree	CR,F	OUPR 5399
Malpighiaceae			
Banisteriopsis angustifolia (A.Juss.) B.Gates	Subshrub	CR	OUPR 7788
Banisteriopsis campestris (A.Juss.) Little	Liana	CR	OUPR 7789
Banisteriopsis gardneriana (A.Juss.) W.R.Anderson & B.Gates	Liana	F	OUPR 1459
Byrsonima clausseniana A.Juss.	Liana	CR,F	OUPR 1228
Byrsonima intermedia A.Juss.	Shrub	CR	OUPR 1277
Byrsonima ligustrifolia A.Juss.	Tree	F	OUPR 20264
Byrsonima spinensis W.R.Anderson	Tree	F	SP 253566
Byrsonima variabilis A.Juss.	Shrub	CR	OUPR 12584
Heteropterys byrsonimifolia A.Juss.	Shrub	CR	SP 253567
Heteropterys intermedia (A.Juss.) Griseb.	Shrub	CR	NY 909452
Heteropterys umbellata A.Juss.	Shrub	CR	OUPR 1278
Heteropterys sp1	Shrub	F	OUPR 3664
Mascagnia bierosa (A.Juss.) W.R.Anderson	Liana	F	NY 1131507
Peixotoa tomentosa A.Juss.	Subshrub	CR	OUPR 1369
Peixotoa sp	Shrub	CR	OUPR 9068
Tetrapterys microphylla (A.Juss.) Nied.	Subshrub	CR	RB 277643
Tetrapterys sp1	Liana	F	OUPR 1460
Tetrapterys sp2	Subshrub	CR	RB 277643
Malvaceae			
Abutilon ibarrense Kunth	Shrub	CR	MO 3038566
Abutilon inaequilaterum A.St.-Hil.	Shrub	CR	OUPR 4854
Abutilon peltatum K.Schum.	Shrub	F	OUPR 4849
Byttneria scabra L.	Subshrub	CR	OUPR 21420
Ceiba sp	Tree	F	OUPR 7432
Krapovickasia macrodon (A.DC.) Fryxell	Subshrub	CR	OUPR 1232
Luehea divaricata Mart. & Zucc.	Tree	F	OUPR 9073
Luehea paniculata Mart. & Zucc.	Tree	CR,F	OUPR 23176
Melochia pilosa (Mill.) Fawc. & Rendle	Subshrub	AA,CR	OUPR 24560
Pavonia communis A.St.-Hil.	Subshrub	CR	NY 407051
Pavonia montana Garcke ex Gürke	Shrub	CR	OUPR 4819
Pavonia sagittata A.St.-Hil.	Subshrub	CR	OUPR 4931
Pavonia schiedeana Steud.	Subshrub	CR	OUPR 1428
Pavonia schrankii Spreng.	Subshrub	CR	UEC 177669
Pavonia viscosa A.St.-Hil.	Shrub	CR,F	OUPR 6796
Peltaea acutifolia (Gürke) Krapov. & Cristóbal	Subshrub	CR	NY 00942549
Peltaea obsita (Mart. ex Colla) Krapov. & Cristóbal	Subshrub	CR	NY 942549
Peltaea polymorpha (A.St.-Hil.) Krapov. & Cristóbal	Shrub	F	OUPR 1405
Sida acuta Burm.f.	Subshrub	CR	NY 942698
Sida cordifolia L.	Subshrub	F	RB 399018
Sida glaziovii K.Schum.	Subshrub	CR	RB 398966

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Sida linifolia Cav.	Subshrub	CR	OUPR 19282
Sida rhombifolia L.	Subshrub	CR	OUPR 1231
Sida spinosa L.	Subshrub	CR	OUPR 13585
Sida tuberculata R.E.Fr.	Subshrub	CR	RB 342260
Sida viarum A.St.-Hil.	Subshrub	CR	SPF 74422
Triumfetta rhomboidea Jacq.	Subshrub	CR	OUPR 12576
Triumfetta semitriloba Jacq.	Subshrub	CR	NY 996627
Waltheria indica L.	Subshrub	CR	OUPR 13618
Wissadula periplocifolia (L.) C.Presl. ex Thwaites	Subshrub	CR	OUPR 5007
Marantaceae			
Goeppertia ackermannii (Körn.) Borchs. & S.Suárez	Herb	F	OUPR 14075
Mayacaceae			
Mayaca sellowiana Kunth	Herb	CR	OUPR 7585
Melastomataceae			
Acisanthera variabilis (Naud.) Triana	Subshrub	CR	NY 2137296
Behuria glutinosa Cogn.	Shrub	CR	OUPR 20229
Cambessedesia corymbosa Mart. & Schrank ex DC.	Subshrub	CR	UB 19511
Cambessedesia espora (A.St.-Hil. ex Bonpl.) DC.	Subshrub	CR	OUPR 23542
Cambessedesia fasciculata (Spreng.) Fidanza & A.B. Martins	Subshrub	CR	NY 1549811
Cambessedesia hilariana (Kunth.) DC.	Subshrub	CR	OUPR 1292
Chaetostoma armatum (Spreng.) Cogn.	Subshrub	CR	RB 515229
Clidemia capitellata (Bonpl.) D.Don	Shrub	CR	BHCB 68080
Clidemia urceolata DC.	Shrub	CR	RB 515524
Comolia sertularia Triana	Shrub	CR	OUPR 21337
Eriocnema acaulis (Cham.) Triana EN**	Subshrub	F	UEC 104907
Eriocnema fulva Naudin VU*,**	Subshrub	CR	OUPR 5819
Fritzschia anisostemon Cham. VU*	Subshrub	CR	OUPR 20231
Fritzschia erecta Cham.	Subshrub	CR	OUPR 8419
Lavoisiera alba Mart. & Schrank ex DC.	Subshrub	CR	HUFU 24943
Lavoisiera caparaoensis Schwacke ex Schwacke & Cogn.	Subshrub	CR	BHCB 67488
Lavoisiera imbricata DC.	Subshrub	CR	OUPR 7382
Lavoisiera pulcherrima DC.	Shrub	CR	OUPR 1440
Leandra aurea (Cham.) Cogn.	Shrub	F	OUPR 5160
Leandra australis (Cham.) Cogn.	Shrub	F	OUPR 1420
Leandra cancellata Cogn.	Shrub	CR	UPCB (Reginato, M. 1175)
Leandra carassana (DC.) Cogn.	Shrub	F	OUPR 515473
Leandra coriacea Cogn.	Subshrub	CR	HUFU 57647
Leandra dendroides (Naudin) Cogn.	Subshrub	F	RB 515390
Leandra erostrata (DC.) Cogn.	Subshrub	CR	RB 515387
Leandra fluminensis Cogn.	Subshrub	F	RB 515378
Leandra foveolata (DC.) Cogn.	Subshrub	CR	HUFU 57652
Leandra fragilis Cogn.	Shrub	CR	OUPR 20218
Leandra glabrata Cogn.	Shrub	CR	RB 515472
Leandra lacunosa Cogn.	Shrub	CR	RB 515478
Leandra lutea Cogn.	Shrub	F	NY 2099130
Leandra melastomoides Raddi	Shrub	CR,F	OUPR 5831
Leandra polychaeta Cogn.	Shrub	CR	UPCB (Reginato, M. 1173)
Leandra purpurascens (DC.) Cogn.	Shrub	F	NY 02098871
Leandra quinquedentata (DC.) Cogn.	Shrub	CR	RB 515474
Leandra sulfurea Cogn.	Shrub	CR	RB 149572
Leandra sp1	Shrub	F	OUPR 1238
Leandra sp2	Shrub	F	OUPR 1419
Leandra sp3	Subshrub	CR	OUPR 17297
Leandra sp4	Subshrub	CR	OUPR 5833
Leandra sp5	Shrub	F	OUPR 19285
Leandra sp6	Shrub	F	OUPR 20164

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Marcetia cordigera DC.	Subshrub	CR	OUPR 5756
Marcetia taxifolia (A.St.-Hil.) DC.	Subshrub	CR	OUPR 1386
Miconia albicans (Sw.) Triana	Subshrub	CR	OUPR 6577
Miconia brunnea DC.	Tree	F	UEC 150710
Miconia calvescens Schrank & Mart. ex DC.	Shrub	F	OUPR 1421
Miconia chartacea Triana	Tree	F	OUPR 1949
Miconia corallina Spring.	Shrub	F	OUPR 1233
Miconia cubatanensis Hoehne	Shrub	F	UPCB 71895
Miconia cyathanthera Triana	Shrub	F	OUPR 3705
Miconia discolor DC.	Tree	F	OUPR 19079
Miconia flammea Casar.	Shrub	F	RB 469741
Miconia ibaguensis (Bonpl.) Triana	Shrub	F	RB 469741
Miconia inconspicua Miq.	Tree	F	UPCB 71896
Miconia ligustroides (DC.) Naudin	Shrub	F	OUPR 1417
Miconia macrophylla (Pav. ex D. Don) Triana	Shrub	F	OUPR 1263
Miconia minutiflora (Bonpl.) DC.	Shrub	F	OUPR 4814
Miconia paniculata (DC.) Naudin	Shrub	F	NY 1738436
Miconia pennipilis Cogn.	Shrub	CR	OUPR 6566
Miconia rimalis Naudin.	Shrub	F	OUPR 1975
Miconia sellowiana Naudin	Shrub	F	RB 358485
Miconia shepherdii R.Goldenb. & Reginato	Tree	F	RB 555987
Miconia stelligera Cogn.	Shrub	F	OUPR 6581
Miconia tentaculifera Naudin	Shrub	F	OUPR 1366
Miconia theizans (Bonpl.) Cogn.	Shrub	CR,F	OUPR 21330
Miconia trianae Cogn.	Shrub	CR	OUPR 1234
Miconia willdenowii Klotzsch ex Naudin	Shrub	F	SPF203103
Miconia sp1	Shrub	F	OUPR 1422
Miconia sp2	Shrub	CR	OUPR 13614
Miconia sp3	Shrub	F	OUPR 20290
Microlicia arenariaefolia DC.	Subshrub	CR	RB 41804
Microlicia avicularis Mart. ex Naudin	Subshrub	CR	OUPR 9986
Microlicia confertiflora Naudin	Subshrub	CR	OUPR 12815
Microlicia cordata (Spreng.) Cham.	Subshrub	CR	OUPR 6788
Microlicia crenulata (DC.) Mart.	Subshrub	CR	OUPR 20462
Microlicia euphorbioides Mart.	Subshrub	CR	OUPR 9996
Microlicia fasciculata Mart. ex Naudin	Subshrub	CR	HUFU 57696
Microlicia glazioviana Cogn. EN**	Subshrub	CR	OUPR 8802
Microlicia graveolens DC.	Subshrub	CR	HUFU 57687
Microlicia isophylla DC.	Subshrub	CR	OUPR 6056
Microlicia macrophylla Naudin	Subshrub	CR	OUPR 9968
Microlicia multicaulis Mart. ex Naudin	Subshrub	CR	RB 515443
Microlicia parvifolia Naudin	Subshrub	CR	NY 926143
Microlicia serpyllifolia D.Don	Subshrub	CR	OUPR 6396
Microlicia sp1	Subshrub	CR	OUPR 6783
Microlicia sp2	Subshrub	CR	OUPR 7785
Microlicia sp3	Subshrub	CR	OUPR 9985
Ossaea cinnamomifolia (Naudin) Triana	Shrub	F	OUPR 2143
Ossaea congestiflora (Naudin) Cogn.	Shrub	CR	OUPR 6652
Ossaea coriacea (Naudin) Triana	Shrub	F	OUPR 5917
Pleroma aemula P.J.F.Guim., A.L.F.Oliveira & R.Romer	Tree	F	RB 524426
Rhynchanthera grandiflora (Aubl.) DC.	Shrub	CR	OUPR 6652
Siphanthera arenaria (DC.) Cogn.	Subshrub	CR	HUFU 57654
Siphanthera paludosa (DC.) Cogn.	Subshrub	CR	OUPR 23552
Tibouchina canescens (D.Don) Cogn.	Tree	F	OUPR 1236
Tibouchina cardinalis (Humb. & Bonpl.) Cogn.	Shrub	CR	OUPR 6160
Tibouchina cerastifolia Cogn.	Shrub	CR	UEC 187651
Tibouchina cisplatensis Cogn.	Subshrub	CR	OUPR 6162
Tibouchina collina (Naud.) Cogn.	Subshrub	CR	UPBC 70958

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Tibouchina dendroides (Naudin) Cogn.	Shrub	CR	OUPR 6175
Tibouchina estrellensis (Raddi) Cogn.	Tree	F	OUPR 6178
Tibouchina fissinervia (Schrank & Mart. ex DC.) Cogn.	Shrub	F	OUPR 1324
Tibouchina formosa Cogn.	Shrub	CR	OUPR 23555
Tibouchina fothergillae (Schrank & Mart. ex DC.) Cogn.	Tree	F	OUPR 19076
Tibouchina frigidula (DC.) Cogn.	Subshrub	CR	OUPR 6198
Tibouchina gardneriana (Triana) Cogn.	Tree	F	HUFU 57919
Tibouchina gracilis (Bonpl.) Cogn.	Subshrub	CR	OUPR 6794
Tibouchina herbacea (DC.) Cogn.	Subshrub	CR	RB 524414
Tibouchina heteromalla (D.Don) Cogn.	Subshrub	CR	OUPR 6138
Tibouchina hieracioides (DC.) Cogn.	Subshrub	CR	HUFU 53795
Tibouchina martialis (Cham.) Cogn.	Shrub	F	OUPR 1267
Tibouchina martiusiana (DC.) Cogn.	Shrub	CR,F	OUPR 1321
Tibouchina moricandiana Baill.	Shrub	F	MBM 233920
Tibouchina mutabilis (Vell.) Cogn.	Tree	F	RB 40788
Tibouchina semidecandra (Schrank & Mart. ex DC.) Cogn.	Shrub	CR,F	OUPR 6777
Tibouchina stenocarpa (Schrank & Mart. ex DC.) Cogn.	Shrub	CR,F	NY 941804
Tibouchina thereminiana (DC.) Cogn.	Shrub	CR	RB 98503
Tibouchina trichopoda (DC.) Baill.	Shrub	CR	OUPR 6488
Tibouchina valtherii Cogn.	Tree	F	RB 524426
Tibouchina sp	Shrub	CR	OUPR 4805
Trembleya calycina Cham. EX*, EN**	Shrub	CR	OUPR 6274
Trembleya laniflora (D.Don) Cogn.	Shrub	CR	OUPR 1365
Trembleya parviflora (D.Don) Cogn.	Shrub	CR,F	OUPR 1378
Trembleya pentagona Naudin	Shrub	CR	OUPR 6425
Trembleya phlogiformis DC.	Shrub	CR	OUPR 1325
Trembleya tridentata Naudin	Shrub	CR	OUPR 1506
Meliaceae			
Cabralea canjerana subsp. *canjerana* (Vell.) Mart.	Tree	CR,F	OUPR 5531
Cabralea canjerana subsp. *polytricha* (A. Juss.) T.D.Penn.	Shrub	CR	OUPR 19180
Menispermaceae			
Abuta sp	Liana	F	OUPR 7397
Cissampelos andromorpha DC.	Liana	F	OUPR 8991
Monimiaceae			
Macropeplus ligustrinus (Tul.) Perkins	Shrub	F	OUPR 5470
Macropeplus schwackeanus (Perkins) I.Santos & Peixoto VU*	Tree	F	BHCB 122307
Mollinedia glabra (Spreng.) Perkins	Tree	F	OUPR 21698
Moraceae			
Dorstenia urceolata Schott	Subshrub	F	OUPR 1291
Ficus sp	Tree	F	OUPR 19179
Sorocea bonplandii (Baill.) W.C.Burger, Lanj. & de Boer	Tree	F	OUPR 21335
Myrtaceae			
Blepharocalyx salicifolius (Kunth) O.Berg	Shrub	CR	RB 276290
Calyptranthes pulchella DC.	Shrub	CR,F	BHCB (FF Carmo 3484)
Campomanesia adamantium (Cambess.) O.Berg	Shrub	CR	OUPR 1980
Campomanesia guaviroba (DC.) Kiaersk.	Shrub	F	BHCB (M.O. Bünger 543)
Campomanesia pubescens (DC.) O.Berg.	Shrub	CR	OUPR 19392
Campomanesia rufa (O.Berg) Nied.	Shrub	CR,F	OUPR 21145
Campomanesia simulans M.L.Kawas.	Tree	F	OUPR 20248
Eucalyptus grandis W.Hill	Tree	F	OUPR 20898
Eugenia brasiliensis Lam.	Shrub	F	SP 313342
Eugenia cerasiflora Miq.	Tree	F	BHCB 144673
Eugenia florida DC.	Shrub	F	BHCB 144297
Eugenia longipedunculata Nied.	Shrub	CR,F	OUPR 21660
Eugenia ligustrina (Sw.) Willd.	Tree	CR,F	OUPR 24025
Eugenia mosenii (Kausel) Sobral	Tree	F	OUPR 1435
Marlierea angustifolia (O.Berg) Mattos	Tree	F	SP 313316

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Marlierea antonia (O.Berg) D.Legrand	Tree	F	OUPR 1492
Marlierea excoriate Mart.	Tree	F	OUPR 21702
Marlierea laevigata (DC.) Kiaersk.	Tree	F	BHCB 144296
Marlierea tomentosa Cambess.	Tree	F	UEC 157769
Marlierea obscura O.Berg	Tree	CR,F	OUPR 19162
Marlierea sp	Shrub	CR	OUPR 15617
Myrceugenia alpigena (DC.) Landrum	Shrub	CR,F	OUPR 5721
Myrceugenia miersiana (Gardner) D.Legrand & Kausel	Tree	F	OUPR 19166
Myrceugenia ovata (Hook. & Arn.) O.Berg	Tree	F	BHCB 107934
Myrcia amazonica DC.	Tree	CR,F	OUPR 6207
Myrcia crocea Kiaersk.	Tree	F	OUPR 21705
Myrcia eriopus DC.	Shrub	CR,F	OUPR 24032
Myrcia eriocalyx DC.	Shrub	CR,F	OUPR 22798
Myrcia guianensis (Aubl.) DC.	Shrub	CR	OUPR 3733
Myrcia hartwegiana (O.Berg) Kiaersk.	Shrub	CR,F	OUPR 22797
Myrcia hebepetala DC.	Shrub	F	OUPR 3819
Myrcia laruotteana Cambess.	Shrub	F	OUPR 23794
Myrcia lutescens Cambess.	Tree	F	BHCB 144300
Myrcia montana Cambess.	Shrub	CR	OUPR 21668
Myrcia mutabilis (O.Berg) N.Silveira	Tree	CR	OUPR 24034
Myrcia obovata (O.Berg) Nied.	Shrub	CR,F	OUPR 4186
Myrcia pubiflora DC.	Subshrub	CR,F	OUPR 21666
Myrcia pulchra (O.Berg) Kiaersk.	Shrub	CR	OUPR 21671
Myrcia retorta Cambess.	Shrub	CR,F	OUPR 1432
Myrcia rufipes DC.	Shrub	F	OUPR 19169
Myrcia splendens (Sw.) DC.	Tree	CR,F	OUPR 3569
Myrcia subalpestris DC.	Tree	F	MBM 227913
Myrcia subcordata DC.	Shrub	CR	OUPR 11332
Myrcia subverticillaris (O. Berg) Kiaersk.	Shrub	CR,F	OUPR 22801
Myrcia tomentosa (Aubl.) DC.	Shrub	F	OUPR 6208
Myrcia vauthieriana O.Berg	Tree	F	OUPR 19172
Myrcia venulosa DC.	Shrub	CR,F	OUPR 22296
Myrciaria floribunda (H.West ex Willd.) O.Berg	Shrub	CR,F	OUPR 24035
Myrciaria glanduliflora (Kiaersk.) Mattos & D.Legrand	Shrub	CR	VIC 20862
Plinia cauliflora (Mart.) Kausel	Tree	F	BHCB (M.O. Bünger 544)
Psidium firmum O.Berg	Shrub	CR	OUPR 22799
Psidium myrtoides O.Berg	Tree	F	BHCB 161191
Psidium robustum O.Berg	Shrub	CR	OUPR 5810
Psidium rufum Mart. ex DC.	Tree	F	OUPR 20248
Siphoneugena crassifolia (DC.) Proença & Sobral	Shrub	F	OUPR 19164
Siphoneugena densiflora O.Berg	Shrub	CR,F	OUPR 19163
Siphoneugena dussii (Krug & Urb.) Proença	Shrub	CR	OUPR 22329
Siphoneugena kiaerskoviana (Burret) Kausel	Tree	CR	RB 276300
Nyctaginaceae			
Guapira opposita (Vell.) Reitz	Shrub	F	OUPR 17709
Guapira tomentosa (Casar.) Lundell	Shrub	CR	OUPR 1412
Neea sp	Shrub	CR	OUPR 17734
Ochnaceae			
Luxemburgia nobilis Eichler ex Engl.	Shrub	CR	OUPR 6730
Luxemburgia octandra A.St.-Hill.	Shrub	CR	OUPR 6816
Ouratea floribunda (A.St.-Hil.) Engl.	Tree	F	OUPR 2265
Ouratea multiflora (Pohl) Engl.	Shrub	CR	RB 60921
Ouratea semiserrata (Mart. & Nees) Engl.	Shrub	CR	OUPR 1372
Sauvagesia erecta L.	Subshrub	CR	OUPR 5695
Sauvagesia vellozii (Vell. ex A.St.-Hil.) Sastre	Subshrub	CR,F	UB 185430
Oleaceae			
Jasminum mesnyi Hance	Shrub	AA	OUPR 19375

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Onagraceae			
Fuchsia coccinea Dryand.	Shrub	CR	RB 50293
Fuchsia regia (Vell.) Munz	Shrub	CR,F	OUPR 1265
Ludwigia anastomosans (DC.) H.Hara	Shrub	CR	OUPR 1243
Ludwigia octovalvis (Jacq.) P.H.Raven	Subshrub	CR,F	NY 1066833
Orchidaceae			
Acianthera prolifera (Herb. ex Lindl.) Pridgeon & M.W.Chase	Herb	CR	OUPR 1444
Acianthera teres (Lindl.) Borba	Herb	CR	OUPR 22796
Anacheilium allemanoides (Hoene) Pabst, Moutinho & A.V.Pinto	Herb	F	OUPR 9621
Bifrenaria aureofulva Lindl.	Herb	F	BHCB 125888
Bifrenaria tyrianthina (Lodd.) Rchb.f.	Herb	CR	OUPR 9623
Cattleya bradei (Pabst) Van den Berg	Herb	CR	OUPR 3940
Cattleya cinnabarina (Bateman ex Lindl.) Van den Berg	Herb	CR	OUPR 7311
Cattleya crispata (Thunb.) Van den Berg	Herb	CR	OUPR 8730
Cattleya longipes (Rchb.f.) Van den Berg	Herb	CR	OUPR 8730
Christensonella subulata (Lindl.) Szlach. *et al.*	Herb	CR	OUPRPR 20226
Cleistes libonii (Rchb.f.) Schltr.	Herb	CR	OUPR 3943
Cleistes metallina (Barb. Rodr.) Schltr.	Herb	CR	OUPR 15540
Cleistes montana Gardner	Herb	CR	OUPR 1442
Cyrtopodium sp	Herb	CR	OUPR 9056
Dichaea cogniauxiana Schltr.	Herb	CR,F	BHCB 125897
Epidendrum chlorinum Barb.Rodr.	Herb	CR	BHCB 125899
Epidendrum dendrobioides Thunb.	Herb	CR	OUPR 3944
Epidendrum denticulatum Barb. Rodr.	Herb	CR	OUPR 3954
Epidendrum martianum Lindl.	Herb	F	OUPR 20465
Epidendrum rupicolum Cogn.	Herb	CR	OUPR 3951
Epidendrum secundum Jacq.	Herb	CR,F	OUPR 8732
Epidendrum xanthinum Lindl.	Herb	CR,F	OUPR 3937
Epistephium praestans Hoehne	Herb	CR	OUPR 1445
Erythrodes sp	Herb	CR	OUPR 22039
Galeandra junceoides Barb.Rodr.	Herb	CR	BHCB 40137
Gomesa planifolia (Lindl.) Kl. & Rchb.f.	Herb	CR	OUPR 3950
Gomesa ramosa (Lindl.) M.W.Chase & N.H.Williams	Herb	CR	OUPR 3953
Gomesa recurva R.Br.	Herb	CR	OUPR 3950
Gomesa warmingii (Rchb.f.) M.W.Chase & N.H.Williams VU*	Herb	CR	OUPR 3952
Grobya amherstiae Lindl.	Herb	CR	OUPR 3942
Habenaria caldensis Kraenzl.	Herb	CR	OUPR 3938
Habenaria hydrophila Barb.Rodr.	Herb	CR	RB 98594
Habenaria imbricata Lindl.	Herb	CR	BHCB 120315
Habenaria itaculumia Garay CR**	Herb	CR	OUPR 20469
Habenaria parviflora Lindl.	Herb	CR	OUPR 3947
Habenaria petalodes Lindl.	Herb	CR	OUPR 8253
Habenaria rodeiensis Barb.Rodr.	Herb	CR	OUPR 1443
Habenaria rupestris Poepp.& Endl.	Herb	CR	OUPR 22046
Habenaria rupicola Barb.Rodr.	Herb	CR	OUPR 9715
Habenaria secundiflora Barb.Rodr.	Herb	CR	OUPR 14317
Habenaria subviridis Hoehne & Schltr.	Herb	CR	OUPR 3948
Habenaria trifida Kunth	Herb	CR	UB 19884
Habenaria sp	Herb	CR	OUPR 18245
Hadrolaelia brevipedunculata (Cogn.) Chiron & V.P.Castro VU**	Herb	CR	OUPR 13864
Hadrolaelia coccinea (Lindl.) Chiron & V.P.Castro VU*	Herb	CR,F	OUPR 13866
Koellensteinia tricolor (Lindl.) Rchb.f.	Herb	F	OUPR 19293
Malaxis excavata (Lindl.) Kuntze	Herb	F	OUPR 14273
Pelexia oestrifera (Rchb.f. & Warm.) Schltr.	Herb	CR	BHCB 125691
Pelexia sp	Herb	CR	OUPR 1821
Prescottia glazioviana Cogn.	Herb	CR	OUPR 3945
Prescottia montana Barb.Rodr.	Herb	CR	OUPR 3946
Prescottia plantaginifolia Lindl. ex Hook.	Herb	CR	RB 193666

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Prosthechea allemanoides (Hoehne) W.E.Higgins	Herb	CR	BHCB 125925
Prosthechea moojenii (Pabst) W.E.Higgins	Herb	CR	RB 182939
Prosthechea vespa (Vell.) W.E.Higgins	Herb	CR	R 222290
Prostechea sp	Herb	CR	RB 193659
Scuticaria hadwenii (Lindl.) Planch.	Herb	CR	OUPR 13969
Skeptrostachys congestiflora (Cogn.) Garay	Herb	CR	OUPR 1447
Xylobium foveatum (Lindl.) Nicholson	Herb	CR	OUPR 3941
Zygopetalum brachypetalum Lindl.	Herb	CR	RB 98634
Zygopetalum maculatum (Kunth) Garay	Herb	CR,F	RB 206763
Zygopetalum triste Barb.Rodr.	Herb	CR	RB 193667
Zygopetalum sp	Herb	CR	RB 193664
Orobanchaceae			
Buchnera lavandulacea Cham. & Schltdl.	Subshrub	CR	OUPR 12816
Castilleja arvensis Schltdl. & Cham.	Subshrub	F	OUPR 7747
Escobedia grandiflora (L.f.) Kuntze	Subshrub	CR	OUPR 1320
Esterhazya macrodonta (Cham.) Benth.	Subshrub	CR	OUPR 1705
Esterhazya splendida J.C.Mikan	Subshrub	CR	OUPR 13474
Velloziella spathacea (Oliv.) Melch.	Subshrub	CR	OUPR 1269
Passifloraceae			
Passiflora haematostigma Mart. ex Mast.	Liana	CR	OUPR 1416
Passiflora porophylla Vell.	Liana	CR	IAC 53474
Passiflora speciosa Gardner	Liana	F	OUPR 5156
Passiflora villosa Vell.	Liana	CR	OUPR 23350
Passiflora sp	Liana	CR	OUPR 20522
Phyllanthaceae			
Phyllanthus fastigiatus Mart. ex Müll.Arg.	Subshrub	CR	OUPR 20172
Phyllanthus klotzschianus Müll.Arg.	Subshrub	CR	OUPR 1304
Phyllanthus niruri L.	Subshrub	CR	OUPR 6395
Phyllanthus obtusatus (Bilb.) Müll.Arg.	Subshrub	CR	NY 1064830
Phyllanthus roselus (Müll. Arg.) Müll.Arg.	Subshrub	CR	RB 277727
Phytolaccaceae			
Phytolacca thyrsiflora Fenzl ex J.A.Schmidt	Subshrub	CR,F	OUPR 7467
Pinaceae			
Pinus sp	Tree	F	OUPR 22826
Piperaceae			
Ottonia sp	Shrub	F	OUPR 20256
Peperomia alata Ruiz & Pav.	Subshrub	CR	RB 399012
Peperomia blanda (Jacq.) H.B.K.	Subshrub	F	OUPR 6907
Peperomia cernuum Vell.	Shrub	F	NY 1021340
Peperomia corcovadensis Gardner	Subshrub	CR	BHCB 8246
Peperomia palcipila C.DC.	Subshrub	CR	OUPR 6889
Peperomia subrubrispica C.DC.	Subshrub	CR	RB 331623
Peperomia tenella (Sw.) A.Dietr.	Subshrub	CR	RB 54549
Peperomia tetraphylla (G.Forst.) Hook. & Arn.	Subshrub	CR	OUPR 7368
Piper aduncum L.	Shrub	CR	OUPR 20294
Piper baptisianum C.DC.	Shrub	F	OUPR 1496
Piper cernuum Vell.	Shrub	F	UB 113500
Piper coccoloboides (Kunth) Kunth ex C.DC.	Shrub	F	OUPR 18261
Piper corcovadensis (Miq.) C.DC.	Shrub	F	OUPR 1312
Piper crassinervium Kunth	Shrub	F	OUPR 18262
Piper damazii C.DC.	Shrub	F	OUPR 1311
Piper lhotzkyanum Kunth	Shrub	CR	RB 357134
Piper regnellii (Miq.) C.DC.	Shrub	F	OUPR 18260
Piper richardiifolium (Kunth) Kunth ex C.DC.	Subshrub	F	MO 3079243
Piper solmsianum C.DC.	Shrub	CR	RB 54631
Piper tectoniaefolium (Kunth) Kunth ex C.DC.	Shrub	F	RB 331625
Piper umbellatum L.	Shrub	F	OUPR 6909
Piper sp1	Shrub	F	OUPR 1310

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Piper sp2	Shrub	F	OUPR 6774
Piper sp3	Shrub	F	OUPR 20253
Plantaginaceae			
Plantago guilleminiana Decne.	Herb	CR	NY 423565
Plantago tomentosa Lam.	Herb	CR	OUPR 12586
Scoparia dulcis L.	Subshrub	CR	OUPR 25230
Stemodia lobata J.A.Schmidt VU*	Subshrub	CR	OUPR 17291
Stemodia microphylla J.A.Schmidt	Herb	CR	OUPR 17733
Stemodia trifoliata (Link) Rchb.	Subshrub	CR	VIC 54548
Poaceae			
Andropogon bicornis L.	Herb	CR	OUPR 15614
Andropogon ingratus Hack.	Herb	CR	CEN 28917
Andropogon leucostachyus Kunth.	Herb	CR	OUPR 22791
Andropogon ternatus (Spreng.) Nees	Herb	CR	MBM 45496
Andropogon virgatus Desv. ex Ham.	Herb	CR	OUPR 20463
Andropogon virginicum (L.) Spreng.	Herb	CR	OUPR 1498
Apochloa chnoodes (Trin.) Zuloaga & Morrone	Herb	CR	BHCB 72595
Apochloa lorea (Trin.) Zuloaga & Morrone	Herb	CR	OUPR 17748
Apochloa molinioides (Trin.) Zuloaga & Morrone	Herb	CR	NY 381729
Apochloa poliophylla (Renvoize & Zuloaga) Zuloaga & Morrone	Herb	CR	OUPR 7291
Aristida recurvata Kunth	Herb	CR	NY 861291
Aristida torta (Nees) Kunth	Herb	CR	OUPR 17149
Aulonemia sp	Herb	CR	UEC 171751
Axonopus aureus P.Beauv.	Herb	CR	MO 861610
Axonopus fissifolius (Raddi) Kuhlm.	Herb	CR	NY 861744
Axonopus pellitus (Nees ex Trin.) Hitchc. & Chase	Herb	CR	OUPR 15600
Axonopus polystachyus G.A.Black	Herb	CR	MO 1577833
Axonopus pressus (Nees ex Steud.) Parodi	Herb	CR	OUPR 15599
Axonopus siccus (Nees) Kuhlm.	Herb	CR	OUPR 20177
Axonopus sp	Herb	CR	OUPR 15599
Cenchrus polystachios (L.) Morrone	Herb	CR	OUPR 1501
Chascolytrum calotheca (Trin.) Essi,Longhi-Wagner & Souza-Chies	Herb	CR	OUPR 15956
Chusquea attenuata (Döll) L.G.Clark EN**	Herb	CR	MBM 27517
Chusquea nutans L.G.Clark	Herb	CR	OUPR 16241
Chusquea pinifolia (Nees) Nees	Herb	CR	BHCB 8211
Colanthelia sp	Herb	CR	BHCB 107925
Danthonia sp	Herb	CR	OUPR 16613
Dichanthelium sabulorum (Lam.) Gould & C.A.Clark	Herb	CR	MO 1919711
Dichanthelium sciurotoides (Zuloaga & Morrone) Davidse	Herb	F	OUPR 6763
Dichanthelium stigmosum (Trin.) Zuloaga	Herb	F	BHCB 133922
Dichanthelium superatum (Hack.) Zuloaga	Herb	F	NY 864620
Digitaria hololeuca Henrard	Herb	CR	UB 14109
Digitaria insularis (L.) Mez ex Ekman	Herb	CR	OUPR 16608
Digitaria pampinosa Henrard EN**	Herb	CR	NY 862167
Digitaria sejuncta (Hack. ex Pilg.) Henrard	Herb	CR	NY 862170
Echinochloa crusgalli (L.) P.Beauv.	Herb	CR	OUPR 15952
Echinolaena inflexa (Poir.) Chase	Herb	CR	OUPR 16612
Elionurus muticus (Spreng.) Kuntze	Herb	CR	MBM 27827
Eragrostis articulata (Schrank) Nees	Herb	CR	NY 862503
Eragrostis pilosa (L.) P.Beauv.	Herb	CR	OUPR 1497
Eragrostis rufescens Schrad. ex Schult.	Herb	CR	OUPR 12768
Eragrostis solida Nees	Herb	CR	OUPR 22785
Eriochrysis holcoides (Nees) Kuhlm.	Herb	CR	OUPR 13512
Eriochrysis sp	Herb	CR	BHCB 721
Ichnanthus bambusiflorus (Trin.) Döll	Herb	CR	OUPR 10775
Ichnanthus calvescens (Nees ex Trin.) Döll	Herb	CR	OUPR 20228
Ichnanthus pallens (Sw.) Munro ex Benth.	Herb	CR	OUPR 6764
Isachne sp	Herb	CR	OUPR 17559

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Melinis minutiflora P.Beauv.	Herb	CR	OUPR 15615
Merostachys fischeriana Rupr. ex Döll	Herb	CR,F	HUEFS 147721
Merostachys sp	Herb	F	OUPR 24844
Mesosetum ferrugineum (Trin.) Chase	Herb	CR	OUPR 14124
Otachyrium versicolor (Döll) Henrard	Herb	CR	OUPR 22782
Panicum campestre Nees ex Trin.	Herb	CR	NY 863761
Panicum sellowii Nees	Herb	F	OUPR 6762
Parodiolyra micrantha (Kunth) Davidse & Zuloaga	Herb	F	OUPR 1499
Paspalum conjugatum P.J.Bergius	Herb	CR	NY 862363
Paspalum coryphaeum Trin.	Herb	CR	OUPR 23303
Paspalum eucomum Nees ex Trin.	Herb	CR	OUPR 15612
Paspalum glaucescens Hack.	Herb	CR	MO 2969469
Paspalum hyalinum Nees ex Trin.	Herb	CR	OUPR 22784
Paspalum juergensii Hack.	Herb	CR	SP 199550
Paspalum mandiocanum Trin.	Herb	CR	MO 2972192
Paspalum multicaule Poir	Herb	CR	OUPR 22784
Paspalum pilosum Lam.	Herb	CR	OUPR 22790
Paspalum polyphyllum Nees ex Trin.	Herb	CR	OUPR 12819
Paspalum pumilum Nees	Herb	CR	NY 895924
Paspalum thrasyoides (Trin.) S.Denham	Herb	CR	MBM 27480
Paspalum sp	Herb	CR	OUPR 22793
Pseudechinolaena polystachya (Kunth) Stapf	Herb	F	OUPR 6762
Rugoloa pilosa (Sw.) Zuloaga	Herb	CR,F	OUPR 7270
Schizachyrium sanguineum (Retz.) Alston	Herb	CR	OUPR 22786
Schizachyrium tenerum Nees	Herb	CR	OUPR 22787
Setaria parviflora (Poir.) M.Kerguelen	Herb	CR	MO 2985867
Setaria tenacissima Schrad.	Herb	CR	NY 906251
Sporobolus ciliatus J.Presl	Herb	CR	RB 239283
Sporobolus piliferus (Trin.) Kunth	Herb	CR	NY 896366
Steinchisma laxum (Sw.) Zuloaga	Herb	CR	NY 863853
Trichanthecium cyanescens (Nees ex Trin.) Zuloaga & Morrone	Herb	CR	OUPR 15621
Trichanthecium pseudisachne (Mez) Zuloaga & Morrone	Herb	CR	OUPR 22789
Trichanthecium wettsteinii (Hack.) Zuloaga & Morrone	Herb	CR	OUPR 22783
Polygalaceae			
Asemeia monninoides (Kunth) J.F.B.Pastore & J.R.Abbott	Subshrub	CR	RB 398973
Asemeia violacea (Aubl.) F.B.Pastore & J.R.Abbott	Subshrub	CR	OUPR 13589
Caamembeca laureola (A.St.-Hil. & Moq.) J.F.B.Pastore	Subshrub	CR	NY 634708
Caamembeca oxyphylla (DC.) J.F.B.Pastore	Subshrub	CR	OUPR 1246
Polygala cuspidata DC.	Subshrub	CR	BHCB 8271
Polygala glochidiata Kunth	Subshrub	CR	BHCB 122262
Polygala paniculata L.	Subshrub	CR	OUPR 13595
Polygala timoutou Aubl.	Subshrub	CR	OUPR 13601
Polygonaceae			
Fallopia convolvulus (L.) A.Löve	Subshrub	CR	OUPR 7765
Homalocladium platycladum (F. Muell.) L.H.Bailey	Subshrub	CR	OUPR 9076
Persicaria capitata (Buch.-Ham. ex D.Don) H.Gros	Subshrub	AA	OUPR 19311
Persicaria hydropiperoides (Michx.) Small	Subshrub	CR	NY 863998
Persicaria meisneriana (Cham. & Schltdl.) M.Gómez	Subshrub	CR	BHCB 8288
Portulacaceae			
Portulaca mucronata Link	Subshrub	AA	NY 3413603
Primulaceae			
Ardisia solanacea (Poir.) Roxb.	Subshrub	CR	OUPR 20271
Cybianthus itacolomyensis M.Lisboa & Badini	Shrub	F	OUPR 5856
Lysimachia arvensis (L.) U.Manns & Anderb.	Subshrub	AA	OUPR 6376
Myrsine congesta (Sw.) Pipoly EN**	Shrub	F	RB 39981
Myrsine coriacea (Sw.) R.Br. ex Roem. & Schult.	Shrub	F	OUPR 1315
Myrsine emarginella Miq.	Shrub	CR	RB 380828
Myrsine gardneriana A.DC.	Tree	F	OUPR 19154

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Myrsine lancifolia Mart.	Shrub	CR	OUPR 12581
Myrsine lineata (Mez) Imkhan.	Tree	F	RB 277645
Myrsine squarrosa (Mez) M.F.Freitas & Kin.-Gouv.	Shrub	CR	OUPR 1484
Myrsine umbellata Mart.	Shrub	CR,F	OUPR 1399
Myrsine villosissima Mart. EN**	Shrub	F	OUPR 5555
Proteaceae			
Euplassa rufa (Loes.) Sleumer	Shrub	F	OUPR 25098
Panopsis multiflora (Schott ex Spreng.) Ducke EN*	Tree	F	OUPR 1249
Roupala montana Aubl. var. montana	Shrub	CR	OUPR 1314
Roupala montana var. *brasiliensis* (Klotzsch) K.S.Edwards	Tree	F	OUPR 13429
Ranunculaceae			
Clematis dioica L.	Liana	F	OUPR 1355
Rhamnaceae			
Crumenaria sp	Subshrub	CR	BHCB 41485
Reissekia smilacina (Sm.) Steud.	Liana	F	OUPR 20272
Rosaceae			
Eriobotrya japonica (Thunb.) Lindl.	Tree	AA	OUPR 19309
Pirus communis L.	Tree	AA	OUPR 7479
Prunus sellowii Koehne	Tree	F	NY 922649
Rubus brasiliensis Mart.	Shrub	F	OUPR 1373
Rubus erythrocladus Mart. ex Hook.f.	Shrub	F	OUPR 1873
Rubus rosifolius Sm.	Subshrub	AA	OUPR 12585
Rubiaceae			
Amaioua guianensis Aubl.	Tree	F	BHCB 41498
Augusta longifolia (Spreng.) Rehder	Shrub	CR	OUPR 17752
Bathysa australis (A.St.-Hil.) K.Schum.	Shrub	F	OUPR 19289
Bathysa cuspidata (A.St.-Hil.) Hook.f. ex K.Schum.	Shrub	F	OUPR 1356
Borreria capitata (Ruiz & Pav.) DC.	Subshrub	CR	OUPR 12796
Borreria verticillata (L.) G.Mey.	Subshrub	AA,CR	OUPR 1250
Coccocypselum aureum (Spreng.) Cham. & Schltdl.	Subshrub	CR,F	OUPR 6792
Coccocypselum erythrocephalum Cham. & Schltdl.	Subshrub	CR,F	OUPR 6792
Coccocypselum lanceolatum (Ruiz & Pav.) Pers.	Subshrub	F	RB 526340
Coccocypselum pedunculare Cham. & Schltdl.	Subshrub	CR	RB 324887
Coccocypselum sp	Subshrub	F	OUPR 23254
Cordiera concolor (Cham.) Kuntze	Tree	F	IAC 52606
Cordiera elliptica (Cham.) Kuntze	Tree	F	OUPR 19173
Coussarea nodosa (Benth.) Müll.Arg.	Shrub	F	NY 396634
Coussarea sp	Tree	F	OUPR 19174
Declieuxia fruticosa (Will. ex Roem. & Schult.) Kuntze	Subshrub	CR	RB 190168
Diodia saponariifolia (Cham. & Schltdl.) K.Schum.	Subshrub	CR	NY397199
Emmeorhiza umbellata (Spreng.) K.Schum.	Subshrub	CR	BHCB 41487
Galianthe brasiliensis (Spreng.) E.L.Cabral & Bacigalupo	Subshrub	CR	NY 950040
Galianthe verbenoides (Cham. & Schltdl.) Griseb.	Subshrub	CR	OUPR 19379
Galium hirtum Lam.	Subshrub	CR	OUPR 19385
Galium hypocarpium (L.) Endl. ex Griseb. subsp. *hypocarpium*	Subshrub	CR	OUPR 1483
Galium noxium (A.St.-Hil.) Dempster	Subshrub	CR	SP 140254
Galium sellowianum (Cham.) Walp.	Subshrub	CR	OUPR 19383
Galium sp	Subshrub	CR	RB 318466
Hillia parasitica Jacq.	Shrub	CR	RB 318466
Ixora brevifolia Benth.	Shrub	CR	RB 110957
Manettia luteorubra (Vell.) Benth.	Liana	CR	OUPR 1285
Palicourea marcgravii A.St.-Hil.	Shrub	F	OUPR 1402
Palicourea tetraphylla Cham. & Schltdl.	Subshrub	F	OUPR 1262
Posoqueria acutifolia Mart.	Shrub	CR	OUPR 19384
Posoqueria latifolia (Rudge) Schult.	Shrub	CR,F	OUPR 19382
Psychotria hispidula Standl. ex Steyerm.	Shrub	CR	OUPR 19380
Psychotria paludosa Müll.Arg. EN**	Subshrub	F	OUPR 7395
Psychotria purpurascens Müll.Arg.	Shrub	F	BHCB 41496

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Psychotria sessilis Vell.	Shrub	CR	RB 324889
Psychotria stachyoides Benth.	Subshrub	F	UEFS 35882
Psychotria subtriflora Müll.Arg.	Shrub	F	RB 324891
Psychotria vellosiana Benth.	Tree	F	OUPR 19175
Psychotria sp1	Shrub	F	OUPR 6765
Psychotria sp2	Shrub	F	RB 40224
Psyllocarpus laricoides Mart. & Zucc.	Subshrub	CR	OUPR 7285
Remijia ferruginea (A.St.-Hil.) DC.	Shrub	CR	OUPR 8333
Rudgea nodosa (Cham.) Benth.	Shrub	F	RB 324880
Rudgea sessilis (Vell.) Müll.Arg. EN**	Shrub	CR	OUPR 1317
Rutaceae			
Dictyoloma vandellianum A.Juss.	Tree	F	OUPR 1763
Zanthoxylum rhoifolium Lam.	Tree	F	OUPR 2274
Salicaceae			
Abatia americana (Gardner) Eichler	Shrub	CR,F	OUPR 13612
Casearia arborea (Rich.) Urb.	Shrub	CR	OUPR 9078
Casearia lasiophylla Eichler	Tree	CR	OUPR 15311
Casearia selloana Eichler	Shrub	CR,F	OUPR 6424
Casearia sylvestris Sw.	Tree	F	OUPR 8049
Xylosma serrata (Sw.) Krug & Urb.	Shrub	CR	OUPR 5155
Sapindaceae			
Cupania emarginata Cambess.	Tree	F	OUPR 1368
Cupania vernalis Cambess.	Tree	F	OUPR 1487
Matayba marginata Radlk.	Shrub	CR	OUPR 8344
Paullinia carpopoda Cambess.	Liana	CR,F	OUPR 1438
Serjania elegans Cambess.	Liana	F	OUPR 1254
Serjania fuscifolia Radlk.	Liana	F	OUPR 1388
Serjania sp	Liana	F	OUPR 4804
Sapotaceae			
Sarcaulus brasiliensis (A.DC.) Eyma	Tree	CR,F	OUPR 22774
Scrophulariaceae			
Buddleja brasiliensis J.Jacq.	Subshrub	CR	OUPR 19312
Siparunaceae			
Siparuna arianeae V.Pereira	Shrub	F	OUPR 18915
Siparuna brasiliensis (Spreng.) A.DC.	Shrub	F	OUPR 1316
Siparuna guianensis Aubl.	Shrub	F	OUPR 20285
Smilacaceae			
Smilax elastica Griseb.	Liana	F	RB 409613
Smilax oblongifolia Pohl ex Griseb.	Liana	CR	OUPR 16579
Smilax quinquenervia Vell.	Liana	CR	OUPR 5776
Smilax sp1	Liana	CR	OUPR 9444
Smilax sp2	Liana	F	OUPR 9974
Solanaceae			
Acnistus arborescens (L.) Schltdl.	Tree	F	OUPR 7376
Aureliana brasiliana (Hunz.) Barboza & Hunz.	Tree	F	OUPR 25103
Aureliana fasciculata (Vell.) Sendtn.	Shrub	F	OUPR 20213
Aureliana picta (Mart.) I.M.C.Rodrigues & Stehmann.	Shrub	CR,F	OUPR 23971
Aureliana velutina Sendtn.	Shrub	CR,F	OUPR 25141
Browallia americana L.	Subshrub	AA	OUPR 24835
Brunfelsia brasiliensis (Spreng.) L.B.Sm. & Downs	Shrub	CR,F	OUPR 25118
Brunfelsia hydrangeiformis (Pohl) Benth.	Shrub	F	OUPR 25687
Brunfelsia uniflora (Pohl) D.Don	Shrub	F	OUPR 10426
Capsicum schottianum Sendtn.	Shrub	CR,F	OUPR 24870
Capsicum sp	Shrub	CR,F	OUPR 25113
Cestrum bracteatum Link & Otto	Shrub	CR,F	OUPR 25138
Cestrum schlechtendahlii G.Don	Shrub	F	OUPR 25300
Dyssochroma viridiflora (Sims) Miers	Shrub	CR,F	OUPR 25114
Nicotiana alata Link & Otto	Subshrub	AA	MBM 233808

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Nicotiana bonariensis Lehm.	Subshrub	CR	OUPR 25602
Nicotiana langsdorfii Weinm.	Subshrub	AA	OUPR 24834
Physalis pubescens L.	Subshrub	AA	OUPR 24898
Schwenckia americana Rooyen ex L.	Subshrub	CR,F	OUPR 24945
Solanum americanum Mill.	Subshrub	CR,F	OUPR 25140
Solanum argenteum Dunal	Shrub	CR,F	OUPR 25088
Solanum campaniforme Roem. & Schult.	Shrub	F	OUPR 25219
Solanum cernuum Vell.	Shrub	F	OUPR 25194
Solanum cinnamomeum Sendtn.	Tree	F	OUPR 24854
Solanum cladotrichum Dunal	Tree	CR,F	OUPR 20085
Solanum granulosoleprosum Dunal	Tree	CR,F	OUPR 25086
Solanum inodorum Vell.	Liana	F	OUPR 25212
Solanum intermedium Sendtn.	Shrub	CR,F	OUPR 25083
Solanum leptostachys Dunal	Shrub	CR,F	OUPR 24796
Solanum luridifuscescens Bitter	Shrub	F	OUPR 25589
Solanum lycocarpum A.St.-Hil.	Shrub	CR,F	OUPR 25192
Solanum palinacanthum Dunal	Shrub	AA	OUPR 22863
Solanum paniculatum L.	Shrub	AA,CR	OUPR 24897
Solanum pseudocapsicum L.	Subshrub	F	OUPR 25590
Solanum pseudoquina A.St.-Hil.	Tree	F	OUPR 24860
Solanum refractifolium Schltdl.	Subshrub	CR	OUPR 25076
Solanum reptans Bunbury	Subshrub	CR	OUPR 24869
Solanum sisymbriifolium Lam.	Subshrub	F	OUPR 4809
Solanum subumbellatum Vell.	Shrub	CR	OUPR 24941
Solanum swartzianum Roem. & Schult.	Tree	CR,F	OUPR 25139
Solanum vaillantii Dunal	Shrub	CR,F	OUPR 24861
Solanum velleum Thunb.	Tree	CR,F	OUPR 25122
Solanum viarum Dunal	Shrub	F	OUPR 25100
Solanum viscosissimum Sendtn. EN**	Liana	F	OUPR 4806
Styracaceae			
Styrax aureus Mart.	Tree	CR	OUPR 9080
Styrax ferrugineus Nees & Mart.	Tree	CR	UB 157565
Styrax latifolius Pohl	Tree	CR	OUPR 19464
Styrax maninul B.Walln. VU*	Shrub	CR,F	OUPR 24626
Symplocaceae			
Symplocos arbutifolia Casar	Shrub	CR,F	OUPR 24966
Symplocos celastrinea Mart.	Shrub	F	OUPR 19308
Symplocos falcata Brand	Tree	F	OUPR 21357
Theaceae			
Camellia sinensis (L.) Kuntze.	Shrub	CR,F	OUPR 1364
Laplacea fructicosa (Schrad.) Kobuski	Tree	F	OUPR 12210
Thymelaeaceae			
Daphnopsis brasiliensis Mart.	Shrub	F	OUPR 13423
Trigoniaceae			
Trigonia paniculata Warm.	Liana	F	OUPR 7269
Urticaceae			
Boehmeria sp	Subshrub	F	OUPR 19292
Cecropia hololeuca Miq.	Tree	F	OUPR 29493
Cecropia pachystachya Trécul	Tree	F	OUPR 7426
Urera baccifera (L.) Gaudich. ex Wedd.	Shrub	F	OUPR 1362
Velloziaceae			
Barbacenia damaziana Beauverd	Herb	CR	OUPR 3627
Barbacenia flava Mart. ex Schult. & Schult.f.	Herb	CR	OUPR 19297
Barbacenia tomentosa Mart.	Herb	CR	OUPR 3264
Vellozia compacta Mart. ex Schult. & Schult.f.	Subshrub	CR	OUPR 1382
Vellozia graminea Pohl	Herb	CR	OUPR 3626
Vellozia hirsuta Goethart & Henrard	Herb	CR	RB 190101
Vellozia sp	Subshrub	CR	OUPR 1383

Table 1. Continued...

Family / Species	Habit	Habitat	Voucher
Verbenaceae			
Lantana camara L.	Shrub	CR	OUPR 6803
Lantana fucata Lindl.	Subshrub	CR	OUPR 19376
Lantana trifolia L.	Subshrub	CR	OUPR 23691
Lantana sp	Shrub	F	OUPR 20293
Lippia hermannioides Cham.	Shrub	CR	OUPR 15534
Stachytarpheta cayennensis (Rich.) Vahl	Subshrub	CR	OUPR 12580
Stachytarpheta commutata Schauer	Subshrub	CR	OUPR 7398
Stachytarpheta glabra Cham.	Subshrub	CR	OUPR 20180
Stachytarpheta jamaicensis (L.) Vahl	Subshrub	CR	OUPR 20295
Stachytarpheta mexiae Moldenke	Shrub	CR	RB 98723
Verbena litoralis Kunth	Subshrub	CR	NY 956732
Violaceae			
Anchietea exaltata Eichler	Liana	F	OUPR 19300
Anchietea pyrifolia (Mart.) G.Don	Liana	F	OUPR 8248
Viola subdimidiata A.St.-Hil.	Subshrub	F	OUPR 23469
Vitaceae			
Cissus verticillata (L.) Nicolson & C.E. Jarvis	Liana	CR	OUPR 9092
Vochysiaceae			
Qualea lundii (Warm.) Warm.	Tree	F	OUPR 21356
Vochysia dasyantha Warm.	Tree	F	OUPR 4818
Vochysia emarginata (Vahl) Poir.	Shrub	CR	OUPR 7793
Vochysia rectiflora Warm.	Tree	F	OUPR 19343
Vochysia thyrsoidea Pohl	Tree	F	OUPR 1261
Vochysia tucanorum Mart.	Tree	CR,F	OUPR 19176
Winteraceae			
Drimys angustifolia Miers	Shrub	F	MBM 264545
Drimys brasiliensis Miers	Shrub	F	OUPR 6798
Drimys winteri J.R.Forst. & G.Forst.	Tree	F	OUPR 9093
Xyridaceae			
Xyris asperula Mart.	Herb	CR	OUPR 1485
Xyris augusto-coburgii Szyszyl. ex Beck	Herb	CR	OUPR 14470
Xyris graminosa Pohl ex Mart.	Herb	CR	OUPR 15954
Xyris hymenachne Mart.	Herb	CR	RB 277721
Xyris macrocephala Vahl	Herb	CR	OUPR 20230
Xyris peregrina Malme	Herb	CR	OUPR 20459
Xyris plantaginea Mart.	Herb	CR	OUPR 20468
Xyris schizachne Mart.	Herb	CR	RB 275396
Xyris teres Alb.Nilsson	Herb	CR	OUPR 17290
Xyris trachyphylla Mart.	Herb	CR	OUPR 4453
Xyris sp1	Herb	CR	OUPR 16393
Xyris sp2	Herb	CR	OUPR 20461
Zingiberaceae			
Hedychium coronarium J. König	Herb	CR	OUPR 22339

The families with higher species richness were Asteraceae (323), Fabaceae (154), Melastomataceae (131), Poaceae (84), Orchidaceae (62), Myrtaceae (58) and Bignoniaceae (57), represent more than 75% of the total sampled species (Figure 2). The richness of these families was also reported in other *campos rupestres* (Harley & Simmons 1986, Giulietti et al. 1987, 1997, Pirani et al. 1994, Conceição & Giulietti 2002, Conceição & Pirani 2005, Jacobi & Carmo 2012, Messias et al. 2012, Forzza et al. 2013). Fabaceae, Myrtaceae, Melastomataceae and Asteraceae are also species-rich families in Espinhaço Range forests (Kamino et al. 2008). However, comparing with the data presented by these authors, Bignoniaceae and Solanaceae presented higher tree species richness in ISP than in other Espinhaço Range forests. Almeida et al. (2014) studying Asteraceae of ISP, described three new species and considered this Park as the richest Brazilian one for Asteraceae. Many families (29) are represented by only one species, what has been reported in other surveys along the Espinhaço Range (Giulietti et al. 1987, 1997, Pirani et al.1994, 2003).

The *campos rupestres* represent 51% of the total area, followed by the forest with 40% and anthropogenic disturbed areas with 9% (Figure 1). The vegetation seems to be closely associated with geology, geomorphology, depth and moisture of the soil, as well as the degree of anthropogenic impact, resulting in a variety of different physiognomies (Figure 3), similarly to other montane areas (Benites et al. 2007; Schaefer et al. 2016). The *campos*

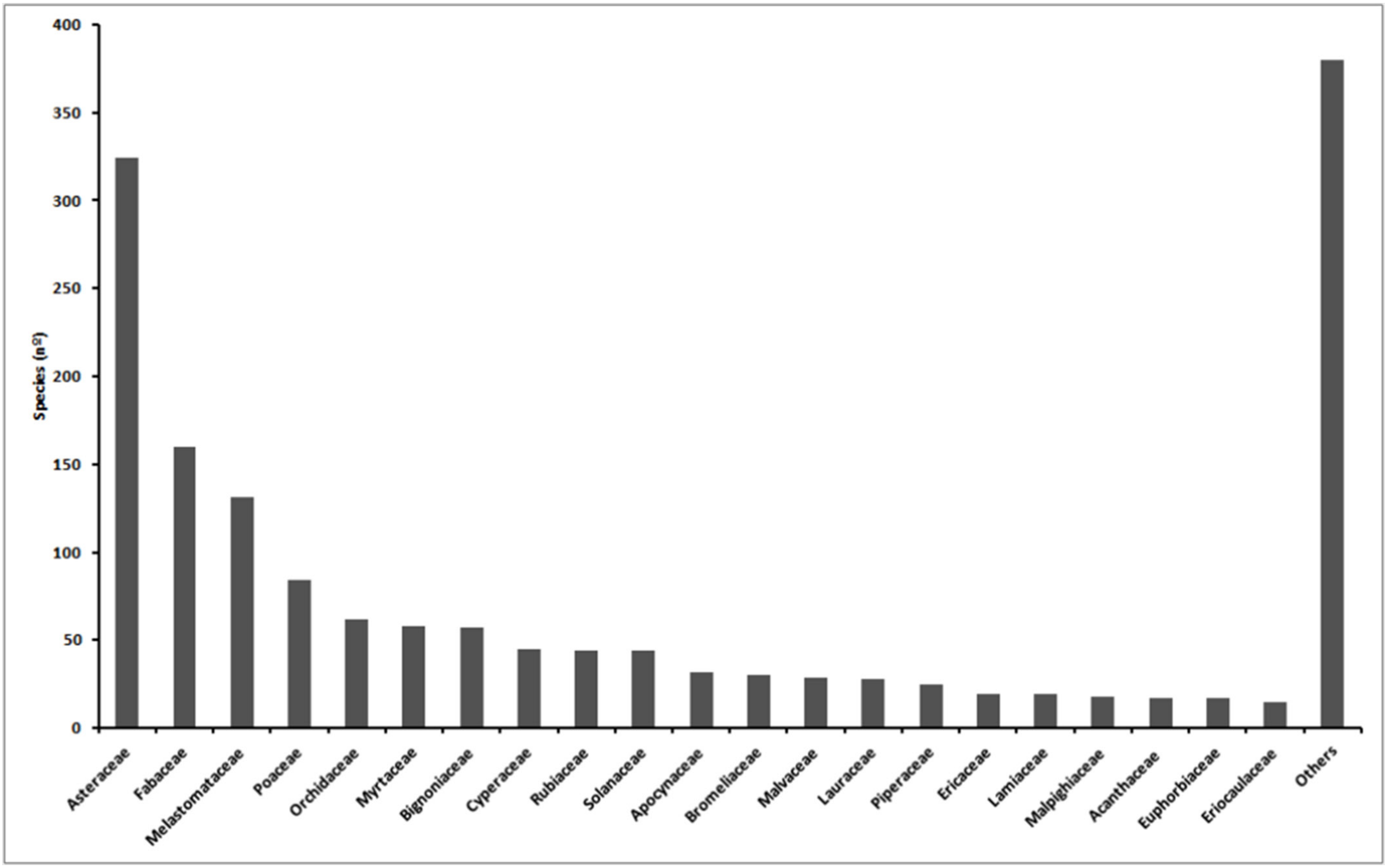

Figure 2. Fanerogamic species-richest families in the Itacolomi State Park, Ouro Preto and Mariana, Minas Gerais, Brazil.

rupestres occur in the highest parts of the ISP, always higher than 1000m, and the forests mostly in the lower ones, from 700m to 1300m, usually along streams and slopes with deeper soil.

The forests at Itacolomi State Park have the same pattern of the forests along the Espinhaço Range (Harley 1995), varying considerably both in composition and structure in response to the geo-climatic conditions, often resulting in a large heterogeneity of patterns, even in a relatively small area. Thus, as described by Kamino et al. (2008), a riparian forest on the valleys may be evergreen, while the adjacent forest on the slope is semideciduous. In fact, Pedreira & Sousa (2011) described a swampy evergreen forest patch in Itacolomi State Park, surrounded by semideciduous forests, with well-drained soils. In spite of the existence of this small scale variation in Itacolomi State Park forest fragments, they were only characterized in this work by the general feature as “montane forest”.

Forest ecosystems with striking seasonality of rainfall or low soil water retention often drive high proportions of deciduous and semideciduous species in their communities (Reich & Borchet 1984, Lenza & Klink 2006). However, the fog events, common in the studied area, may favor the coexistence of species with different phenological strategies (Valim et al. 2013). Thus, care must be taken when generalizing the terminology “seasonal semideciduous forests” (Scolforo et al. 2008) for the forest of this park and similar surrounding areas.

The greatest species richness occurs in *campos rupestres* (almost 70%), with 61% of species occurring exclusively in this phytophysiognomy. The *campo rupestre* is a very old mountaintop ecosystem, being a museum of ancient lineages as well as a cradle of continuing diversification of endemic lineages (Silveira et al. 2016). The astonishing species richness found in the *campos rupestres* is favoured by the high environmental diversity, wide latitudinal and altitudinal range, isolation and influence of different vegetation domains (Giulietti et al. 1997, Jacobi et al. 2007, Silveira et al. 2016).

The forests house about 36% of the species and 7% of the species occur in both physiognomies (forest and *campos rupestres*) like *Eremanthus erythropappus*, *Schinus terebinthifolius*, *Clethra scabra*, *Vochysia tucanorum*, among others. Some species occur just in anthropogenic disturbed areas (1.8%) (Table 1, Figure 4). Some species found in the forests like *Hedyosmum brasiliense*, *Laplacea fructicosa*, *Drimys brasiliensis*, *Weinmamania pauliniiflora* and *Ocotea percoriacea* are typical of Brazilian montane forests (CRIA 2016).

The subshrubs represented 30% of the species, followed by shrubs (25%), herbs (19%), trees (13%), lianas (12%) and few palms and epiphytes (Figure 5). Shrubby species occur in both forest and *campos rupestres*, explaining the higher richness of this habit. In addition, the southern portion of the Espinhaço Range is more strongly influenced by the Atlantic Forest than the central/northern parts, leading to a higher proportion of phanerophytic species in *campos rupestres* (Echternacht et al. 2011). Most of the herbs occur in *campos rupestres*, mainly from Poaceae and Orchidaceae families. The epiphytes seem to be sub sampled since the montane forests of the region are reported as having a greater richness of this life-form (Ferreira, 2011).

Some parts of the forest are mono-dominated by *Eremanthus erythropappus*, known locally as *candeal*, similar to the other areas of Espinhaço Range (Oliveira-Filho & Fluminhan-Filho 1999, Kamino et al. 2008). Most of these areas are in primary succession after fire occurrence.

The environmental impact in the ISP region dates back to the early seventeenth century, with gold mining. Later, with the decline of gold

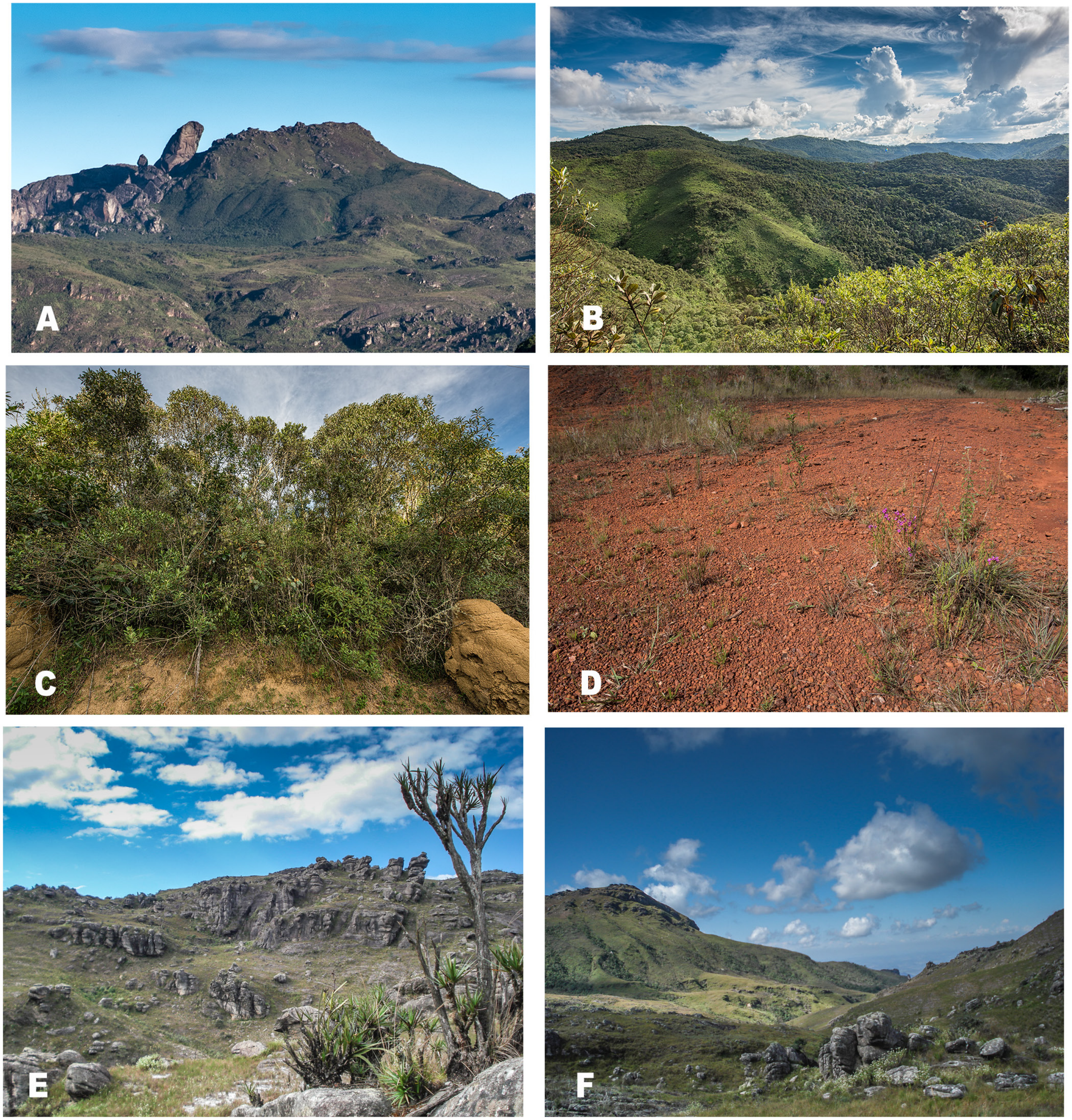

Figure 3. Phytophysiognomies of Itacolomi State Park, Ouro Preto and Mariana, Minas Gerais, Brazil. A. Itacolomi Peak surrounded by *campos rupestres*; B. Partial view of the Itacolomi State Park (ISP), with *campos rupestres* and forest patches; C. Monodominant forest made up of "Candeia" (*Eremanthus erythropappus*); D. Partial view of a ferruginous *campo rupestre*; E-F. Quartzitic *campos rupestres*.

exploration, some farms in this area focused on agriculture, especially tea (*Cammelia sinensis*), *Eucalyptus* plantation for charcoal production and cattle. The tea was introduced in this area in the eighteenth and nineteenth centuries, playing a key role in the local economy. This activity lasted until the 50s, when the plantations were then abandoned (Fujaco et al. 2010). Thus, individuals of this species are still found in the vicinity of the old farm "Fazenda São José do Manso", where there was the largest planted area. Other significant anthropogenic impact occurred in the second half of XX century, with the aluminum (bauxite) mining. In addition, more recently urban sprawl has become one of the main threats alongside the Park, compromising the integrity of the surrounding environment (Fujaco et al. 2010).

Most of the exotic phanerogamic species occur in these anthropogenic disturbed areas. The most aggressive ones are *Eucalyptus grandis*,

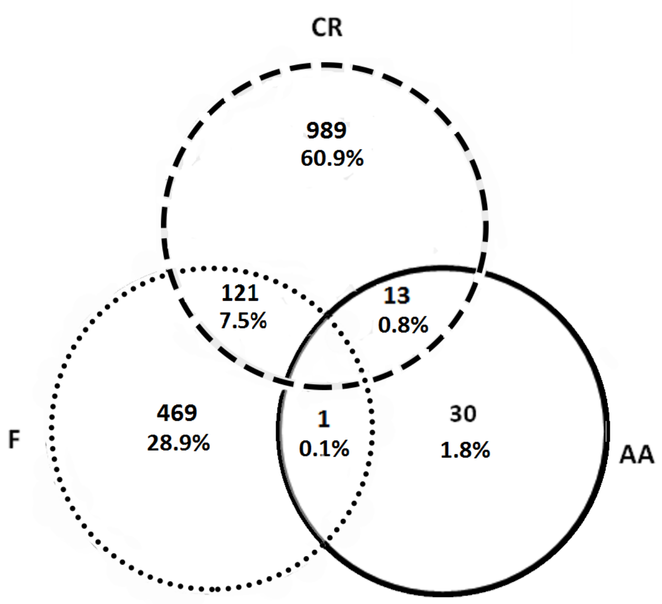

Figure 4. Venn diagram showing the number of shared and exclusive phanerogamic species in the different habitats at Itacolomi State Park, Ouro Preto and Mariana, Minas Gerais, Brazil. AA= Antropogenic disturbed areas, CR= Campos rupestres, F=Montane forests.

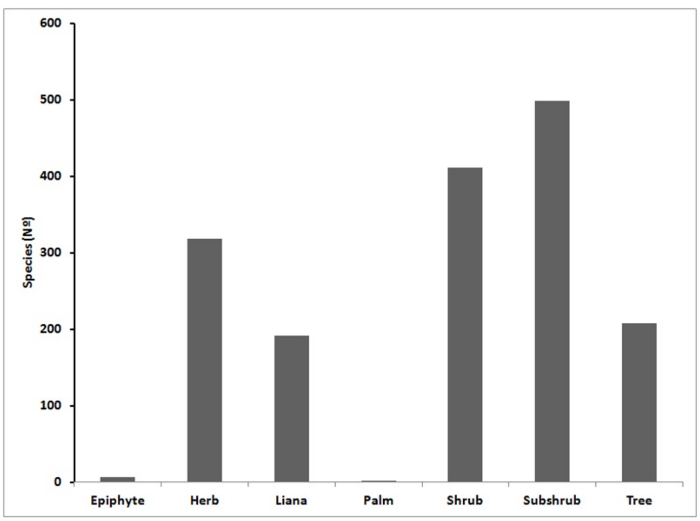

Figure 5. Growth habit of the recorded phanerogamic species of the Itacolomi State Park, Ouro Preto and Mariana, Minas Gerais, Brazil.

Hedychium coronarium and *Melinis minutiflora*. They have shown fast grown and high dispersal ability, easily increasing their population and spreading to other surrounding areas. Other exotic species observed didn't seem to present much competitiveness like *Cammelia sinensis* (tea), some ornamental plants like *Hydrangea macrophylla*, *Impatiens balsamina* and *Rhododendron indicum*. Probably, because most of the cultivated species are not able to grow in these metaliferous and nutrient-poor soils (Fernandes et al. 2016; Schaefer et al. 2016). Little is still known about biological invasion on mountaintop complexes where the threat of invasive species is very substantial (Wilson et al. 2016). Thus, more research is necessary about this subject.

Although *Araucaria brasiliensis* was considered initially as a naturally occurring species in this region (Mello-Barreto 1942, Hueck 1953), the observation of aerial photographs showed that the older individuals of this species were aligned, suggesting they were grown in this area. Further studies are still needed to clarify if this species is native to the Itacolomi State Park area.

A total of 79 species are threatened with extinction, being 57 species listed on the red list of Minas Gerais State (COPAM 1997), 40 figure on the Brazilian Red List (MMA 2014) and two others from IUCN Red List (2015). Most of them are described as vulnerable or endangered. However, some species are listed as critically endangered (CR) like *Stevia hilarii*, *Valeriana organensis*, *Ocotea felix* and *Habenaria itaculumia* on The Brazilian Red List (Table 1). Even though *Trichogonia eupatorioides* (=*Trichogonia martii*) and *Trembleya calycina* are described as probably extinct in the red list of the state of Minas Gerais (COPAM 1997), there are recent collections of these species in the herbarium databases (CRIA 2016). Other surveys along the *Espinhaço* Range have shown the same pattern, with many threatened species (Giulietti & Pirani 1988, Pirani et al. 1994, Echternacht et al. 2011). The *campos rupestres* show a high concentration of endemic species, some of them occur in small population (Giulietti & Pirani 1988, Echternacht et al. 2011). Thus, they are very vulnerable to extinction.

Some species are endemic to the Itacolomi State Park: *Habenaria itaculumia* and *Heterocondylus itacolumiensis*, in the *campos rupestres* and *Cybianthus itacolomyensis* in the forest. Other species are known as endemic to Espinhaço Range, in *campos rupestres*, like: *Chamaecrista dentata*, *C. hedysaroides, C. rotundata*, *Cryptanthus schwackeanus*, *Mimosa aurivillus* var. *aurivillus, Aspilia caudata* and *Richterago hatschbachii* (Flora do Brasil 2016, CRIA 2016).

The high flora diversity of this Park, harboring more than 12% of the Angiosperm species known for Minas Gerais State (BFG 2015), as well as the many endemic and endangered species, shows the value of this conservation unit as one of the most important in the state of Minas Gerais and Brazil. The knowledge of phanerogamic flora of ISP can help the Management Plan of this Unit of Conservation, contribute to future works related in this area and also improve the understanding of the Minas Gerais and Brazilian flora.

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