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Thematic Review

Freshwater mussels from South America: state of the art of Unionida, specially Rhipidodontini

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Abstract: Unionida is the most diverse clade of freshwater bivalves. Among the groups occurring in South America, one with the highest number of species is Rhipidodontini (Hyriidae, Unionida, Paleoheterodonta, Bivalvia). However several issues remains on taxonomy and systematic of this group, leading to problems on species identification, description, as also as a limiting factor to other type of studies (*e.g.,* ecology, conservation,…). In this paper is presented a synthesis of available knowledge about *Diplodon* Spix in Wagner, 1827 and *Rhipidodonta* Mörch, 1853 in South America, as a first step in order to a better understating of Rhipidodontini. The evaluation of different authors exposes the little agreement between them that resulted in a sort of divergent taxonomical opinions. Some comments on ecology, conservation and habitat preferences were made. This work can also encourage future research on taxonomy, systematic, ecology and conservation of freshwater mussels in South America.

Keywords: *Diplodon, Rhipidodonta, Hyriidae, Bivalvia, Freshwater bivalve.*

Bivalves de água doce da América do Sul: estado da arte de Unionida, especialmente Rhipidodontini

Resumo: Unionida é o clado mais diverso de bivalves de água doce. Entre os grupos que ocorrem na América do Sul, um dos com maior número de espécies é Rhipidodontini (Hyriidae, Unionida, Paleoheterodonta, Bivalvia). Porém, diversas questões taxonômicas e sistemáticas ainda incidem sob este grupo, levando a problemas de identificação de espécies, descrição, entre outros, como também tem atuado como limitador de outros tipos de estudos (*e.g.,* ecologia, conservação,..). Neste trabalho é apresentada uma revisão do conhecimento acerca dos gêneros *Diplodon* Spix in Wagner, 1827 e *Rhipidodonta* Mörch, 1853 na América do Sul como um primeiro passo para a melhor compreensão de Rhipidodontini. Avaliando-se diferentes autores, se torna claro a pouca concordância entre eles, resultando em opiniões taxonômicas divergentes. São feitos também alguns comentários sobre ecologia, conservação e preferências ambientais. Este trabalho também deve encorajar futuros trabalhos sobre a taxonomia, sistemática, ecologia e conservação de bivalves de água na América do Sul.

Palavras-chave: *Diplodon, Rhipidodonta, Hyriidae, Bivalvia, Bivalve de água doce.*

Introduction

Mollusca is the second phylum in number of species, with estimates on the number of living species ranging up to 200,000 (Ponder & Lindberg 2008). Bivalvia constitutes one of the most representative groups of this phylum with more than 8,000 species living worldwide. Although most are marine species, about 1,300 live in freshwater in all continents, except Antarctica (Ruppert et al. 2005, Bogan 2008). Several lineages colonized freshwater ecosystems, especially the order Unionida (Paleoheterodonta), as well as some species of Arcida, Mytilida (Pteriomorpha), Venerida, Myida, and Anomalodesmata (Heterodonta), suggesting that bivalve invasions of freshwater environments occurred numerous times (Haag 2012). All living species of Unionida and Sphaeriidae (Heterodonta: Venerida) live exclusively in freshwater (Mansur 2007, Giribet 2008). Freshwater mussels (Unionida) are one of the most endangered animal group due to continuous degradation of their ecosystems (Strayer et al. 2004, Amaral et al. 2008, Pereira et al. 2014). More recently Asian freshwater bivalves like *Limnoperna fortunei* (Dunker, 1857) (Mytilidae) and *Corbicula* spp. (Cyrenidae) that have been introduced to several distant countries and continents including South America caused severe ecological and economical loss (Darrigran & Damborenea 2006, Mansur et al. 2012, Boltovskoy & Correa 2015, Xu et al. 2015).

Bivalves inhabit the bottom substrate, and are important members of freshwater communities performing important ecosystem services (Vaughn 2017). Except for the environmental differences between marine and freshwater organisms, the freshwater species are generally similar to marine ones; although they are less colorful, camouflaged among sand grains and stones (Mansur 2007). Freshwater bivalves can be found in almost all available microhabitats, occupying different niches: burrowers of soft sediments (majority of species), burrowers of compacted sediments (*e.g., Mycetopoda* d'Orbigny, 1835 and *Mycetopodella* Marshall, 1928), wedgers of soft rocks and laterite (*e.g., Bartlettia* Adams, 1867), attached by byssus (*e.g., Byssanodonta* d'Orbigny, 1846 and *Eupera* Bourguignat, 1854) and species cemented to hard substrate (*e.g., Acostaea* d'Orbigny, 1851 and *Etheria* Lamarck, 1807) (Mansur 2007, 2012, Haag 2012, Pereira et al. 2014).

Our main goal was to summarize the knowledge about Unionida (Bivalvia, Paleoheterodonta) in South America, especially regarding Rhipidodontini (Hyriidae).

1. Systematics of Unionida

The taxonomic instability of bivalves results, in part, from the large amount of available names (Bieler & Mikkelsen 2006), which change according to the different characters emphasized by each author. The systematic of Bivalvia was addressed by several authors (Thiele 1934, Newell 1965, Cox et al. 1969, Franc 1960, Schneider 2001, Giribet 2008) and the position of Paleoheterodonta remains quite stable. Paleoheterodonta is usually presented as a "halfway" between Pteriomorphia and Heterodonta (Schneider 2001, Giribet 2008). Bieler et al. (2014) presented a slightly different arrangement where Paleoheterodonta is sister group to Archiheterodonta, and this is sister to a clade composed by Anomalodesmata + Imparidentia, that embraces most bivalves previously in Heterodonta. Unionida is included in Paleoheterodonta and it is a group of usually large-sized mussels that have a peculiar life cycle with a parasitic stage and presents the most successful radiation in freshwaters by bivalves (Graf & Cummings 2006, Haag 2012).

The inner relationships of Unionida are not as clear as the position of Paleoheterodonta. Simpson (1914) proposed only two families in Unionoida (= Unionida): Unionidae and Mutelidae. Most bivalves that are currently recognized as Unionidae, Margaritiferidae and Hyriidae (Figure 1A-C) compose the first group; and the current representatives of Mycetopodidae and Iridinidae are part of the second group (Figure 1D-E). Therefore, Unionidae *sensu* Simpson (1914) encompasses the species with larvae of glochidium type, whereas Mutelidae *sensu* Simpson (1914), those with the lasidium type. Ortmann (1921) recognized three families within the superfamily Naiades: Margaritanidae (= Margaritiferidae), Unionidae and Mutelidae (= Hyriidae + Mycetopodidae + Iridinidae). Based on morphological characteristics of their soft parts, not only on their shells, Ortmann (1911, 1921) noted similarities between hyriids and mutelids, and removed hyriids from Unionidae, establishing them as a subfamily of Mutelidae.

Thiele (1934) classified all freshwater mussels as Unionacea, recognizing four families: Margaritanidae, Unionidae, Mutelidae, and Aetheriidae (Figure 1). That is the first classification scheme that posed an exclusive family for freshwater oysters (Aetheriidae = Etheriidae) (Figure 1F). Thiele (1934) used the same subfamilies of Mutelidae proposed by Ortmann (1921).

These first arrangements of Unionida follow biogeographical patterns: Boreal species grouped in Margaritiferidae (or Margaritanidae) and Unionidae; and Austral species in Mutelidae (= Hyriidae + Mycetopodidae) (Ortmann 1921, Thiele 1934). However, the separation is not clear cut, for example, Thiele (1934) left *Virgus* Simpson, 1900 and other austral insular species of Oceania in Unionidae.

Modell (1942) proposed four families (Mutelidae, Elliptionidae, Margaritiferidae, and Unionidae) with many subfamilies. Elliptionidae comprises the majority of species traditionally allocated in Unionidae. Modell (1942) also suggested a relationship between this group and Mutelidae, wherein all lasidium bearers were grouped together. Modell (1942) as Simpson (1914), placed hyriids within Unionidae. According to Modell (1942), Mutelidae is a basal group that originates all other mussels.

Parodiz & Bonetto (1963) proposed an arrangement in two superfamilies based mainly on the larval type, which was widely accepted by subsequent authors: Unionacea (Unionidae + Margaritiferidae + Hyriidae) with glochidium larva; and Mutelacea (Mutelidae + Mycetopodidae) with lasidium larva. Etheriidae is not included in the classification, as its larval stage was unknown at that time (Bogan & Roe 2008). The larval stage of *Acostaea rivolii* (Deshayes, 1827) (Etheriidae) was later identified as a lasidium (Arteaga-Sogamoso 1994, Bonetto, 1997). Kabat (1997) revised the names used in Unionida claiming that Etherioidea and Iridinidae should be used instead of Muteloidea and Mutelidae. The recent works accepted these suggestions (*e.g.,* Graf & Cummings 2007).

In the beginning of the 21st century, there has been a reevaluation of systematic relationships based on phylogenetic methodologies, including molecular data in some of these analyses. The monophyly of Etheriidae was questioned by Bogan & Hoeh (2000) who considered *Acostea* and *Etheria* (traditionally included in Etheriidae) within Mycetopodidae and, *Pseudomulleria* Anthony, 1907, an Indian freshwater oyster, inside Unionidae. Bogan & Hoeh (2000) proposed multiple origins to cementation among freshwater bivalves, arguing the occurrence of the same process in the non-related Cyrenidae, *Posostrea anomioides* Bogan & Bouchet, 1998. The analysis of Hoeh et al. (2001) is similar to Bogan & Hoeh (2000), suggesting that Hyriidae is a sister group to the remaining Unionida and considering Unionacea (*sensu* Parodiz & Bonetto, 1963) as a paraphyletic group. That scheme implies that the glochidium and the larvae incubation in the inner demibranch (endogenous) are plesiomorphic characteristics of Unionida.

Graf (2000) analyzed the relationships inside Etherioidea, with an emphasis on Hyriidae; suggesting that Hyriidae, Iridinidae and Etheriidae are monophyletic. Unionidae, once more had its monophyly questioned and *Grandidieria* Bourguignat, 1885, traditionally placed in Unionidae is considered a sister group of Etherioidea. Graf & Cummins (2006) suggested that Paleoheterodonta is monophyletic and divided Unionoida in two clades: Unionoidea (Unionidae + Margaratiferidae) and Etherioidea (Hyriidae + Etheriidae + Mycetopodidae + Iridinidae). Unlike other authors (*e.g.,* Bogan & Hoeh, 2000; Hoeh et al. 2001), Graf & Cummings (2006) suggested the monophyly of Unionidae and Etheriidae, condition latter also supported by Whelan et al. (2011). In that scheme, Unionoidea is the

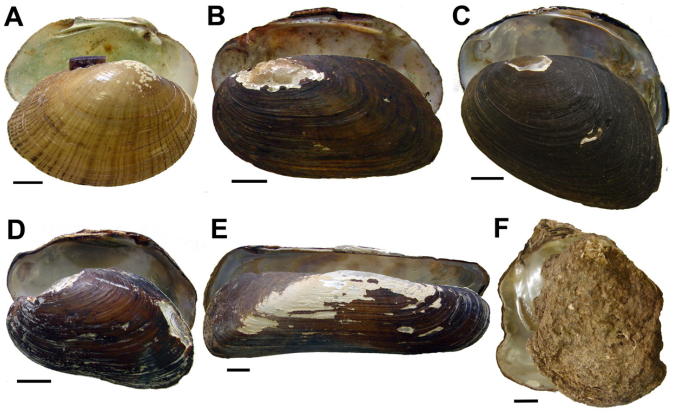

Figure 1. Members of Unionida. A – Unionidae, *Lampsilis fasciola* Rafinesque, 1820, MNRJ (Museu Nacional do Rio de Janeiro) 7468; B – Margaritiferidae, *Margaritifera* sp., MNRJ 32868; C - *Echyridella menziesii* (Dieffenbach, 1843), MNRJ 4374; D – Iridinidae, *Aspatharia pfeifferiana* (Bernardi, 1860), MNRJ HSL 6328; E – Mycetopodidae, *Mycetopoda soleniformis* d'Orbigny, 1835, MNRJ 3841; F – Etheriidae, *Etheria elliptica* Lamarck, 1807, MNRJ HSL 6111. Scale bar = 1 cm.

basal group of Unionida, while Hyriidae is the basal group of Etherioidea. In certain aspects, this arrangement is a return to early schemes of systematic organization (*e.g.,* Ortmann, 1921) with boreal species separated from the austral species. The exceptions are some austral species of Unionidae (Haas 1969, Graf & Cummings 2007).

Nevesskaja (2009) recognized two groups of living freshwater mussels: Unionoidea (Unionidae + Margaratiferidae) and Etherioidea (Mutelidae + Etheriidae), but did not list the genera within each group, so it is impossible to know precisely where Hyriidae and Mycetopodidae stand. As Mutelidae traditionally embrace the species of Iridinidae, Mycetopodidae and Hyriidae (Ortmann 1921, Thiele 1934, Graf 2000); we can suppose that Nevesskaja (2009) considered all the species of these families as belonging to Mutelidae.

A major point of disagreement between different authors is the position of Hyriidae, sometimes grouped with glochidium-bearing species, and sometimes grouped with the other Gondwanic species (Mycetopodidae and Etheriidae) (Bogan & Hoeh 2000, Graf 2000, Graf & Cummings 2006, Bogan 2008). Bieler et al. (2010) adopted an intermediate solution to the problem, dividing the living species of Unionida in three superfamilies (Table 1). In this classification, Hyrioidea (represented only by Hyriidae) occupy an intermediate position between the two other groups, Etherioidea and Unionoidea, reflecting the conflicting data from other authors concerning the position of Hyriidae (Bogan & Hoeh 2000, Hoeh et al. 2001, Graf & Cummins 2006, 2007). Graf et al. (2015) presented Hyriidae as sister to all other freshwater mussel families, in a position quite different from the previously one (Graf & Cummings 2006), however similar (regarding to Hyriidae position) to topology presented by Bogan & Hoeh (2000) and Hoeh et al. (2009).

2. Geographical distribution of South American mussels

Unionida occurs worldwide in different kinds of freshwater habitats except in Antarctica (Graf & Cummings 2006, Bogan 2008). Current estimates recognize approximately 900 species distributed among six families: Hyriidae, Mycetopodidae, Unionidae, Iridinidae and Etheriidae (Graf & Cummings 2006, 2007, Bieler et al. 2010).

Etheriidae is Gondwanic and comprises four species, occurring in Africa, *Etheria elliptica* Lamarck, 1807; India, *Pseudomulleria dalyi* (Smith, 1898) and South America, *Acostea rivolii* and *Bartlettia stefanensis* (Moricand, 1856) (Haas 1969, Graf & Cummings 2006, 2007). The monophyly of this family is disputed and there is no agreement as highlighted by different opinions available (Parodiz & Bonetto 1963, Bogan & Hoeh 2000, Bonetto 1997, Simone 2006, Hoeh et al 2009, Mansur et al. 2012).

Mycetopodidae is Neotropical distributed all over South America east of the Andes and west of Central America all the way to Mexico (Graf & Cummings 2006, Bogan 2008). There are about 30 valid species of Mycetopodidae in 12 (Simone 2006) or 11 genera (Graf & Cummings 2007). Bonetto (1997) also includes *Acostaea* in Mycetopodidae, whereas other authors (Parodiz & Bonetto 1963, Graf 2000) believe that *Leila* Gray, 1840, usually placed in Mycetopodidae, belongs to Iridinidae. The origin of Mycetopodidae is in the Cretaceous (Cox et al. 1969).

There are around 80 species of Hyriidae, occurring throughout Oceania and South America, with only two or three species west of the Andes (Bonetto et al. 1986; Parada & Peredo, 2002; Graf & Cummings, 2007; Bogan, 2008). Hyriidae is monophyletic (Graf et al. 2015) and usually divided in two groups (sub-families), the Hyriinae, which comprises South American species, except by *Hyridella* Swainson, 1840 and some related Australian species; and Velesunioninae, that comprises most Australian species (Graf & Cummings, 2006, 2007; Bieler et al. 2010; Graf et al. 2015). Among Hyriidae seven genera are recognized to South America: *Prisodon* Schumacher, 1817; *Paxyodon* Schumacher, 1817; *Callonaia* Simpson, 1900; *Castalia* Lamarck, 1819; *Castaliella* Simpson, 1900; *Diplodon* Spix in Wagner, 1827 and *Rhipidodonta* Mörch, 1893 (Simone, 2006); and, nine genera to Australia: *Hyridella*; *Cucumerunio* Iredale, 1934; *Echyridella* McMichael & Hiscock, 1958; *Virgus*; *Velesunio* Iredale, 1934; *Alathyria* Iredale, 1934; *Lortilella* Iredale, 1934; *Microdontia* Tapparone Canefri, 1883; *Westralunio* Iredale, 1934 (Graf & Cummings, 2007). It is noteworthy that not all authors agree with the valid status of each of these genera. Simone (2006) considered *Triplodon* Spix in Wagner, 1827 as synonym, unlike Mansur & Pimpão (2008) who described a new species of this genus. The oldest Hyriidae record is from Triassic of New Zealand (Campbell et al. 2003) and from Jurassic of South America (Perea et al. 2009). Molecular clock indicated a Gondwanan origin of Hyriidae (Graf et al. 2015; Santos-Neto et al. 2016).

The Figures 2 to 4, based on the data available in Graf & Cummings (2007), allow a more detailed evaluation of the distribution of the South American species of Hyriidae, Etheriidae and Mycetopodidae. Graf & Cummings (2007) divides the Neotropical region in six areas: Mesoamerica (including Cuba), Transandean (including the basins of rivers Magdalena and Maracaibo), Amazonas-Orinoco (including the Guyanas), Atlantic coastal streams (including the São Francisco River basin), Paraná-Paraguay and Patagonia. The two main families (Hyriidae and Mycetopodidae) are widespread in the region; Mycetopodidae occurs in all regions and Hyriidae in five of them (Figure 2). Etheriidae are limited to three regions. In the regions of Atlantic coastal streams, Paraná-Paraguay and Patagonia prevails species of Hyriidae; in the others regions, there are

Table 1. Relationships of Paleoheterodonta, following Bieler et al. (2010), modified to include only the living taxa of Paleoheterodonta.

Paleoheterodonta	Trigoniida	Trigonioidea	Trigoniidae
	Unionida	Etherioidea	Etheriidae
			Iridinidae
			Mycetopodidae
		Hyrioidea	Hyriidae
		Unionoidea	Unionidae
			Margaritiferidae

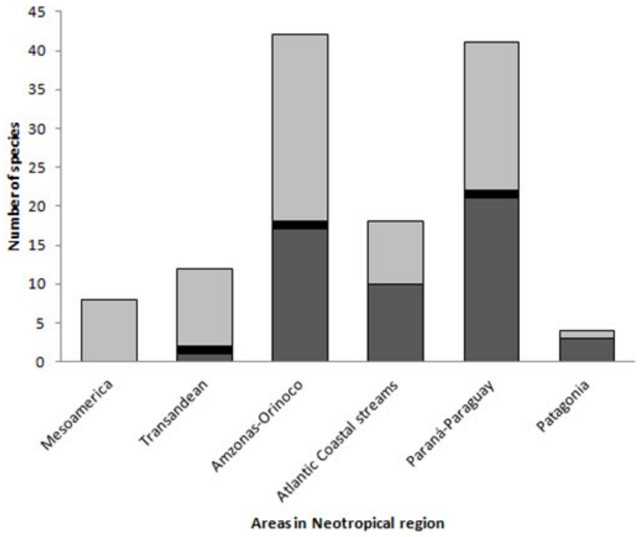

Figure 2. Number of species of Hyriidae and Mycetopodidae in different areas of Neotropical region. Based on the original data by Graf & Cummings (2007). Key: Dark gray – Hyriidae; Black – Etheriidae and Light gray – Mycetopodidae.

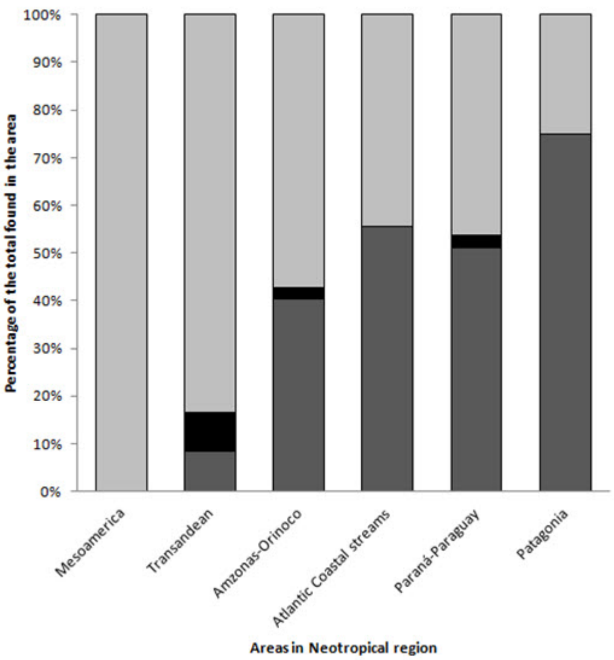

Figure 3. Representativeness of Hyriidae, Mycetopodidae and Etheriidae in the Neotropical region based on the original data by Graf & Cummings (2007). Key: Dark gray – Hyriidae; Black – Etheriidae and Light gray – Mycetopodidae.

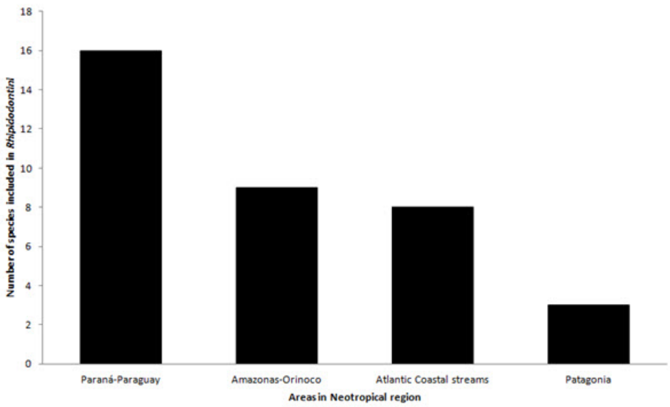

Figure 4. Number of species of Rhipidodontini (*Rhipidodonta* + *Diplodon*) in Neotropical region. The regions that are not presented don´t have any Rhipidodontini species (i.e. Mesoamerica and Transandean). Based on the original data by Graf & Cummings (2007).

more Mycetopodidae species (Figure 2). The areas with the greatest total number of species are Amazonas-Orinoco (42 spp.) and Paraná-Paraguay (41 spp.), the first with the greatest number of Mycetopodidae (24 spp.) and the second with the greatest number of Hyriidae (21 spp.). Pereira et al. (2014) indicated the same areas as those of high diversity and pointed all region east of Andes (except by Northeast Brazil) as phylogenetically structured by Hyriidae and Mycetopodidae.

Comparing the representativeness of each family in different areas, we notice a trend towards a decrease in Mycetopodidae, along with an increase in Hyriidae (Figure 3), from North to South. The extremes are Mesoamerica, without Hyriidae species, and Patagonia where Hyriidae represents more than 70% of Unionida fauna. However, Patagonia is a poor region in mussels richness and this high percentage amounts to only three species. Etheriidae is always a small fraction of total species.

Figure 4 presents the diversity of Rhipidodontini (*Rhipidodonta* + *Diplodon*). There are no representatives of Rhipidodontini in the Mesoamerica and Transandean regions. The unique species of Hyriidae pointed out by Graf & Cummings (2007) to Transandean region is *Castalia multisulcata* Hupé, 1857 that belongs to Castaliini. Rhipidodontini represents most species of Hyriidae in Neotropics resulting in similarities between figure 4 and 2. The difference between the Amazonas-Orinoco region and Atlantic coastal streams region, lower in Figure 2 than in Figure 4, are due to the occurrence of exclusive Amazonian Hyriidae genera like *Callonaia*, *Castaliella* and *Prisodon*. Graf & Cummings (2007) included these genera in other tribes (Hyriini or Castaliini).

3. Taxonomy and systematics of Rhipidodontini

The most important studies concerning the systematics of Rhipidodontini (Figure 5) are shown in Table 2 and Appendix I (see Supplementary material): Simpson (1914), Ortmann (1921), Morretes (1949), Parodiz (1968), Haas (1969), Simone (2006) and Graf & Cummings (2007). The following discussion focused on specific epithet, regardless of the genus or subgenus the author employed. For example, Haas (1969) used *Diplodon* (*Rhipidodonta*) *rhombea* Spix in Wagner, 1827, while Graf & Cummings (2007) used *Rhipidodonta rhombea*; regardless of genus designation, we regarded that both authors considered "*rhombeus*" as a valid species. *Diplodon* is traditionally divided in subgenera, mainly based on features of the shell, and the two most used are *Rhipidodonta* and *Diplodon s.s.*.

Simpson (1914) and Thiele (1934) recognized three subgenera: *Diplodon s.s.*, *Rhipidodonta* (= *Cyclomya* Simpson, 1900) and *Bulloideus* Simpson, 1900 (see Table 2 and Appendix I). Ortmann (1921) and Morretes (1949) recognized two subgenera, *Diplodon* and *Rhipidodonta*. The species placed in *Bulloideus* were usually included in *Rhipidodonta* by the authors that did not use the first subgenera. Haas (1969) recognized four subgenera, adding *Schleschiella* Modell, 1950 to those mentioned previously. Ortmann (1921) was the first to notice differences in glochidium, however, he did not assign those variations to subgenera. The characteristics of glochidium were linked to subgenera by Bonetto (1961, 1965) and Parodiz & Bonetto (1963). Simone (2006) raised *Rhipidodonta* to genus status, an idea followed later by Graf & Cummings (2006, 2007), considering *Diplodon* to encompass the species with parasite glochidium, and *Rhipidodonta* those with non-parasite glochidium. These two genera (*Diplodon* and *Rhipidodonta*) were included in tribe Rhipidodontini (Graf & Cummings 2007), with all species previously arranged in subgenera by other authors (Table 2, Appendix I). Simone (2006) do not presented an explanation to support his decision, and probably for this reason some authors like Pereira et al (2014) don´t followed his suggestions. It is clear that glochidium is a good diagnostic feature in Hyriidae (Parodiz & Boneto 1963, Mansur 1999, Mansur & Silva 1999, Pimpão et al. 2012), but is also necessary to find other characteristics on the morphology of adult specimens as well as on molecular aspects to substantiate this division. It is also important to mention that in South America there are many under-sampled areas and undescribed glochidia of Rhipidodontini. We propose to adopted parsimoniously the suggestion of Simone (2006), using *Rhipidodonta* only to the species that the glochidium is described and without any doubts about identification.

Glochidium is known for 31 nominal species of *Diplodon* and for 17 nominal species of *Rhipidodonta*. In cases where the glochidium type is missing, the genus assignment is based only on adult shell morphology. That is the case of some species recognized in several works as *Diplodon rhombeus* (= *Rhipidodonta rhombea* after Simone 2006) (Figure 5G). There were also some cases of "change" of the glochidium type, after detailed revision; for example, glochidium type in *Diplodon suavidicus* (d´Orbigny, 1835) (Figure 5E) was firstly assigned as non-parasite (Simone 2006) and

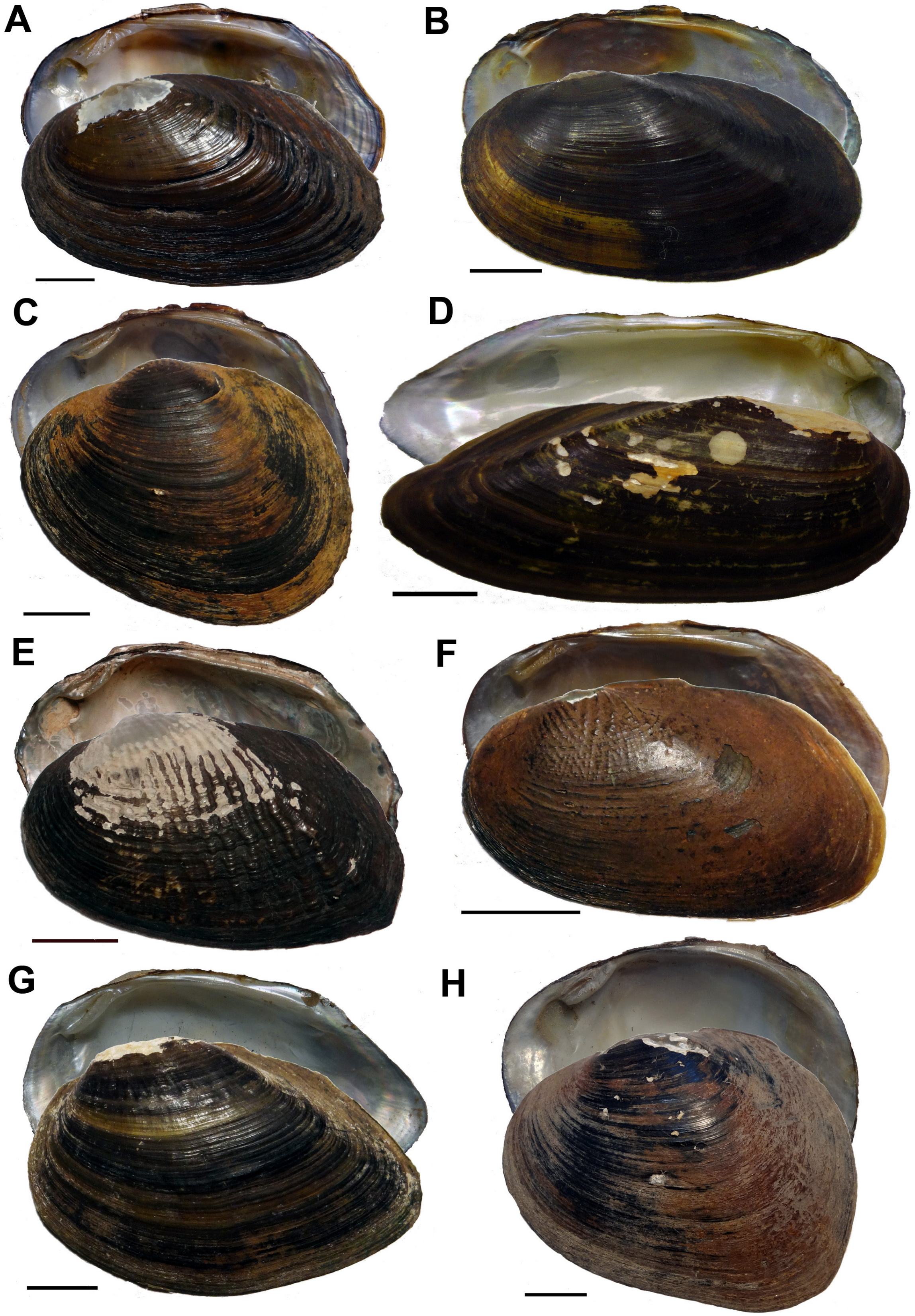

Figure 5. Members of Rhipidodontini. A- *Rhipidodonta charruana* (d´Orbigny, 1835), MHNM (Museo Nacional de Historia Natural – Montivideo) 1210; B – *Diplodon chilensis* (Gray, 1828), LMD (Aquazoo Löbbecke Museum Düsseldorf) w/n (Lisikhe collection); C – *Diplodon fontainianus* (d´Orbigny, 1835), MHNM 6285; D – *Diplodon parallelopipedon* (Lea, 1834), LMD w/n (Lisikhe collection); E – *Rhipidodonta hylaea* (d´Orbigny, 1835), ZMB (Museum für Naturkunde) w/n (Paetel collection); F – *Diplodon multistriatus* (Lea, 1831), MHNM 3966; G – *Diplodon parodizi* Bonetto, 1962, MHNM – Soc. Taguató 670; H – *Diplodon rhombeus* Spix in Wagner, 1827, SMF (Senckenberg Forschungsinstitut und Naturmuseum)11248. Scale bar = 1cm.

later as parasite (Pimpão et al. 2012). Pimpão et al. (2012) successfully used the glochidium to differentiate Amazonian species of Hyriidae, thus proving that glochidium can be powerful in species delimitation.

One concept that appeared in several older works was that of a "group of species" (Simpson 1914, Ortmann 1921, Haas 1930, 1931a,b), abandoned in more recent works (Haas 1969, Simone 2006, Graf & Cummings 2007). A "group of species" includes several species that share some characteristics, usually the shell structure, and the most prominent species (according to the author) lend their name to the group. However, the characteristics of the different groups are not very clear, which led to some overlapping. A group of species does not hold taxonomic status and is used only as a way to organize the species by morphological similarity. All the authors that used "group of species" presented six groups; however, the choice of main species and the composition of each group was different (Table 3). The groups of *Diplodon granosus* (Bruguière, 1792) and *D. delodontus* (Lamarck, 1819) (= *D. lacteolus*) were mentioned by the three authors above (Simpson 1914, Ortmann 1921, Haas 1930, 1931a,b); however, the species included in each group were different according to each author. For example, Simpson (1914) allocated 27 species in the group of *D. granosus*, whereas Ortmann (1921) and Haas (1930, 1931a,b), proposed only one species with three subspecies. The concept of "group of species" was similar to the concept of "super-species" used by Parodiz (1968, 1973), but also not applied in subsequent works (*e.g.*, Simone 2006, Graf & Cummings 2006).

Simpson (1914) presented the highest number of species (80), with seven new species and one new subspecies. Some species names were used only by Simpson (1914) like *Diplodon ampullaceus* (Lea, 1866), *Diplodon aplatus* (Reeve, 1865), *Diplodon effulgens* (Lea, 1856), *Diplodon modestus* (Küster, 1856), *Diplodon quadrans* (Lea, 1859) and *Diplodon rufofuscus* (Lea, 1859). Simpson (1914) also included some Australian species in *Diplodon* (subgenera: *Hyridella*, *Cucumaria* and *Laevirostris*), which were removed by subsequent authors (*e.g.,* Ortmann 1921, Haas 1969). Simpson (1914) synonymized some species names and described the species based mainly on their shell, using few information about the soft parts. *Diplodon dunkerianus* (Lea, 1856) and *D. martensi* (Ihering, 1893) were only presented by Simpson (1914) and Parodiz (1968), among the authors of Table 2. However, there are more recent references to *D. martensi* (Mansur 1970, Vaz et al. 1987, Mansur 1999, Pfeifer & Pitoni 2003) and *D. dunkerianus* (Amaral et al. 2008).

Ortmann (1921) presented a similar number of species compared to later works (Haas 1969, Graf & Cummings 2007); however, the species listed were different. Ortmann based on the Unionida soft-parts morphology, proposed the first phylogenetic relationships of the Unionida families and subfamilies, as well as one of the first schemes to classify the South American naiades, which is still partially accepted. He also brought a wealth of information on the shell comparative morphology and glochidia of Hyriidae, a tool needed to better understand the high degree of polymorphism at the specific level.

The tendency to describe several new species decreases after Ortmann (1921). However, Haas (1916, 1929, 1938, 1966) still described four new species in separate works. This change in point of view is probably due to a better comprehension of shell polymorphism. The study of the naiads starts to acquire its current shape in Haas (1969), when he upgraded and expanded the synonymic lists started by Simpson (1914) and Ortmann (1921). Several species validated by Haas (1969) remained with the same status in subsequent works. For example, out of the 27 species considered valid by Graf & Cummings (2007), 23 received the status of species or subspecies by Haas (1969).

There are 149 species names associated to *Diplodon* and *Rhipidodonta*, excluding fossil species and *nomen nudum* (Parodiz 1968, Bonetto & Tassara 1987), 109 (73.15%) were used in species or subspecies rank by at least one of the authors of Table 2 and Appendix I (Simpson 1914, Ortmann 1921, Morretes 1949, Parodiz 1968, Haas 1969, Simone 2006, Graf & Cummings 2007), leaving out 40 species names (26.85%) that were not used by any of them. Despite the high number of species names used (*i.e.* 109), most of them were used only by one or two authors, 28.86% and 18.80%, respectively (Figure 6, Appendix I).

Table 2. Number of species of Rhipidodontini considered valid by different authors. Noteworthy that for our purposes, a global scope work is almost equal to a South America scope because the recent fauna Rhipidodontini occurs only in South America. * - The author presents some Australian forms as *Diplodon* subgenera (*Hyridella* Swainson, 1840; *Cucumaria* Conrad, 1853; *Laevirostris* Simpson, 1900), that are not included in this table, in order to allow an equal comparison with other authors. ** - Considered doubtful by the author.

Reference	Genera or subgenera	Number of valid species	Geographic scope
Simpson (1914)	*Diplodon* (*Diplodon*); *Diplodon* (*Cyclomya*); *Diplodon* (*Bulloideus*)	80 species + 4 subspecies*	Global
Ortmann (1921)	*Diplodon* (*Diplodon*); *Diplodon* (*Cyclomya*)	28 species	South America
Morretes (1949)	*Diplodon* (*Diplodon*); *Diplodon* (*Rhipidodonta*)	36 species + 2 subspecies	Brazil
Haas (1969)	*Diplodon* (*Diplodon*); *Diplodon* (*Rhipidodonta*); *Diplodon* (*Schleschiella*); *Diplodon* (*Bulloideus*)	22 species + 13 subspecies	Global
Parodiz (1968)	*Diplodon* (*Diplodon*); *Diplodon* (*Rhipidodonta*)	32 species + 6 subspecies	South America
Simone (2006)	*Diplodon*; *Rhipidodonta*	22 (14 spp. in *Diplodon*, 8 spp. in *Rhipidodonta*) + 2 spp. doubtful**	Brazil and nearby areas
Graf & Cummings (2007)	*Rhipidodonta*; *Diplodon*	27 (19 spp. in *Diplodon*, 8 spp. in *Rhipidodonta*)	Global

Table 3. Groups of species of *Diplodon* Spix in Wagner, 1827 presented by different authors.

Author	Groups proposed
Simpson (1914)	*Diplodon lacteolus, D. granosus, D. burroughianus, D. pazi, D. parallelipipedon, D. quadrans*
Ortmann (1921)	*D. hylaeus, D. granosus, D. chilensis, D. charruanus, D. lacteolus, D. ellipticus*
Haas (1930, 1931a,b)	*D. chilensis, D. charruanus, D. hylaeus, D. parallelipipedon, D. delodontus, D. granosus*

Only four species (2.68%) are recognized as valid by all authors, namely: *Diplodon charruanus* (d´Orbigny, 1835), *Diplodon granosus*, *Diplodon hylaeus* and *Diplodon parallelopipedon* (Lea, 1834) (Figure 5A,D,E). Besides these, all the authors cited the "set" *ellipticus* Spix in Wagner, 1827 + *wagnerianus* Simpson, 1900 that refers to the same biological species, though Haas (1969) used *ellipticus* as a subspecies of *Diplodon granosus*. This agreement between the authors could suggest that these species are easily recognizable. This is (probably) true to *Diplodon hylaeus and Diplodon parallelopipedon*, which have peculiar shell characteristics. The others (*charruanus/granosus/ellipticus*) were among the first species described to South America and have priority but were involved in taxonomical problems.

Six names: *D. besckeanus* (Dunker, 1848), *D. burroughianus* (Lea, 1834), *D. suavidicus* (Lea, 1856), *Diplodon gratus* (Lea, 1860), *Diplodon patagonicus* (d'Orbigny, 1835) and *Diplodon fontainianus* (d´Orbigny, 1835) were used by six authors as species or subspecies, representing 4.03% of all names. *Diplodon lacteolus* is clearly a synonymy of *Diplodon delodontus* as already noted by Lea (1836) in the original description. The "set" *lacteolus* + *delodontus* was mentioned by all the authors except for Morretes (1949).

This evaluation illustrates the divergence among authors, a result of different characteristics employed for species differentiation. Even in the species recognized by most authors, there are considerable differences in descriptions and synonymic lists.

Despite the differences in the geographic scope of Simone (2006) and Graf & Cummings (2007), they agree completely about the eight species included in *Rhipidodonta*. The few differences between these authors comprised species included in *Diplodon s.s*. Five species in Graf & Cummings (2007) were not included in Simone (2006), namely: *Diplodon chilensis* (Gray, 1828); *Diplodon flucki* Morrison, 1943; *Diplodon guaporensis* Bonetto & Tassara, 1987; *Diplodon losadae* Haas, 1966 and *Diplodon solidulus* (Philippi, 1869). Despite the differences, Simone (2006) and Graf & Cummings (2007) were the most similar works presented in Appendix I.

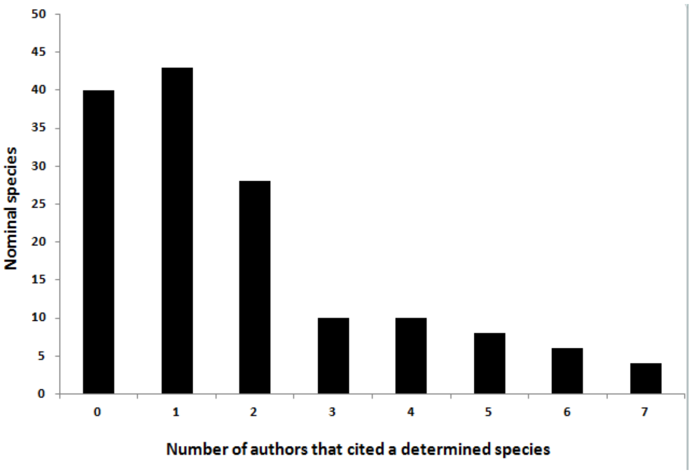

Figure 6. Number of times that a determined nominal species is cited by the evaluated authors (Table 3). See the Appendix I to base data.

4. Identification of Rhipidodontini

Shell characteristics were considered since the first researches on Rhipidodontini (*e.g.*, Simpson 1914). The study of anatomical features was introduced by Ortmann (1921) and recently some molecular studies were done (*e.g.*, Graf & Cummings 2006, Santos-Neto 2016). However, none of these approaches have been exhausted.

The shell outline was used for a long time to differentiate the subgenera of *Diplodon*. For example, the rounded species were asserted to *Rhipidodonta* and the more elongated species to *Diplodon s.s.* This division based only on shell proven to be artificial and not agree with other aspects. Nowadays, the shell structures were still in use and were not described in detail for most species (Miyahira et al. 2013). The most important characteristics of the shell are the umbo position, umbonal sculpture and hinge details. The ultra-structure of the shell was poorly studied in Hyriidae (Bieler et al. 2014) and must to be improved.

Only after the studies of Ortmann (1921) and Parodiz & Bonetto (1963) that provide the basic information on the glochidium type (with or without hooks) it was possible to link larva to subgenera and later to genera. *Diplodon* has glochidium with hooks and an obligate stage as parasite of fishes (Mansur et al. 2012, Pimpão et al. 2012). The life cycle of *D. martensi* was described by Mansur (1999) and remains as the unique species to have the cycle described in Brazil. The glochidium of *Rhipidodonta* is hookless and the life cycle still poorly known. The glochidium develop at the marsupium and the mussel release a juvenile (Wächtler et al. 2001, Mansur & Silva 1999, Mansur et al. 2012, Pimpão et al. 2012). Unfortunately the glochidium type was not described to all species of Rhipidodontini (Table 4). According to Pimpão et al. (2012) the glochidium was useful not only to separate the genera but also to identify species based on morphometrics and a detailed description. In order to avoid unnecessary taxonomical fluctuations, it is recommended that species remains at *Diplodon* until information on glochidia were obtained.

Some details of internal morphology was described only to the following species, *Diplodon charruanus*, *D. pilsbry* Marshall, 1928, *D. besckeanus*, *D. multistriatus* (Lea, 1831), *D. rhombeus fontainianus* and *D. rotundus gratus* (Hebling & Penteado 1974, Alvarenga & Ricci 1981, Mansur & Anflor 1982, Ricci et at 1988, Avelar & Cunha 2009). Meyer et al. (2012, 2014) described the reproductive system of *D. expansus* (Küster, 1856) and *D. ellipticus* from a histological perspective. Considering the reduced knowledge about Rhipidodontini morphology it is difficult to elect good diagnostic features in soft parts. Until now some differences between species were found with success on the outline and morphology of branchiae, position of marsupium, labial palps and stomach.

Table 4. Nominal species of Rhipidodontini with glochidium type described in the literature. (Lea 1869, Ortmann 1921, Bonetto 1954, 1960, 1961, Bonetto & Ezcurra-de-Drago 1965, Alvarenga & Ricci 1979, Bonetto et al. 1986, Mansur & Campos-Velho 1990, Ricci et. al. 1990, Martinez-Escabassiere & Royero 1995, Mansur & Silva 1999, Pimpão et al. 2012). We used *Diplodon* for all species to avoid new combinations without further studies

Glochidium with hooks (*Diplodon s.s.*)	**Hookless glochidium (*Rhipidodonta*)**
D. atratus, D. berthae, D. besckeanus, D. decipiens, D. delodontus, D. ellipticus var. santanus, D. expansus, D. granosus, D. guaranianus, D. firmus, D. fontaineanus, D. frenzeli, D. hartwrighti, D. imitator, D. martensi, D. mogymirim, D. multistriatus, D. obsolescens, D. parallelopipedon, D. parodizi, D. paulista, D. peculiares, D. piceus, D. rhuacoicus, D. rotundus, D. simillimus, D. solidulus, D. suavidicus, D. trivialis, D. vicarius, D. wagnerianus e D. yaguaronis	*D. assuncionis, D. bulloides, D. burroughianus, D. charruanus, D. garbei, D. hasemani, D. hildae, D. hylaeus, D. iheringi, D. koseritzi, D. paranensis, D. peraeformis, D. rhuacoicus, D. subcuadratus, D. suppositus e D. variabilis*

Only four species of *Diplodon* were used in molecular approaches: *D. deceptus* Simpson, 1914, *D. suavidicus*, *D. demeraraensis* (Lea, 1859) and *D. chilensis* (Hoeh & Bogan 2000, Graf et al. 2015, Santos-Neto et al. 2016). All these molecular studies deals with wider questions, inner relations of Rhipidodontini were never investigated in detail.

Analyzing all these information, it is clear that several gaps remain in available knowledge about Rhipidodontini. More data are necessary to provide better species identification and consequently the genera differentiation. Nowadays, the unique secure attribute to separate *Diplodon* and *Rhipidodonta* is the glochidium type. All these data will be necessary to discover the internal relationships of Rhipidodontini.

5. Ecological preferences of Rhipidodontini

The species can occur in lentic or lotic habitats, from small streams to big rivers and lakes; however, they are not common in strong currents. They tolerate a wide granulometric range, but prefer fine sediments, usually rich in organic matter. Some species can occur between or below pebbles, or even in rock cracks. They usually start appearing at a depth of 30 cm. They are sometimes found associated to roots of aquatic plants (Avelar & Cunha 2009). They prefer the final sections of the river, due to the highest amount of nutrients, and are rare or absent in headwaters (Pereira et al. 2011, Miyahira et al. 2017). They can share space with other native freshwater mussels, as is commonly seen in southern Brazil, northern Argentina and Uruguay, apparently without harm to the species (Mansur & Pereira 2006, Pereira et al. 2011, 2014, Mansur et al. 2012). The species of Rhipidodontini are sensitive to environmental changes and suffer with domestic and industrial sewage discharge, the main cause of decrease in populations of freshwater mussels (Strayer et al. 2004; Miyahira et al. 2012; Mansur et al. 2012; Pereira et al. 2014).

6. Threats and the conservation of Unionida

Freshwater mussels are among the most endangered species (Strayer et al. 2004, Bogan 2008, Santos et al. 2009, Miyahira et al. 2012). The main cause of this threat is the habitat change. At the basin of the Paraná River, one of the richest areas in Brazil regarding freshwater mussels, there is a series of 70 reservoirs, turning a long-term survival difficult for mussels (Santos et al. 2009, Mansur et al. 2012, Pereira et al. 2014). The change of lotic to lentic habitats caused by the dams profoundly changes the physical and chemical conditions, affecting not only the mussels but also the host fishes (in the case of parasitic life cycle of *Diplodon*). This situation creates relictual populations of mussels, alive but fated to die, because they cannot reproduce or disperse without fish host. The long life achieved by these mussels allows them to have a long survival, but without any hope (Philipp & Abele, 2009). This situation created an extinction debt that already stated to be paid in North American fauna with several recent extinctions (Haag 2012).

Freshwater mussels were exploited by the mother of pearl button industry for a long time, mainly in North America (Neves 1999, Haag 2012), but also in South America (Beasley 2001, Matos 2007, Clavijo 2017). That exploitation caused the decline of several mussel populations in the USA, especially in the first half of the twenty century (Strayer et al. 2004, Haag 2012). Although usually considered a threat from the olden days, populations of *Paxyodon*, *Triplodon* and *Castalia* in the Brazilian Amazon are still exploited by the button industry (Beasley 2001, Matos 2007).

Recently, one of the major threats to the native bivalves is the introduction of invasive species (Haag 2012, Mansur et al. 2012). Among the most harmful species that were introduced to Brazil are *Limnoperna fortunei*, *Corbicula fluminea* (Müller, 1774), *Corbicula fluminalis* (Müller, 1774) and *Corbicula largillierti* (Philippi, 1844). *Limnoperna fortunei* (golden mussel) has caused the worst damages to native mussels populations, as they grow over any hard substrate, including the shell of native mussels, preventing them to open their valves, causing death by suffocation and starvation (Mansur et al. 2004a, Darrigran & Damborenea 2006, Mansur et al. 2012). Besides the ecological problems, the introduced species can cause several economic losses to industries and energy plants; clogging pipes, filters and other structures (Mansur et al. 2004a,b, Darrigran & Damborenea 2006, Mansur 2007, Darrigran et al. 2007). The damages caused by these bivalves were recently reviewed by Boltovskoy & Correa (2015).

In the 2008 edition of the Brazilian Red Book of Threatened Species, 26 out of the 29 listed molluscs are freshwater mussels, including ten species of *Diplodon* (Amaral et al. 2008). However, a recent re-evaluation of the list (Santos et al. 2015), which strictly used the criteria of IUCN listed only two species as threatened, 11 as Data Deficient and 9 as Near Threatened. It is clear that the environmental conditions in Brazil not improved in these few years. This is actually an evidence of the lack of data and the risk of extinction must be re-evaluated considering the Brazilian reality. The threat to freshwater mussels is a global phenomenon. In North America, there are 73 species critically endangered and 37 probably extinct (Neves 1999, Strayer et al. 2004, Haag 2012).

Knowledge on freshwater mussels of Brazil is not sufficient, with several important data to species extinction evaluation risk missing, such as information about population dynamics and reproductive cycle. This prevents the inclusion of species in IUCN risk categories, unless if distribution evidence is used, the better data that we have. However, even the information about distribution has problems. Many times the distribution of freshwater mussels is assigned to a hydrographic basin as a whole (*e.g.,* Simone 2006, Mansur et al. 2012); however, the situation in the "real world" is quite different, as the distribution of the species is not homogeneous and depends on several environmental factors (Haag 2012, Mansur et al. 2012). The mussels assemblages are patchily distributed and the movements in adult mussels are restricted (Pereira et al 2011, Haag 2012, Vaughn 2017, Miyahira et al. 2017). Thus, the distribution of a mussel can never consider the basin as the whole for evaluation of extinction risks. Pereira et al. 2011 evaluated six sites along a gradient at a stream in the state of Rio Grande do Sul (Brazil); *Anodontites trapesialis* (Lamarck, 1819) and *Anodontites lucidus* (d'Orbigny, 1835) were found in one site; *A. patagonicus* (Lamarck, 1919) in two; and *Diplodon pilsbry* in three. Similar situation is found by Miyahira et al. (2017) in a river at state of Rio de Janeiro (Brazil); *A. trapesialis* and *D. ellipticus* where found respectively in two and three sites out of ten surveyed. Another problem about distribution information is the use of old data obtained in literature and museums records (*e.g.,* Mansur & Pereira 2006, Simone 2006, Miyahira et al. 2013). Thus, the distribution presented in most works is closest to the original, but also includes several places where species do not occur anymore, leading to a wrong evaluation of the risk of extinction.

Moreover, complete morphological information is absent as detailed above and this not affect only the taxonomy, but also conservation. For example, *Diplodon pfeifferi* (Dunker, 1848) is a species recorded only at the state of Rio de Janeiro and listed in 2008 edition of the Brazilian Red Book (Amaral et al. 2008) but some authors include this species in the synonym of *D. granosus* (Simpson 1914, Haas 1969, Simone 2006), a species not listed as threatened. It is clear that the correct identification of these two species is not only a problem of taxonomy.

It is necessary to improve the evaluation of risk to our mussels, some recommendations are done: 1) detail the distribution of the species relating, when possible, to environmental factors; 2) separate the old (or museum) records from current records; 3) collect in sub-sampled areas; 4) improve our knowledge on morphology and genetics to solve the taxonomical questions; 5) study the population dynamics of the species and 6) study the species reproduction cycle.

7. Summary of studies on Brazilian freshwater mussels

The first studies on South American Unionida fauna occurred during the time of great expeditions; several naturalists/collectors came to or received material from South America. One of the byproducts of this activity was that most part of South American type specimens are currently keep in European museums. Ironically, few species of naiads were described by South American researches, some exceptions are Bonetto (1962), Bonetto & Tassara (1987) and Mansur & Pimpão (2008). In this phase, the descriptions were based mainly on the shells and, the species nowadays recognized as *Diplodon* or *Rhipidodonta,* were placed in the genus *Unio* Retzius, 1788 and commonly any variation of the shell was described as a new species, resulting in a large number of species names.

To this descriptive phase, a new phase in the study of South American freshwater mussels followed, that encompassed the organization and analysis of these names, with the aim to determine what really correspond to a biological species. Ihering (1893), a Deutsch zoologist established in Brazil, made the first attempt to organize some Brazilian species, and an improved work was published later (Ihering, 1910). He also published a series of studies about Brazilian mussels (*e.g.,* Ihering, 1890, 1891), including some specimens from little studied states of Brazil, like Goiás (Ihering, 1904). Morretes (1949) is the first catalogue about freshwater mussels of Brazil made by a Brazilian researcher. Marshall (1917, 1922, 1923, 1926, 1927) described a series of South American species and proposed two new genera, *Diplodontites* Marshall, 1922 and *Mycetopodella*, both still in use (Simone 2006, Graf & Cummings 2007). Ortmann received a large amount of specimens from South America with soft parts that allowed him to describe new species and produced the above mentioned catalogue (Ortmann, 1921). Haas (1930, 1931a,b) published a catalog about South American species in a series of fully illustrated works. Haas (1969) is a landmark in the study of freshwater bivalves not only in South America but also in the world, and remains as the most "modern" global catalog of Unionida species with synonymic lists. At the same time Cox et al. (1969) presents a scheme including fossil groups.

The formation of South American freshwater mussel researches finally starts in the 1950's. Argentino A. Bonetto from the 1950's onwards published a series of papers concerning the mussel fauna of South America. From his extensive bibliography we can mention some of his studies about Rhipidodontini: dealing with diversity and anatomy (Bonetto 1954, 1962, 1964, 1965, 1967, Bonetto & Mansur 1970), larval stages (Bonetto 1961, 1965, Bonetto & Ezcurra 1965), museum collection revision (Bonetto 1973) and factors that affect mussel distribution (Bonetto et al. 1962, Bonetto & Di Persia 1975). The author also described two new species of *Diplodon* (Bonetto 1962, Bonetto & Tassara 1987) and one subgenus (Bonetto et al. 1986).

Amongst the most important papers published by Juan J. Parodiz about mussels are the compendium of available names for *Diplodon* (Parodiz 1968) and a study about the hybridization of *Diplodon delodontus* (Parodiz 1973). However, his masterpiece was the catalog about continental fossil molluscs (Parodiz 1969). Parodiz & Bonetto (1963) suggested a systematic arrangement of Unionida families based on larval type (see details above).

In Brazil, the studies of freshwater mussels intensified during the 1960's. Zanardini (1965) published a note about the occurrence and distribution of *Diplodon* and *Anodontites* Bruguière, 1792 in the state of Paraná. A greater increase in knowledge about freshwater mussel fauna began with the works of Mansur (1970) that presented the catalog of Hyriidae and Mycetopodidae of the state of Rio Grande do Sul. She published works dealing with specimens from southern Brazil (Mansur 1972, 1973, Mansur & Anflor 1982, Mansur & Pereira 2006) as well as from the Amazon River basin (Mansur & Valer 1992, Mansur & Pimpão 2008, Pimpão & Mansur 2009) and Pantanal (Serrano et al. 1998, Callil & Mansur 2005, 2007). There are also two technical works, one identifying Southern and Southeastern genera of mussels (Mansur et al. 1987) and another on how to obtain and identify glochidia (Mansur & Campos-Velho 1991). Recently, her studies are mainly concerned with non-native bivalves (Mansur et al. 2012). The morphological aspects of Mycetopodidae were also studied in southern South America (Veitenheimer-Mendes 1973a,b, Veintenheimer-Mendes & Mansur 1978a,b, 1979).

Other researchers also studied freshwater mussels in Brazil. At the state of Rio de Janeiro, L.C. Alvarenga and C.N. Ricci studied the soft parts and glochidium of *Diplodon multistriatus* (Ricci et al. 1988, 1990), as well as the morphology of soft parts, glochidium and shell variation of *Diplodon besckeanus* (Alvarenga & Ricci 1977a,b, 1981). In the field of functional anatomy, there are the works of Wagner Avelar (Avelar & Santos 1992, Avelar 1993, Avelar & Cunha 2009) and Nilton Hebling (Hebling & Penteado 1974, Hebling 1976) concerning Hyriidae and Mycetopodidae species. Simone (1994, 1997) described the morphology of two species of *Anodontites*. Simone (2006) published an illustrated catalogue of molluscs species of Brazil (details above).

There are few studies on ecology, population dynamics and reproduction in Brazil. As the taxonomic and systematic issues have not been properly handled for most species, this often becomes an obstacle for ecological approaches. Henry & Simão (1985) analyzed the distribution of a population of *Diplodon delodontus expansus* (Küster, 1856) in the state of São Paulo. Beasley (2001) presents strategies for managing hyriids from the Amazon River basin. Meyer et al. (2010) evaluated the population structure and sexual proportion in a population of *Diplodon expansus*. Beasley et al. (2005) presented the reproductive cycle of *Paxyodon syrmatophorus* (Meuschen, 1781), while Avelar & Mendonça (1998) presented the gametogenesis of *Diplodon rotundus gratus*. Tomazelli et al. (2003) suggested the potential use of *A. trapesialis* as biological sentinel and the life cycle of this species were investigated by Callil & Mansur (2007) and Callil et al (2012). Lopes et al. (2011) identified the parasite interaction in *Diplodon suavidicus* parasited by *Hysterothylacium* sp. (Nematoda). Recently the first phylogenetic approach of Hyriidae in Brazil was presented by Santos-Neto et al. (2016).

Important faunal surveys about freshwater mussels exist in other South American countries: French Guyana (Drouet 1859, Massemin et al. 2010), Suriname (Verhout 1914), Venezuela (Baker 1930, Lasso et al. 2009, Cummings & Mayer 2011), Peru (Ramírez et al. 2003), Paraguay (Quintana 1982), Argentina (Rumi et al. 2008), Uruguay (Corsi 1901, Olazarri 1966, Scarabino & Mansur 2007, Clavijo 2009) and Chile (Parada & Peredo 2002).

8. Conclusions

The large number of works concerning freshwater mussels in South America can give a false idea that the knowledge about these species is deep, but most of works are discreet and deal with one or two species. Comparative approaches of all kinds (morphological, ecological or molecular) are virtually absent. Even basic information is missing, as many species have not been studied beyond the original description. There have been some recent advances in Unionida systematic and the scheme with six or five families looks well established. However, the relationships inside the groups (*e.g.,* families, genera) are poorly known and Rhipidodontini is not an exception. More data were needed to a better comprehension of the species and try to definitively solve questions like the status of *Rhipidodonta*. It is also an important step in order to reveal the real diversity of this group. The absence of good taxonomic and systematic information has been a limiting factor for biological and ecological studies, preventing appropriated extinction risk evaluation. The interactions of native with the invasive species need to be better understood, considering that the dispersion of the latter is notorious and harmful for native species. Integrating all this information is essential to development of appropriate conservation strategies for freshwater mussels in South America.

Supplementary material

The following online material is available for this article:

Appendix I – Taxonomical arrangement of principal revisions of Rhipidodontini. E – species; SE – subspecies; NA – not used by the author as species or subspecies.

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Igor Christo Miyahira: conception and design, data acquisition, data analysis, text editing, critical revision

Maria Cristina Dreher Mansur: conception and design, data analysis, text editing, critical revision

Sonia Barbosa dos Santos: conception and design, data analysis, text editing, critical revision

Conflicts of interest

The authors declare to have no conflict of interest.

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The mating behavior of *Leucothyreus marginaticollis* Blanchard, 1843 (Coleoptera: Scarabaeidae: Rutelinae)

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Abstract: In Brazil, there are several species of the genus *Leucothyreus* Macleay, 1819 occurring throughout the country; however, there are only a few studies describing their biological aspects, times of occurrence, associations of adults and juveniles with native or cropped plants, as well as their mating behavior. Thus, this study aimed to assess the mating behavior of *Leucothyreus marginaticollis* Blanchard, 1843. The study took place in an experimental area of the State University of Mato Grosso do Sul, in Aquidauana, MS, Brazil. Firstly, we sampled adult insects using light traps, from October to November 2013. Simultaneously, we registered the flight period in the field. Afterward, in the laboratory, males and females were separated and then combined into couples for observations of the mating steps. A large number of the adults mated, which lasted on average 22.71 min and occurred from 7 to 11 pm. After mating, part of the males released the females and the other part remained attached to them, which must have happened as an attempt to inhibit other males from mating with them. Moreover, a few females refused to mate with the established male, reinforcing the fact that the pairing of couples occurs after chemical identification among adults. In the field, the largest amount of adults was collected from 8 to 10 pm. Regarding eating habits, adults feed mostly on acerola leaves and flowers (*Malpighia emarginata* DC, Malpighiaceae), mastic gum leaves (*Myracrodruon urundeuva* Allemão, Anacardiaceae), grapes (*Vitis vinifera* L., Vitaceae), apples (*Malus domestica* Borkh., Rosaceae), and bananas (*Musa* sp. L., Musaceae). To the end of the experiment, we could clarify the mating steps of *L. marginaticollis* in order to assist in further extraction and identification of sexual pheromone.

Keywords*:* *Chemical communication, eating habit, Geniatini, Scarabaeoidea, white grubs.*

Comportamento de cópula de *Leucothyreus marginaticollis* Blanchard, 1843 (Coleoptera: Scarabaeidae: Rutelinae)

Resumo: No Brasil várias são as espécies de *Leucothyreus* Macleay, 1819 que ocorrem nas diferentes regiões, entretanto, poucos são os trabalhos que descrevem os aspectos biológicos, épocas de ocorrência, associação de adultos e imaturos com plantas nativas ou cultivadas, e comportamento de cópula. Assim o presente trabalho apresentou como objetivo estudar o comportamento de cópula de *Leucothyreus marginaticollis* Blanchard, 1843. Os estudos foram conduzidos na fazenda experimental da Universidade Estadual de Mato Grosso do Sul em Aquidauana, MS, sendo realizadas coletas de adultos com armadilha luminosa de outubro a novembro de 2013. Com as coletas foram analisados os horários de voo em campo, e em laboratório machos e fêmeas foram separados, posteriormente foram formados casais e as etapas que envolvem o comportamento de cópula, verificadas. Vários adultos realizaram cópula, a qual durou em média 22,71 minutos e ocorreram das 19 às 23 horas. Após a cópula o macho se soltava da fêmea ou permanecia sobre essa, provavelmente para impedir que outro macho realizasse a cópula. Algumas fêmeas não aceitaram os machos para cópula, indicando que a formação de casais ocorre após o reconhecimento químico entre os adultos. O horário de maior coleta dos adultos em campo foi das 20 às 22 horas. Adultos podem se nutrir de folhas e flores de acerola (*Malpighia emarginata* DC, Malpighiaceae), folhas de aroeira (*Myracrodruon urundeuva* Allemão, Anacardiacea), frutos de uva (*Vitis vinifera* L., Vitaceae), maçã (*Malus domestica* Borkh., Rosaceae) e banana (*Musa* sp. L., Musaceae), ampliando-se as informações sobre o hábito alimentar dos adultos. Foram elucidadas as etapas relacionadas ao comportamento de cópula de *L. marginaticollis*, a qual pode auxiliar nos processos de extração e identificação de feromônio sexual.

Palavras-chaves*:* *Comunicação química, hábito alimentar, Geniatini, Scarabaeoidea, corós.*

Introduction

A total of 164 species has been identified in the genus *Leucothyreus* Macleay, 1819. These species can be found from the southern Mexico to Uruguay (Jameson & Hawkins 2005). In Brazil, Morón (2004) reported 83 species of this genus in several regions. Adult dimensions range from 8.0 to 18.0 mm, being in general of dark brown, brown, or black coloration (Jameson & Hawkins 2005).

Just a few species stand out as crop pests, however causing significant losses. In Colombia, larvae of *L. femoratus* Burmeister, 1844 are listed as crop and pasture pests (Pardo-Locarno et al. 2003, 2005, 2006), as well as for African oil palm (*Elaeis guineensis* Jacquin, Arecaceae) (Martinez & Plata-Rueda 2013, Martinez et al. 2013). In Mexico, Ramírez-Salinas & Castro-Ramírez (2000) labeled *Leucothyreus* species as major pests for corn (*Zea mays* L., Poaceae).

In Brazil, only a few species are recorded as belonging to the genus *Leucothyreus*. One of these records was made by Puker et al. (2011), who reported *L. albopilosus* Ohaus, 1917 in lesions of *Eucalyptus citriodora* Hook (Myrtaceae). Pereira et al. (2013) sampled larvae of *L. alvarengai* Frey, 1976 and of *L.* aff. *semipruinosus* Ohaus, 1917 in crop succession systems of soybeans (*Glycine max* (L.) Merr., Fabaceae) and maize (*Zea mays* L., Poaceae). Yet Rodrigues & Pereira (2014) observed larvae of *Leucothyreus* sp. and of *L.* aff. *ambrosius* Blanchard, 1850 in a succession system of soybeans and cotton (*Gossypium hirsutum* L., Malvaceae).

The biological aspects of certain species are already known. The lifecycle of *L. femoratus* is completed within 170.4 days (Martínez & Plata-Rueda 2013), while those of *L. alvarengai* and of *L.* aff. *semipruinosus* are fulfilled in less than one year (Pereira et al. 2013). For *L. ambrosius*, the end of the lifecycle is reached within 173.3 days (Gomes et al. 2014); yet for *L. albopilosus*, it lasts 185.5 days (Ferreira et al. 2016).

When it comes to mating behavior, little information is known about this genus. As an example, we may cite the study by Ferreira et al. (2016) for *L. albopilosus*, which detailed the several steps involving this aspect, as well as the possible release of sexual pheromone by females.

In view of such scarcity of information on the genus *Leucothyreus*, we developed a study on the mating behavior of *L. marginaticollis* Blanchard, 1843. This species (cited as *L. dorsalis*) ends its lifecycle within 273.5 days (Rodrigues et al., 2010) and there are reports stating that juveniles feed on palm roots (*Acrocomia aculeata* (Jacq.) Lodd. ex Mart., Arecaceae) (Puker et al. 2009). In brief, the present study aimed to elucidate the mating behavior of *L. marginaticollis* for purposes of future extraction and isolation of its sex pheromone.

Material and Methods

The studies were carried out at the experimental farm of the State University of Mato Grosso do Sul (UEMS), in Aquidauana, MS, Brazil (lat. 20° 28’; long. 55° 48’). The local climate is *Aw* subtype according to the Köppen (1948) classification. The rainy season goes from October to March, with an average temperature of 28 ºC; and the dry one is from April to September, with an average temperature of 22ºC. The average annual rainfall is about 1,400 mm.

At first, adults of *L. marginaticollis* were collected with the aid of a light trap, “Luiz de Queiroz” model, between October and November 2013. Adults were collected from 6 p.m. to 6 a.m. the next day. The adults were identified by comparing specimens deposited in the collection of the laboratory of entomology, UEMS Aquidauana, wich had been identified by Dr. Sérgio Ide (Instituto Biológico, São Paulo, SP, Brazil). The sampled insects were taken to the Laboratory of Entomology, State University of Mato Grosso do Sul (UEMS). Five adult couples were deposited at the Museu de Zoologia da Universidade de São Paulo (USP). Then, males and females were separated, being placed individually in 50 mL plastic containers. Half of the volume of these containers was filled with soil, being closed with a voile-type fabric. Sex differentiation was performed visually through protarsomers, which are enlarged in males (Figure 1).

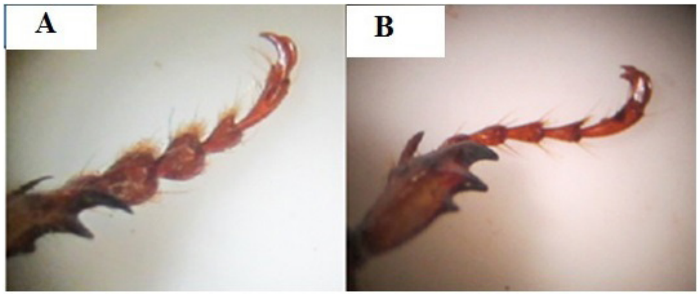

Figure 1. Protarsomers of *Leucothyreus marginaticollis* Blanchard (1843): A) Male; and B) Female.

In the laboratory the study of mating behavior was carried out from 05 to 28 October. At dusk, as soon as the adults emerged from the soil, observations began and, after that, couples (male and female) were paired and transferred to 1,000 mL plastic containers, as the method described by Rodrigues et al. (2014). Each container held two couples, which were used for the evaluation of mating outcome. The containers were filled with soil, for about one-third of its volume. Then, pieces of fruits, leaves, and flowers of different plant species were provided to the insects, so we could analyze their feeding habits. During the study period, 70 couples (n=70) were paired; and 20 females (n=20) were dissected to check for egg development.

The observation room was in the dark as stated by Facundo et al. (1999); and, in order to visualize and record the mating behavior of males and females, we used a camcorder (Canon SX160 IS).

From October 28 to November 4, 2013, adult flight time was monitored with the aid of a light trap, which was inspected at 60 minute intervals by counting the number of sampled insects. Flight time data were transformed into $\sqrt{x+1}$ and subjected to analysis of variance (ANOVA). The means compared by the Scott-Knott’s test ($P < 0.01$). For these statistics, we used ASSISTAT software package (Silva & Azevedo 2002). Data of temperature (° C), rainfall (mm), and solar radiation (kJ/m^2) for Aquidauana, MS were gathered from the Brazilian Institute of Meteorology (INMET).

Results

The adults kept in the observation containers remained during the daytime sheltered in the soil. At dusk, from 5:50 p.m. to 8:20 p.m., a small portion of the clypeus of insects was protruded from the soil surface, remaining at this position for on average 25.85 ± 12.40 (10-50) minutes. At that time, antennae remained raised with clubs open, probably in search for environmental chemical information. Next, adults left the soil for flying and walking inside containers for about 26.25 ± 7.7 (15-40) minutes. After this, the adults stood still and hence couples were paired. After being paired, several steps related to the mating behavior could be recorded and described (Figures 2 and 3a).

For most of the pairs, there were no approach responses for adults (n = 47), which remained either walking or feeding (n = 42), and some of them returned to the interior of the soil (n = 5).

On the other hand, approach responses were observed for 23 pairs of adults. In these cases, males found females by touching them on the back (n = 17) or on the sides (n = 6) using antennas and protarsomers (Figures 3b and 3c). After meeting, males mounted on females, positioning themselves to start the mating process (Figure 3d). From that moment on, females showed two kinds of behavior: part of them walked and rolled on the ground moving their elytra to get rid of the males (n = 9) and refuse to mate, while the other part accepted the male (n = 14) and allowed copulation (Figure 3e).

At the beginning of the mating, males bent their bodies until reaching female pygidium and then inserting their aedeagus. During this event, some females (n = 4) walked and others stood still (n = 10). The mating process lasted on average 22.71 ± 4.71 (15-31) minutes, occurring between 7 p.m. and 11 p.m., with twelve of them being recorded between 8 p.m. and 10 p.m. At the end of the mating, males retracted their aedeagus in 14.86 ± 3.08 (12-21) seconds.

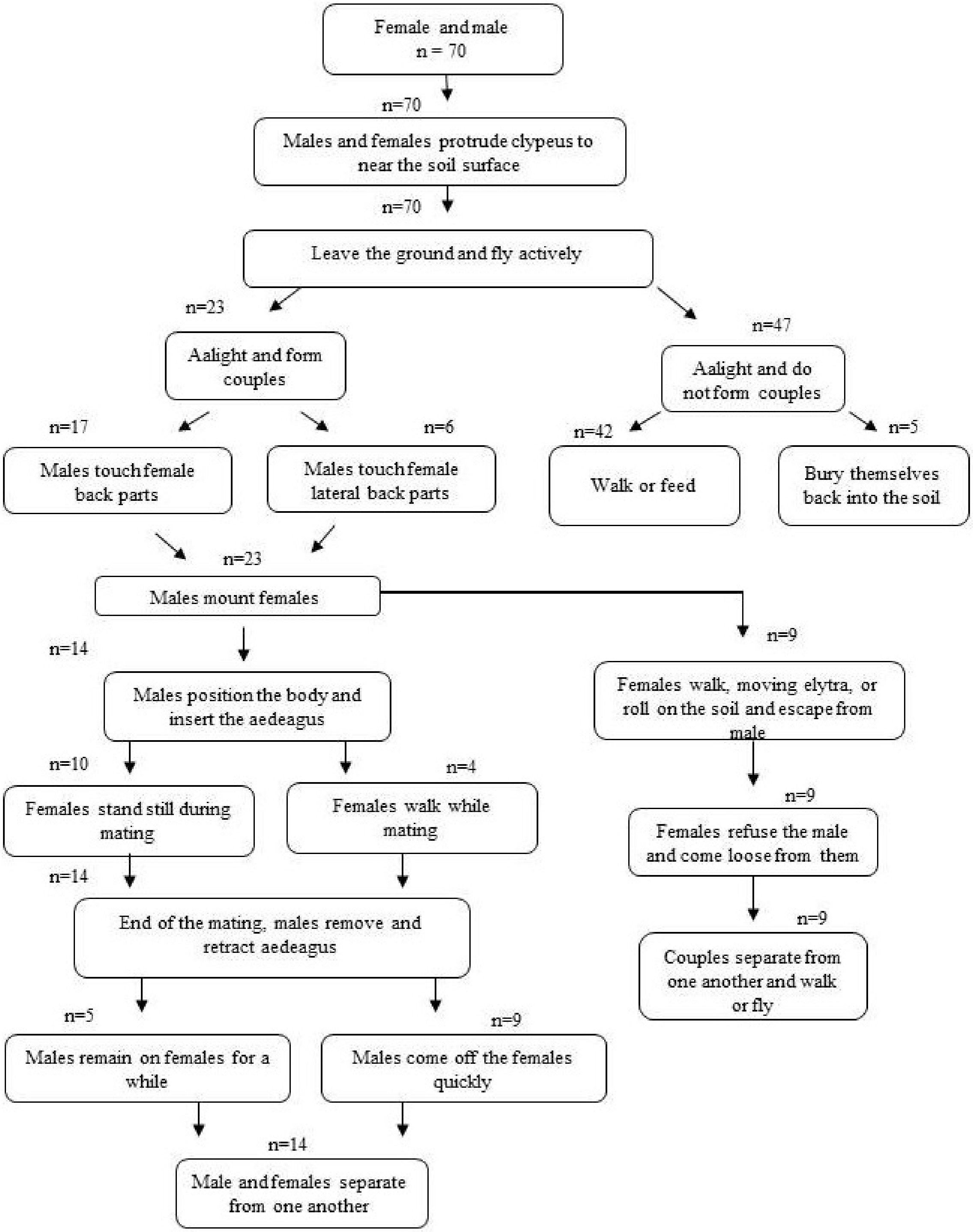

Figure 2. Ethogram of the mating behavior of *Leucothyreus marginaticollis* Blanchard (1843) (n=70 pairs), in the laboratory.

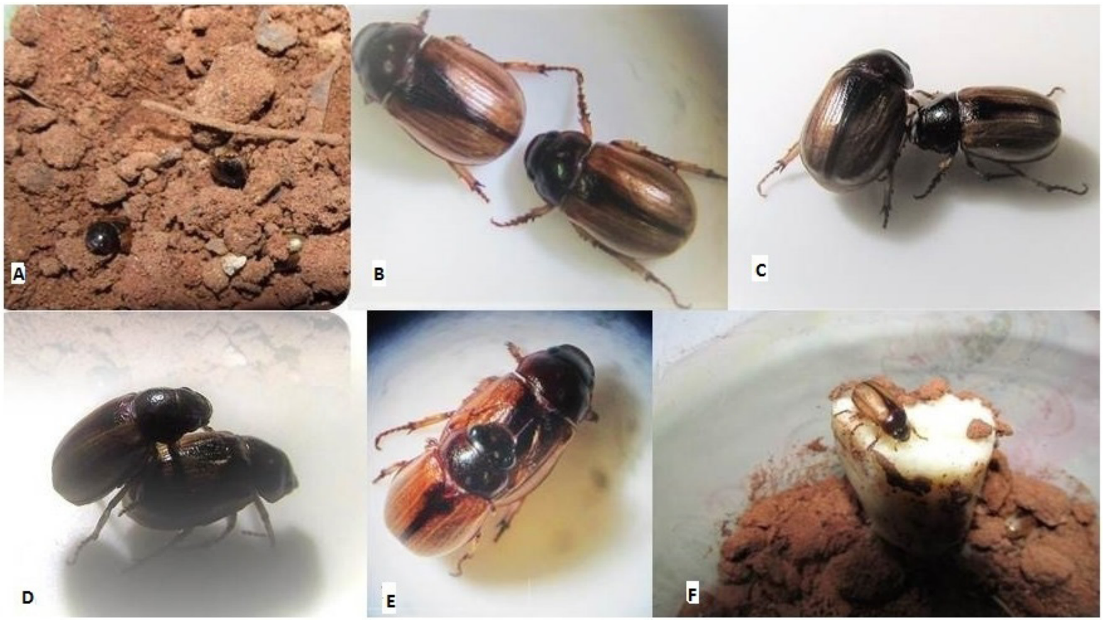

Figure 3. *Leucothyreus marginaticollis* Blanchard, 1843. A) Adults coming off the soil. B) Male meets female and touches the back body. C) Male meets the female and touches the side of her body. D) Male climbs on the female and stands for mating. E) Couple in mating. F) Adult feeding on piece of banana fruit.

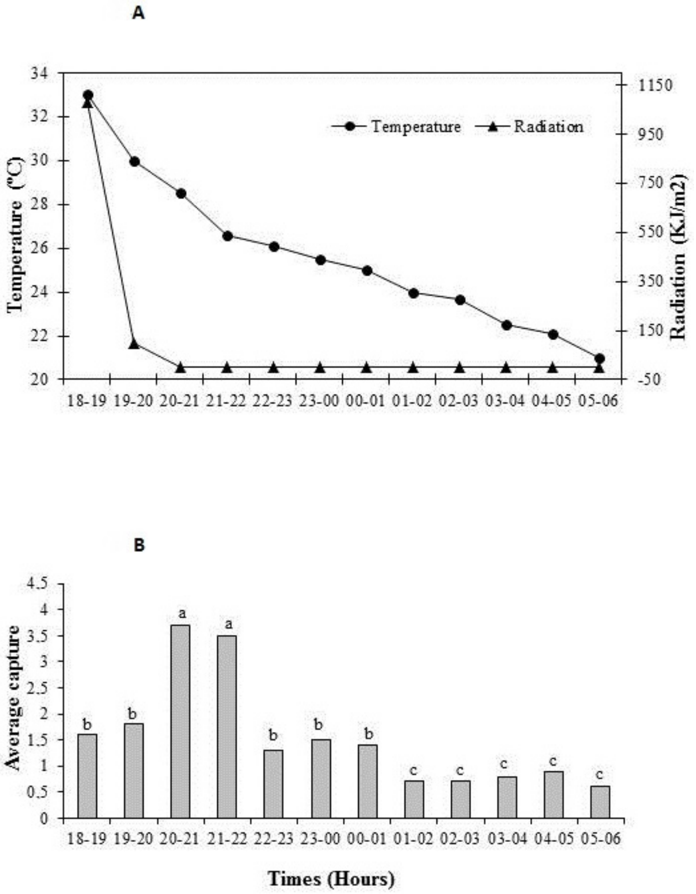

Figure 4. A) Averages of temperature (ºC) and radiation (KJ/m^2) gathered a weather station (INMET). B) Mean number of adults of *Leucothyreus marginaticollis* Blanchard (1843) at different observation times. Means followed by the same letters do not differ from each other by the Scott-Knott's test ($p<0.01$), data were converted into $\sqrt{x+1}$. Data collected from October 28 to November 4 of 2013.

After retracting the aedeagus, some males immediately dismounted the females (n = 9) and others (n = 5) remained mounted for around 140.2 ± 8.84 (130-152) seconds. Afterward, they walked, flew, or fed and later returned to the interior of the ground.

Among the several types of leaves, flowers, and fruits provided for the feeding of adults, we noted a preference for flowers of acerola (*Malpighia emarginata* DC, Malpighiaceae), leaves of mastic gum (*Myracrodruon urundeuva* Allemão, Anacardiaceae), and fruits of grapevine (*Vitis vinifera* L., Vitaceae), apple (*Malus domestica* Borkh., Rosaceae), and banana (*Musa* sp. L., Musaceae) (Figure 3f).

Regarding the flight time, the first adults began to be sampled by the light trap from 6 p.m., which coincided with the beginning of the scotophase (dark period), from 6 p.m. to 7 p.m., when radiation decreased from 1,080 to 100 kJ/ m^2 (Figure 4). Therefore, a reduction or absence of sunlight may stimulate adults to initiate flight activities. During flying hours, the mean temperature ranged from 33 to 21 °C (Figure 4).

All dissected females had developing eggs. We found an average of 8.8 ± 5.02 (2-17) (n = 20) developing eggs, proving that during the time of collection, females are within the reproductive period.

Discussion

The adults of *L. marginaticollis* in designing the clypeus near the surface of the ground, demonstrate the use of the visual stimuli, then with moving of antennae are detecting chemical information of the environment. For some species of Scarabaeidae, a number of antennal sensilla have been detected in the antennae, being more numerous in males, such as for the species *Phyllophaga obsoleta* (Blanchard, 1851) (Romero-Lópes et al. 2004) and *P. anxia* (LeConte, 1850) (Ochieng et al. 2002). These organs are probably related to the detection of sexual pheromone released by females.

In the laboratory, *L. marginaticollis* flight began by the time the insects left the soil, which might have occurred for many purposes as dispersal, foraging or even for breeding. Such behavior was also recorded for *L. albopilosus* (according to Ferreira et al. 2016) and *Anomala testaceipennis* Blanchard, 1851 (Rodrigues et al. 2014).

Facundo et al. (1999) reported that adults of *Exomala orientalis* Waterhouse, 1875 leave the ground but do not fly, and females rub the third pair of legs against the abdomen, what might be to the releasing of sex pheromone to attract males (Facundo et al. 1999). In our study, no similar behavior was observed for *L. marginaticollis* females concerning the releasing of sexual pheromone for males attraction.

After accepting the males, females remained for on average 22.71 minutes mating. For *L. albopilosus*, mating lasts for 19.45 minutes on average (Ferreira et al. 2016). Both *L. marginaticollis* and *L. albopilosus* have a similar mating time since, according to Ferreira et al. (2016), for the latter it occurs from 7:00 p.m. to 11:00 p.m. Martínez et al. (2013) also reported that adults of *L. femoratus* perform mating from 8:00 p.m. to 11:00 p.m..

Interestingly, several females of *L. marginaticollis* refused to mate with males, escaping from them during male mating attempts. Similar behaviors were observed for *L. albopilosus* (according to Ferreira et al. 2016), *A. testaceipennis* (according to Rodrigues et al. 2014), and *Liogenys fusca* Blanchard, 1850 (Rodrigues et al. 2016).

As stated by Ferreira et al. (2016), adults of *L. albopilosus* were collected in larger numbers from 8 p.m. to 10 p.m., which was also the period of greatest occurrence of *L. marginaticollis* in this study.

Adults of *L. marginaticollis* fed on leaves, flowers, and fruits of various plant species, showing that they have a wide range of food sources. Adults of *L. albopilosus* can feed on banana fruits (*Musa* sp., Musaceae) and mango flowers (*Hancornia speciosa* Gomes, Apocynaceae). According to Rodrigues et al. (2014), adults of *A. testaceipennis* feed on flowers of oiti (*Licania tomentosa* (Benth.) Fritsch., Chrysobalanaceae) and flowers of laurel (*Cordia glabrata* (Martius) A.DC., Boraginaceae).

In the present study, we obtained a great deal of information on the mating behavior of *L. marginaticollis*, as well as information regarding the flight time, feeding habits, widening the existing background on the genus *Leucothyreus*.

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Author's Contribution

Kleyton Rezende Ferreira: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Sérgio Roberto Rodrigues: Contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Inventory

Anurans of the Parque Estadual do Mirador, a remnant of Cerrado in the state of Maranhão, Northeastern Brazil

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Abstract**:** The Cerrado is one of the most threatened biomes in Brazil. It is estimated that much of its original vegetation has been replaced by some type of human use. This is quite worrying, mainly in the northern part where the number of wildlife inventories is insufficient and creates the false impression of low diversity in the region. The Parque Estadual do Mirador-PEM, located in the south-central region of the state of Maranhão, presents vegetation typical of the Cerrado biome and corresponds to one of 46 priority areas for conservation in the state. Herein, we describe the species richness and composition of the anurofauna from the PEM and analyze the influence of different types of vegetation in its formation. Our inventory was conducted from December 2013 to February 2015, using the active search and auditory census methods on breeding sites in different water bodies of the park. We recorded 31 anuran species belonging to five families (species number in parentheses): Leptodactylidae (14), Hylidae (12), Bufonidae (3), Microhylidae (1) and Phyllomedusidae (1). The rarefaction curve and species richness estimators indicated that the sampling effort was enough to record most of the species in the region. The richness of anurans in the PEM was higher than reported by other authors for several areas of Cerrado. Most species have a wide distribution in Brazil or are strongly associated with the Caatinga or Amazon biomes. Only about 19% are endemic to the Cerrado biome. This study is the first to inventory the anurans species of the south-central region of state of Maranhão and provides important data on amphibian communities from the northern part of the Brazilian Cerrado.

Keywords: *inventory, Amphibia, Anura, conservation unit, species richness.*

Anfíbios anuros do Parque Estadual do Mirador, um remanescente de Cerrado no estado do Maranhão, nordeste do Brasil

Resumo**:** O Cerrado é um dos biomas mais ameaçados do Brasil. Estima-se que grande parte da cobertura vegetal original deste bioma tenha sido substituída por alguma forma de utilização humana. Este fato é bastante preocupante, principalmente na porção norte, onde o número de inventários faunísticos é insuficiente e gera a falsa impressão de baixa diversidade na região. O Parque Estadual do Mirador-PEM, localizado na região centro-meridional do Maranhão, apresenta vegetação típica do Cerrado e corresponde a uma das 46 áreas prioritárias para conservação no estado. Aqui, nós descrevemos a riqueza e a composição da anurofauna no PEM, analisando a influência dos diferentes tipos vegetacionais na sua formação. Inventariamos a anurofauna de dezembro de 2013 a fevereiro de 2015, utilizando métodos de procura ativa e censo auditivo em sítios reprodutivos de diferentes corpos d'água do parque. Registramos 31 espécies de anuros, pertencentes a cinco famílias (números de espécies entre parênteses): Leptodactylidae (14), Hylidae (12), Bufonidae (3), Microhylidae (1) e Phyllomedusidae (1). A curva de rarefação e os estimadores de riqueza indicaram que o esforço amostral foi suficiente para registrar a maior parte das espécies da região. A riqueza de anuros no PEM foi maior que a registrada por outros autores em diversas áreas de Cerrado. A maioria das espécies possuem ampla distribuição no Brasil ou são fortemente associadas aos biomas Caatinga ou Amazônia. Somente cerca de 19% são endêmicas do bioma Cerrado. O presente trabalho representa o primeiro estudo a inventariar as espécies de anfíbios anuros na região Centro-Sul do Maranhão e fornece dados importante sobre as comunidades de anfíbios da porção norte do Cerrado brasileiro.

Palavras-chave: *inventário, Amphibia, Anura, unidade de conservação, riqueza de espécies.*

Introduction

The Cerrado is the second largest biome in Brazil, covering about a quarter of national territory, and extends from the state of Paraná to northern of the state of Maranhão (Ribeiro & Walter 2008), including transition areas in the state of Piauí. Despite its great extension, it is believed that more than a half of the areas originally occupied by this biome have been lost in recent years due to uncontrolled human occupation and agribusiness expansion (Klink & Machado 2005).

Except for Pampas biome, the Cerrado biome borders with all other Brazilian biomes, forming ecotonal areas of mutual influence. This mutual influence added to the historical, climatic and geomorphological processes (Silva & Bates 2002) are responsible for the formation of a complex mosaic of phytophysionomies, ranging from open fields (fields or savannas) to forest formations (Ribeiro & Walter 2008). These factors contribute to the formation of different types of microhabitats, allowing coexistence of species with different ecological niches, placing the Cerrado biome as holding 5% of the animals and plants in the world and accounting for about one third of Brazilian biodiversity (Brasil 2015). Furthermore, high rates of endemism and high level of threat make this biome one of 25 biodiversity hotspots for conservation priorities. Despite the global recognition of biological importance and conservation priorities of the Cerrado, conservation units protect only 9.4% of its area (IBAMA 2009) and at least 1173 species of animals are currently at extinction risk (MMA 2014).

The state of Maranhão has about 65% of its territorial extension covered by the Cerrado biome (IBGE 2004), which corresponds to almost 216,000 km^2, and has one of the best-preserved Cerrado areas of the northern portion of the biome, being considered an important priority area for biodiversity conservation (Diniz-Filho et al. 2005b). The state has also large ecotone areas (transitions between Amazon-Cerrado formations and Cerrado-Caatinga) creating ecological corridors with high potential for species endemism (Barreto 2007, Silva et al. 2013), but that are rarely considered in environmental policy definitions. Furthermore, the Brazilian Ministry of the Environment (Ministério do Meio Ambiente), through Ordinance No. 09 from 27 January 2007, established actions for the implementation of priority areas for biodiversity conservation of the Brazilian biomes, and identified 46 priority areas for conservation in the state of Maranhão, 28 of them of extremely high priority (MMA 2007).

Although of great importance for biodiversity conservation, studies in the northern part of the Brazilian Cerrado regarding amphibian communities are still insufficient, mainly in the state of Maranhão (Barreto et al. 2007, Brasileiro et al. 2008, Andrade 2017), which creates a false impression of low species diversity in the region (Diniz-Filho et al. 2005a). The south-central portion of Maranhão, which encompasses the northern sector of the Cerrado biome, has large priority areas for faunal inventory (MMA 2007), especially by presenting knowledge gaps on various taxonomic groups, which further reinforces the need to obtain basic information about anuran fauna in the region. Moreover, this region is located in an important Brazilian ecotone, which suffer strong influence of the other biomes, as such Amazonia and Caatinga.

In this scenario, faunal inventories are important tools to collect information on the biology and species richness, providing basic requirements for development of ecological models necessary for the adoption of effective conservation measures (Gotelli & Colwell 2001). Furthermore, the constant impact on amphibian populations caused by the intense anthropic activities in the Cerrado biome reinforces the need for taxonomic inventories (Ribeiro-Júnior & Bertoluci 2009). Therefore, this study aims to inventory the anuran fauna of the Parque Estadual do Mirador-PEM, an important remnant of Cerrado environment in the northern portion of the Cerrado biome, Northeastern Brazil.

Material and Methods

1. Study area

The Parque Estadual do Mirador (PEM) is located in the central-meriodional region of the state of Maranhão (06°10'- 06°42' S and 44°43'- 45°54' W, 425 asl, WGS84 datum), Brazil, between the headwaters of the Itapecuru and Alpercartas rivers (Figure 1), and encompasses the municipalities of

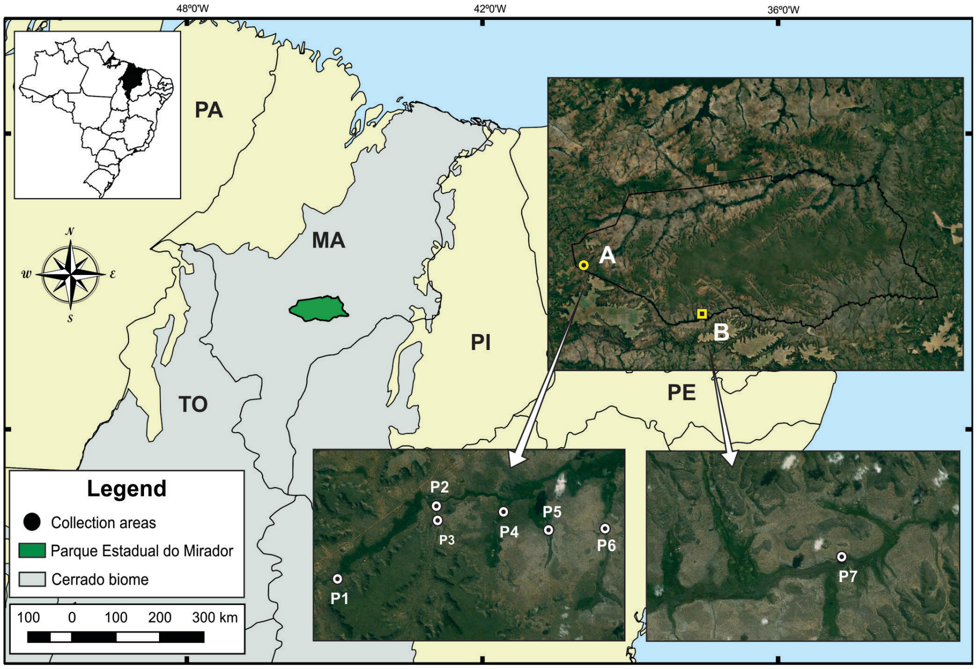

Figure 1. Map featuring the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil (in green). On the left, northernmost Brazilian areas of the Cerrado biome, in gray; in the upper-right corner, sampling areas (A-B) within the park; underneath: A - Geraldina's advanced checkpoint (P1-P6), B - Zé Miguel's advanced checkpoint (P7).

Mirador, Formosa da Serra Negra and Fernando Falcão. Created in 1980 by State Decree nº. 641 of June 20, the PEM has the Cerrado *sensu lato* as predominant vegetation (Conceição & Castro 2009) on red-yellow latosols associated with quartz sands and lithic soils, stony and rocky. With an initial area of 700,000 ha, the PEM was expanded by Law nº. 8.958 of May 8, 2009, having now a total area of 766,781.00 ha (Maranhão 2009). The climate is Aw' type (tropical sub-humid dry) with annual rainfall 1,200-1,400 mm and average temperatures ranging from 19.5 ° to 33 ° C (Alcântara 2004).

We sampled seven points distributed between two areas. The area A (advanced monitoring station Geraldina) has six sampling points: P1 - permanent pond amidst the riparian forest; P2 - temporary pond with large amounts of aquatic vegetation inside; P3 - temporary pond in an open field; P4 - permanent stream in dirty field area with presence of spaced trees; P5 - permanent stream amidst the gallery forest; P6 - permanent stream in open area. The area B (advanced monitoring station Zé Miguel) has only one sampling point: P7 - swamp area, located near the banks of the Rio Itapecuru (see details in Table 1). The sampled sites presented different types of vegetation, varying from grasslands to areas of large trees and palm trees (Figure 2).

2. Data collection

Were conducted, bimonthly, eight field expeditions in each sampling area from December 2013 to February 2015, comprising both rainy and dry seasons. We used two sampling methods: active visual search and auditory census in different microhabitats used by frogs (Heyer et al. 1994). These methods are inexpensive and considered the most efficient to record the largest species number in the shortest time (Valdujo et al. 2009). Three researchers carried out field activities for three consecutive days, starting around 6 p.m and ending at 12 p.m, resulting in a sampling effort of 144 hours/person. Furthermore, opportunistic records of vocalizations, or individuals found outside sampling points, were included in the list of species.

Identification of specimens were carried out through vocalization and/or comparison with specimens housed at the Herpetological Collection of the Universidade Federal do Maranhão and Delta do Parnaíba Zoological Collection housed at the Universidade Federal do Piauí, Campus Parnaíba, Piauí, Brazil. Voucher specimens (Appendix 1) were collected under permit granted by "Secretaria de Estado do Meio Ambiente e Recursos Naturais do Maranhão" (SEMA-MA #008/2013) and were deposited at the Delta do Parnaíba Zoological Collection (CZDP I1). No collected species is listed on the Brazilian list of Threatened Species (MMA 2014). The species distribution and taxonomic arrangement follow Frost (2017).

3. Data analysis

We evaluated the efficiency of anuran sampling methods in the PEM from using individual-based accumulation curves (Gotelli & Colwell 2001). We calculated the expected species richness using the nonparametric estimators Jacknife 1 and Bootstrap (Magurran 2004). The analysis was performed in EstimateS software v.8.0.0 with 10,000 randomizations (Colwell 2006).

To compare the anuran species composition of PEM with studies performed in areas of Cerrado and in other biomes (Amazon, Atlantic Forest, Caatinga and Pantanal), we performed a hierarchical cluster analysis using the unweighted pair-group method with averaging (UPGMA) (Magurran 2004). This analysis optimizes intra-group homogeneity clustering the most similar samples (different localities) based on the species composition similarities (Silva et al. 2011). The studies were chosen randomly or according to the proximity to the PEM, and only six studies per biome were selected. Despite the disparities between types of environments and sampling methods generate difference in the anuran species composition (Pombal Jr. 1995), and the arbitrary chose of studies to insert a bias in our comparisons, the analysis was performed to demonstrate influence of other biomes on the anuran species composition of the PEM. We used the Jaccard similarity index (Magurran 2004) to construct the similarity matrix based in a presence and absence matrix with 328 anuran species. To reduce differences caused by taxonomic problems, we excluded from matrix the species without specific identification ("gr.", "aff." and "sp."), considering only species with identification to be confirmed ("cf."). The cluster analysis was performed on the software Past 3.06 (Hammer et al. 2001).

Data from anuran composition was taken from the following studies carried out in different regions of Brazil: **Cerrado** – Serra do Gado Bravo-SGB, Maranhão (Barreto et al. 2007), Tocantins river basin, southwestern Maranhão (Brasileiro et al. 2008), Parque Nacional das Emas-PNE, Goiás (Kopp et al. 2010), Estação Ecológica Serra Geral do Tocantins-EESGT, Tocantins and Bahia (Valdujo et al. 2011), Estação Ecológica Uruçuí-Una-EEUU, Piauí (Dal Vechio et al. 2013), Piracanjuba and Peixe river basins, southeastern Goiás (Santos et al. 2014); **Caatinga** – Boa Vista and São João do Cariri, Paraíba (Vieira et al. 2007), Planalto da Ibiapaba, Ceará (Loebmann & Haddad 2010), Chapada do Araripe, Ceará (Ribeiro et al. 2012), Estação Ecológica Raso da Catarina-EERC, Bahia (Garda et al. 2013), Ilha Grande, Delta do Rio Parnaíba, Piauí (Andrade et al. 2014), Parque Nacional do Catimbau-PNC, Pernambuco (Pedrosa et al. 2014); **Amazon** – Reserva Ducke, Amazonas (Lima et al. 2005), Reserva Experimental Catuaba-REC, Acre (Souza et al. 2008), Altamira, Pará (Knispel & Barros 2009), Rio Preto da Eva, Amazonas (Ilha & Dixo 2010), Reserva Extrativista do Rio Gregório-RERG, Amazonas (Pantoja & Fraga 2012), Querência, Mato Grosso

Table 1. Environmental description of the sampled habitats of the Parque Estadual do Mirador (PEM), south-central region of the state of Maranhão, Northeastern Brazil.

Sampled habitats	Coordinates	Description
P1	6°37'49.60" S 45°52'44.00" W	Large pond surrounded by medium to large trees and the presence of aquatic weeds and small islands formed by emergent vegetation within the pond.
P2	6°36'10.6" S 45°50'32.0" W	Small pond surrounded by spaced vegetation, few trees and many herbaceous plants, with large amount of herbaceous and aquatic vegetation within the water.
P3	6°36'27.65" S 45°50'29.58" W	Pond formed in a flooded field, located in a spaced arboreal vegetation area, with little shrub vegetation inside.
P4	6°36'13.30" S 45°49'1.50" W	Stream located in a "campo sujo" (grassland with sparse shrubs) area. The marginal vegetation is shrub type with opened areas and emergent vegetation.
P5	6°36'37.30" S 45°47'59.20" W	Stream within the gallery forest, with the presence of large palm trees. It features flat edge and soaked in much of its length and accumulation of leaf litter on the ground.
P6	6°36'34.20" S 45°46'43.90" W	Stream within an opened field area with the presence of some palm trees. The marginal vegetation is formed by herbaceous plants.
P7	6°47'28.20" S 45°28'25.10" W	Flooded field formed periodically during the rainy season, presenting a large extension and covered by emergent vegetation and few trees fairly spaced.

Figure 2. Sampled environments in the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil. Collection points: A = P1, B = P2, C = P3, and D = P7.

(Bitar et al. 2012); **Atlantic Forest** – Área de Preservação Permanente Mata do Buraquinho-APPMB, Paraíba (Santana et al. 2008), Reserva Ecológica Rio das Pedras-RERP, Rio de Janeiro (Carvalho-e-Silva et al. 2008), Serra do Ouro Branco-SOB, Minas Gerais (São Pedro & Feio 2011), Parque Estadual Carlos Botelho-PECB, São Paulo (Forlani et al. 2010), Jequié, Bahia (Silva et al. 2013), Reserva de Serra Bonita-RSB, Bahia (Dias et al. 2014); **Pantanal** – Corumbá, Mato Groso do Sul (Ávila & Ferreira 2004), Serras do Entorno do Pantanal Sul-SEPS, Mato Groso do Sul (Gordo & Campos 2005), Estação Ecológica Nhumirim-EEN, Mato Grosso do Sul (Gordo & Campos 2003), Parque Nacional da Serra da Bodoquena-PNSB, Mato Groso do Sul (Uetanabaro et al. 2007), Fazenda Baía da Pedra-FBP, Mato Grosso (Pansonato et al. 2011).

Results and Discussion

We recorded 31 species of anuran amphibians distributed in 11 genera and five families (species number in paretheses): Leptodactylidae (14), Hylidae (12), Bufonidae (3), Microhylidae (1) and Phyllomedusidae (1) (Table 2; Figure 3-4). From this total, 29 species had their occurrence recorded from visual encounters and two species (*Elachistocleis* sp. and *Boana boans*) were only recorded through vocalization. Two species (*Leptodactylus petersii* and *L. mystaceus*) were visually observed outside the sampled points. Among the species recorded in the PEM, 80.6% (n = 25) were considered as least concern (LC) according to IUCN Red List Categories and Criteria (IUCN 2012). The LC category includes generalist and abundant species not qualified in another category (Critically Endangered, Endangered, Vulnerable or Near Threatened) (IUCN 2012). *Pseudopaludicola canga* and *P. jaredi* present deficient data (DD) due the lack of appropriate informations on their abundances, distributions and ecological requirements (IUCN 2012). Conservation status was not provided for *Dendropsophus* sp., *Scinax* aff. *ruber*, *Adenomera* sp. and *Elachistocleis* sp. since they were not identified at the specific level (Table 2). Considering the individuals number, we recorded a total of 892 inividuals which *Scinax fuscomarginatus* (n = 83; 9.30%), *Boana multifasciata* (n = 76; 8.52%) and *Dendropsophus rubicundulus* (n = 68; 7.62%) were

Table 2. Anurans recorded in the Parque Estadual do Mirador (PEM), municipalities of Mirador, Formosa da Serra Negra and Fernando Falcão, state of Maranhão, Northeastern Brazil. Sampling points: P1 - P7 (see point characterization in Material and Methods); *Record of specimens outside the respective sampling point. Habitat: A - open formations, F - forest formations. Geographic distribution: CAM - species occurring in the Cerrado and Amazon biomes; CC - species recorded in the Cerrado and Caatinga biomes; E - endemic to the Cerrado biome; W - wide distribution. Sampling method: A - Acoustic, V - Visual. N - number of individuals recorded. Number of species per family in parentheses. CS - Conservation status of the species classified according to IUCN: LC - least concern, DD - data deficient.

Taxon	Samp. point	Habit.	Geogr. dist.	Samp. method	N	CS
BUFONIDAE (3)						
Rhinella jimi (Stevaux, 2002)	P1, P5, P6	A	CC	A/V	7	LC
Rhinella mirandaribeiroi (Gallardo, 1965)	P2*, P6	A	CC	A/V	13	LC
Rhinella ocellata (Günther, 1858)	P2	A	E	A/V	4	LC
HYLIDAE (12)						
Dendropsophus branneri (Cochran, 1948)	P1, P2	F	W	A/V	48	LC
Dendropsophus minutus (Peters, 1872)	P1-P3	A	W	A/V	56	LC
Dendropsophus rubicundulus (Reinhardt & Lütken, 1862)	P2, P3, P5	A	CC	A/V	68	LC
Dendropsophus soaresi (Caramaschi & Jim, 1983)	P3, P5	F	CC	A/V	56	LC
Dendropsophus sp.	P1, P7*	F	-	A/V	12	-
Boana boans (Linnaeus, 1758)	P2*, P7	F	CAM	A	4	LC
Boana multifasciata (Günther, 1859)	P1, P2, P4-P7	A/F	CAM	A/V	76	LC
Boana punctata (Schneider, 1799)	P1, P2, P4-P7*	A/F	W	A/V	50	LC
Osteocephalus taurinus Steindachner, 1862	P1, P5	F	CAM	A/V	14	LC
Scinax fuscomarginatus (Lutz, 1925)	P1-P3	A	W	A/V	83	LC
Scinax nebulosus (Spix, 1824)	P1, P2, P4-P6	A/F	CAM	A/V	30	LC
Scinax aff. *ruber*	P3, P5, P7	A	-	A/V	55	-
LEPTODACTYLIDAE (14)						
Adenomera hylaedactyla (Cope, 1868)	P2, P3, P6	A/F	CAM	A/V	10	LC
Adenomera saci Carvalho & Giaretta, 2013	P7	A	E	A/V	41	LC
Adenomera sp.	P1*, P6, P7	A	-	A/V	4	-
Leptodactylus fuscus (Schneider, 1799)	P2-P5	A	W	A/V	34	LC
Leptodactylus mystaceus (Spix, 1824)	P1*	-	W	V	1	LC
Leptodactylus petersii (Steindachner, 1864)	P5		CAM	V	2	LC
Leptodactylus sertanejo Giaretta & Costa, 2007	P7	A	E	A/V	31	LC
Leptodactylus troglodytes Lutz, 1926	P3	A	CC	A/V	2	LC
Leptodactylus vastus Lutz, 1930	P3, P5	A	CC	V	3	LC
Physalaemus centralis Bokermann, 1962	P3, P6	A	E	A/V	29	LC
Physalaemus cuvieri Fitzinger, 1826	P3, P5	A	W	A/V	31	LC
Pseudopaludicola canga Giaretta & Kokubum, 2003	P1, P2, P5, P7	A	CAM	A/V	51	DD
Pseudopaludicola jaredi Andrade, Magalhães, Nunes-de-Almeida, Veiga-Menoncello, Santana, Garda, Loebmann, Recco-Pimentel, Giaretta & Toledo, 2016	P7	A	E	A/V	8	DD
Pseudopaludicola mystacalis (Cope, 1887)	P1, P3, P5	A	W	A/V	37	LC
MICROHYLIDAE (1)						
Elachistocleis sp.	P5, P7	A	-	A	7	-
PHYLLOMEDUSIDAE (1)						
Pithecopus azureus (Cope, 1862)	P1*-P5	A/F	E	A/V	24	LC

the most abundant species, accounting for 25.45% of the total number of sampled individuals (Figure 5).

Out of 31 recorded species, 19.3% are endemic to Cerrado, 29% are distributed in at least one of the other biomes, mainly in the Caatinga and Amazon biomes, and 41.9% of them have wide distribution in Brazil (Valdujo et al. 2011, Frost 2017). All species have previous records for the state of Maranhão. Recently, three endemic species of the Cerrado hitherto unrecorded for the state had their geographical distributions extended until the limits of the PEM: *A. saci* (Araújo et al. 2015), *L. sertanejo* (Lima et al. 2015) and *P. jaredi* (Andrade et al. 2016a). We recorded these species calling under dense vegetation in a flooded field covered by emergent vegetation, located near the banks of the Rio Itapecuru, within a typical Cerrado area (sampling point P7). *Pseudopaludicola jaredi* was recently described, being currently recognized three isolated populations in the states of Ceará, Rio Grande do Norte and Maranhão (Andrade et al. 2016a, b), occurring syntopically with *P. canga* in the PEM (Andrade et al. 2016a). In this case, the development of studies to access the actual distribution and conservation status of this species is important.

The anuran fauna of the PEM consists for the most part by species typical of open formations (Cerrado-Caatinga-Chaco complex) and often found in the Cerrado biome (Valdujo et al. 2011, Frost 2017). Our results support other studies conducted in Cerrado areas (Brasileiro et al. 2008, Oda et al. 2009, Roberto et al. 2013, Dal Vechio et al. 2013, Santoro & Brandão 2014, Dória et al. 2015), which affirm that the amphibian communities are dominanted by tolerant and generalist species. However, we recorded also typical species of forested areas, as *Osteocephalus taurinus* and *B. boans*, common species in riparian forests of the Cerrado and the Amazon biomes (Lima et al. 2005, Jungfer et al. 2013, Matavelli et al. 2013, Freitas et al. 2017). *Boana boans* was restricted to the Amazon Basin, but Matavelli et al. (2013) recorded the first occurrence of this species

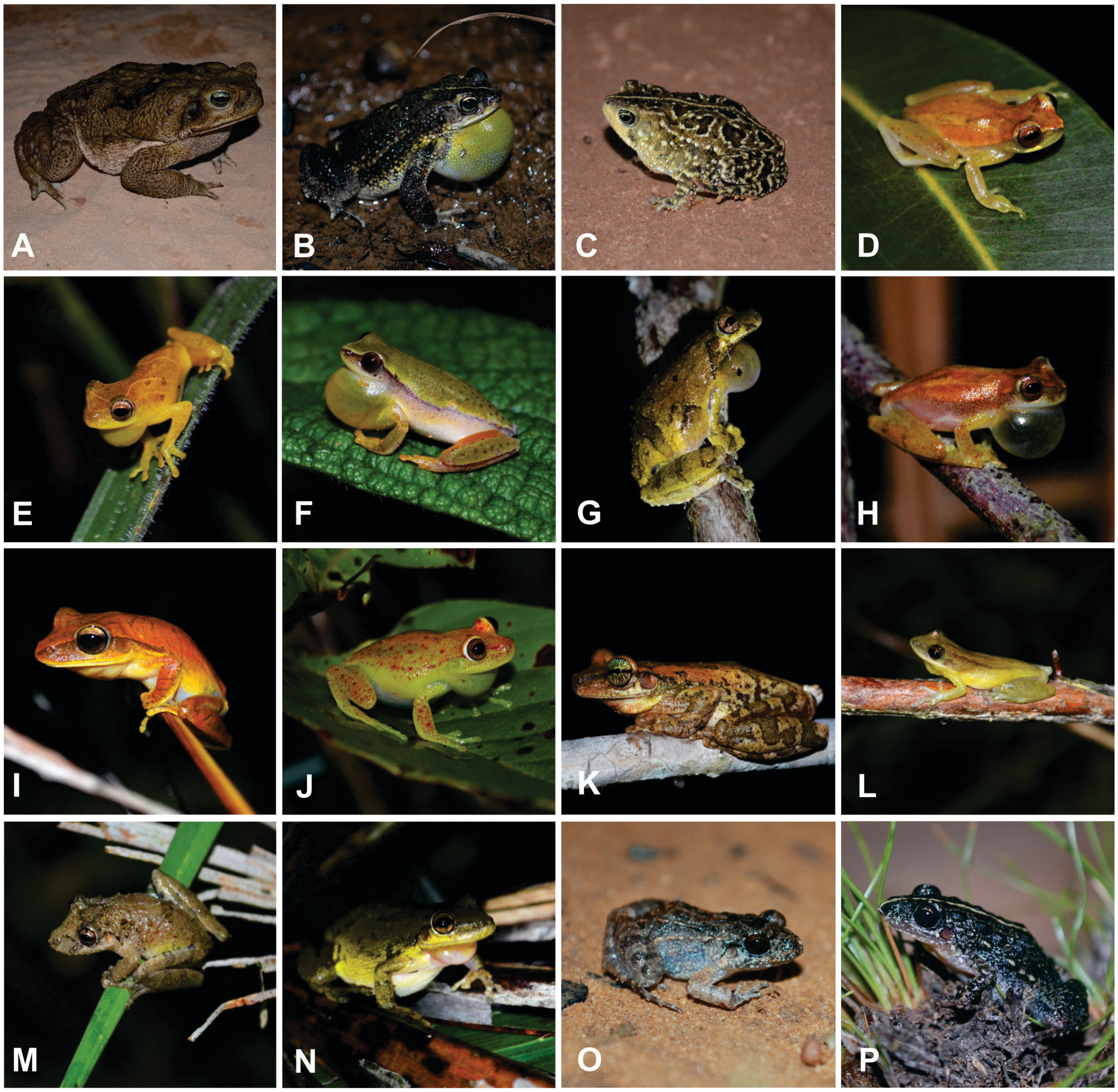

Figure 3. Anurans recorded in the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil. In parentheses, the institutional registration number and the specimen snout-vent length (SVL) for each vouchered specimen. A - *Rhinella jimi* (unvouchered specimen); B - *R. mirandaribeiroi* (CZDP I1 520; SVL 55.63 mm); C - *R. occelata* (CZDP I1 521; 40.70 mm); D - *Dendropsophus braneri* (CZDP I1 604; SVL 16.46 mm); E - *D. minutus* (CZDP I1 533; SVL 22.61 mm); F - *D. rubicundulus* (CZDP I1 535; SVL 21.65 mm); G - *D. soaresi* (CZDP I1 625; SVL 32.07 mm); H - *Dendropsophus* sp. (CZDP I1 607; SVL 19.97 mm); I - *Boana multifasciata* (CZDP I1 525; SVL 44.72 mm); J - *B. punctata* (CZDP I1 529; SVL 29.79 mm); K - *Osteocephalus taurinus* (CZDP I1 615; SVL 71.29 mm); L - *Scinax fuscomarginatus* (CZDP I1 532; SVL 22.76 mm); M - *S. nebulosus* (CZDP I1 611; SVL 26.82 mm); N - *Scinax* aff. *ruber* (CZDP I1 539; SVL 39.59 mm); O - *Adenomera hylaedactyla* (CZDP I1-613; SVL 16.98 mm); P - *A. saci* (CZDP I1 456; SVL 23.05 mm).

in Cerrado areas in the state of Maranhão. Recently, Freitas et al. (2017) recorded the presence of this species in the REBIO Gurupi, a Biological Reserve of the Amazon biome in the northwestern Maranhão. We present here the third record of *B. boans* in the state of Maranhão, filling the distribution gap between the REBIO Gurupi region and the municipality of Mata Roma (Matavelli et al. 2013, Freitas et al. 2017). This indicates that this species has a wide distribution in forest areas in the Amazon and Cerrado biomes. In addition, we present the second record of *Rhinella ocellata* for the state of Maranhão, filling a distribution gap between its first occurrence in the northeast region of the state (Matavelli et al. 2014), and its closest record in the Estação Ecológica Uruçuí-Una, state of Piauí (Dal Vechio et al. 2013).

The accumulation curve presented a strong tendency towards stabilization (Figure 6), indicating that the methods used herein were effective to sample large part of the PEM species. Furthermore, according to the estimators used, it is expected the addition of only two species on observed richness (observed richness = 31 ± 0.79; Jacknife 1 = 33.95 ± 1.67; Bootstrap = 33.34). The species richness of PEM was higher than that reported by other authors in different areas of the Brazilian Cerrado: Barreto et al. (2007; 23 spp.), Silva-Leite et al. (2008; 22 spp.), Oda et al. (2009; 29 spp.), Kopp et al. (2010; 25 spp.), Araujo & Almeida-Santos (2011; 27 spp.), Dal Vechio et al. (2013; 26 spp.) and Dória et al. (2015; 22 spp.), and quite similar to the number of species observed by Valdujo et al. (2009; 32 spp.) in the municipality of São Desidério, Western Chapadão region of the state of Bahia (See Table 3 for comparison between the different localities of the Cerrado). Barreto et al. (2007) has reported the presence of only 23 frog species in a study on the anuran fauna of the Serra do Gado Bravo, River Basin Balsas, state of Maranhão, neighboring region to the PEM.

Figure 4. Anurans recorded in the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil. See caption of the figure 3 for additional information. A - *Adenomera* sp. (CZDP I1 537; SVL 18.42 mm); B - *Leptodactulus fuscus* (CZDP I1 541; SVL 37.79 mm); C - *L. mystaceus* (CZDP I1 543; SVL 44.42 mm); D - *L. petersii* (CZDP I1 627; 42.88 mm); E - *L. sertanejo* (CZDP I1 544; 48.77 mm); F - *L. troglodytes* (CZDP I1 545; SVL 47.48 mm); G - *L. vastus* (CZDP I1 547; SVL 107.76 mm); H - *Physalaemus centralis* (CZDP I1 562; SVL 34.79 mm); I - *P. cuvieri* (CZDP I1 564; SVL 27.09 mm); J - *Pseudopaludicola canga* (CZDP I1 548; SVL 15.32 mm); K - *P. jaredi* (CZDP I1 624; SVL 16.63 mm); L - *P. mystacalis* (CZDP I1 554; SVL 14.89 mm); M - *Pithecopus azureus* (CZDP I1 560; SVL 31.04 mm).

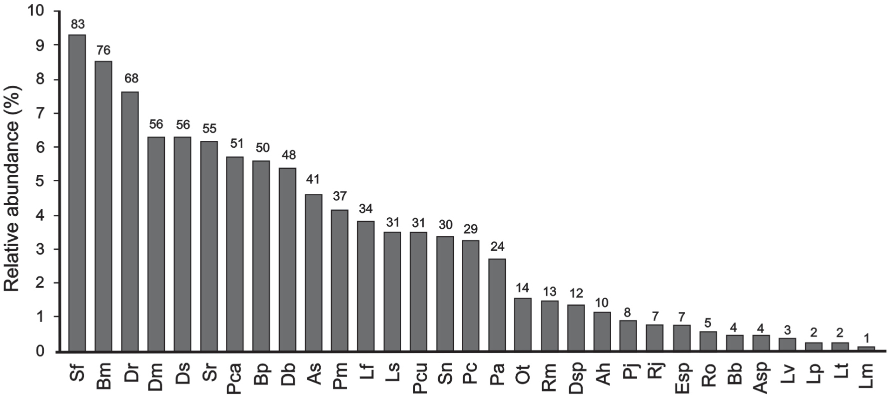

Figure 5. Relative abundance of anuran species in the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil. The total number of recorded individuals is indicated above each column. Anuran species: Rj - *Rhinella jimi*; Rm - *R. mirandaribeiroi*; Ro - *R. occelata*; Db - *Dendropsophus braneri*; Dm - *D. minutus*; Dr - *D. rubicundulus*; Ds - *D. soaresi*; Dsp - *Dendropsophus* sp.; Bb - *Boana boans*; Bm - *B. multifasciata*; Bp - *B. punctata*; Ot - *Osteocephalus taurinus*; Sf - *Scinax fuscomarginatus*; Sn - *S. nebulosus*; Sr - *Scinax* aff. *ruber*; Ah - *Adenomera hylaedactyla*; As - *A. saci*; Asp - *Adenomera* sp.; Lf - *Leptodactulus fuscus*; Lm - *L. mystaceus*; Lp - *L. petersii*; Ls - *L. sertanejo*; Lt - *L. troglodytes*; Lv - *L. vastus*; Pc - *Physalaemus centralis*; Pcu - *P. cuvieri*; Pca - *Pseudopaludicola canga*; Pj - *P. jaredi*; Pm - *P. mystacalis*; Esp - *Elachistocleis* sp.; Pa - *Pithecopus azureus*.

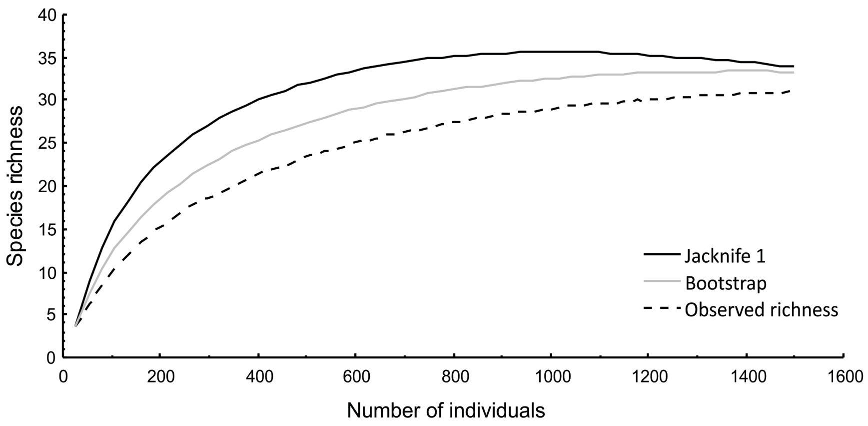

Figure 6. Accumulation curves for anurans sampled in the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil, based on the number of individuals representing the observed (Sobs) and estimated species richness (Bootstrap and Jacknife 1). Curves constructed from 10,000 randomizations.

Table 3. Localities with amphibian inventories in the Cerrado biome, including the Parque Estadual do Mirador (PEM). Spp. - Number of species; SE - sampling effort (days/month); NS - number of sampling sites; SM - sampling method (1 - interception and pitfall traps, 2 - sampling in breeding site; 3 - active search; 4 - casual encounter); DP - distance in kilometers from PEM.

Localities	Spp.	SE (days/month)	NS	SM	DP (Km)	Reference
Balsas-MA	23	40/4	8	1, 3	125	Barreto et al. (2007)
Parque Estadual de Terra-Ronca-GO	22	-/12	4	3	780	Silva-Leite et al. (2008)
Ingazeira Farm-BA	22	30/4	10	1, 3	470	Dória et al. (2015)
Parque Nacional das Emas-GO	25	-/16	12	2	1500	Kopp et al. (2010)
Estação Ecológica Uruçuí-Una-PI	26	66/4	12	1, 3, 4	280	Dal Vechio et al. (2013)
Estação Ecológica de Assis-SP	27	35/7	13	1, 2, 3, 4	1820	Araujo & Almeida-Santos (2011)
Municipality of Niquelândia-GO	29	36/12	14	1, 2, 3	870	Oda et al. (2009)
Parque Estadual do Mirador-MA	**31**	**24/8**	**7**	**2, 3, 4**	**-**	**Present study**
São Desidério-BA	32	12/2	4	2, 3	630	Valdujo et al. (2009)
Southeast of Maranhão	33	28/4	11	1, 2, 3, 4	220	Brasileiro et al. (2008)
Estação Ecológica Nova Roma-GO	36	20/7	8	1, 3	800	Campos & Lage (2014)
Estação Ecológica Serra Geral do Tocantins-TO/BA	37	20/3	8	1, 2, 3	540	Valdujo et al. (2011)
Barro Alto-GO	39	151/24	5	2, 3	985	Gambale et al. (2014)

Even in other environmental protected areas, the species richness was lower than that observed in this study, such as the studies carried out in the Parque Estadual Terra Ronca, state of the Goiás (Silva-Leite et al. 2008), in the Parque Nacional da Emas, state of Goiás (Kopp et al. 2010) and in the Estação Ecológica Uruçuí-Una, state of Piauí (Dal Vechio et al. 2013), in which were recorded 22, 25 and 26 amphibian species, respectively. Our results support the idea that the low anuran diversity of the northern Cerrado biome reflects the small number of studies in these areas (Diniz-Filho et al. 2004, 2005a; Valdujo et al. 2011). In addition, it is evident the importance of PEM in terms of anuran diversity if compared to other areas of the Brazilian Cerrado.

On the other hand, increasing the sampled areas the number of species tends to increase, as observed by Brasileiro et al. (2008) and Melo et al. (2013), which recorded 33 and 36 species, respectively, in studies carried out in various sampling sites in the states of Maranhão and Goiás. Santos et al. (2014) registered 40 species of amphibians in 17 municipalities of the Piracanjuba and Peixe rivers basins in the southeast of Goiás. In the same way, long-term studies conducted to detect small spatio-temporal variations in the species composition between environments of a same region enable the registration of a greater species number and allows determining the actual species richness in the sampled environments (Valdujo et al. 2011, Gambale et al. 2014). In addition, the use of several collection methods is critical to ensure the registration of species with different reproductive behaviors and/or different habits (terrestrial vs. arboreal vs. aquatic habits).

By studying the anuran fauna of underexplored areas, it is possible to find new species occurrences or even new species. Furthermore, it is usual to record species with taxonomic problems, such as the species of the genus *Scinax*. In the PEM, we recognized at least two species populations closely related to *S. ruber*, but to prevent future taxonomic problems, we adopted here a more conservative position recognizing them only as *Scinax* aff. *ruber*, as adopted by other authors (e.g. Valdujo et al. 2011, Dal Vechio et al. 2013, Roberto et al. 2013, Andrade et al. 2014). Similarly, acoustic differences between species of the genus *Elachistocleis* are almost undetectable to human ear, which prevented the identification of the species from this genus in the PEM because they were recorded only by their vocalizations and because there is the possibility that occur at least three species in region: *E. piauiensis*, *E. carvalhoi* and *E. bumbameuboi* (Caramaschi 2010). Furthermore, we believe that *Dendropsophus* sp. and *Adenomera* sp. are still undescribed species, being this latter morphologically similar to population samples obtained in several areas of the Cerrado and Caatinga biomes (e.g., Loebmann & Haddad 2010, Dal Vechio et al. 2013, Roberto et al. 2013).

Cluster analysis evidenced differences in species composition between the different biomes, revealing similarities lower than 30% in most of the comparisions (Figure 7). Despite the anuran community of PEM be composed also by species occurring in other types of environments, we observed the grouping of studies carried out in the Cerrado biome. The anuran species composition of the PEM presented a greater similarity with the study conducted in the Estação Ecológica Uruçuí-Una-EEUU (Dal Vecchio et al. 2013), located about 250 km in the southwest Piauí, and to the study conducted in Serra do Gado Bravo, located about 125 km in municipality of Balsas, southern state of Maranhão. Out of 26 species of anurans recorded in the EEUU, about 60% (16 species) (*D. minutus*, *D. rubicundulus*, *D. soaresi*, *B. multifasciata*, *O. taurinus*, *Pithecopus azureus*, *S. fuscomarginatus*, *L. fuscus*, *L. petersii*, *L. troglodytes*, *L. vastus*, *Physalaemus centralis*, *P. cuvieri*, *R. jimi*, *R. mirandaribeiroi* and *R. ocellata*) were also common to the PEM. Six species (*D. nanus*, *Trachycephalus typhonius*, *Rhaebo guttatus*, *R. veredas*, *Dermatonotus muelleri* and *E. carvalhoi*) were exclusive to the EEUU. Out of 23 species recorded in the Serra do Gado Bravo, about 48% (11 species) were also present in the PEM and in the EEUU (*D. minutus*, *D. rubicundulus*, *B. multifasciata*, *L. fuscus*, *L. petersii*, *L. vastus*, *P. cuvieri*, *R. jimi*, *R. ocellata*, *R. mirandaribeiroi* and *P. azureus*). Seven other species were exclusive to Serra do Gado Bravo (*R. margaritifera*, *B. albopunctata*, *B. raniceps*, *D. nanus*, *D. walfordi*, *Barycholos ternetzi* and *E. piauienseis*).

Although this hypothesis has not been tested, we believe that the similarity pattern among the PEM, SGB and EEUU could be explained by biogeographic, historical and climatic factors that resulted in the formation of similar vegetation types (Colli et al. 2002). The three protected areas are located in high and steep areas of interfluvial, forming extensive gallery forests in drained areas (Conceição & Castro 2009, Dal Vecchio et al. 2013) that allows displacement of species between patches of vegetation (Rodrigues 2005) favoring species composition similarity among these areas. Additionaly, the PEM presents several types of vegetation formations (typical cerrado, cerradão, veredas, dirty fields, flooded fields, open fields and riparian forests) and is inserted in the Uruçuí-Mirador biodiversity corridor, which can explain the great species richness and species composition. The Uruçuí-Mirador corridor, still in the implementation phase, has an estimated initial area of about 13.4 million hectares, distributed among the states of Piauí, Maranhão and Tocantins (Conceição & Castro 2009, Dal Vecchio et al. 2013).

We also observed the occurrence of typical species from other biomes within the PEM, such as *B. boans* and *P. canga* (typical species of the Amazon biome), and *L. troglodytes* and *L. vastus* (typical species of

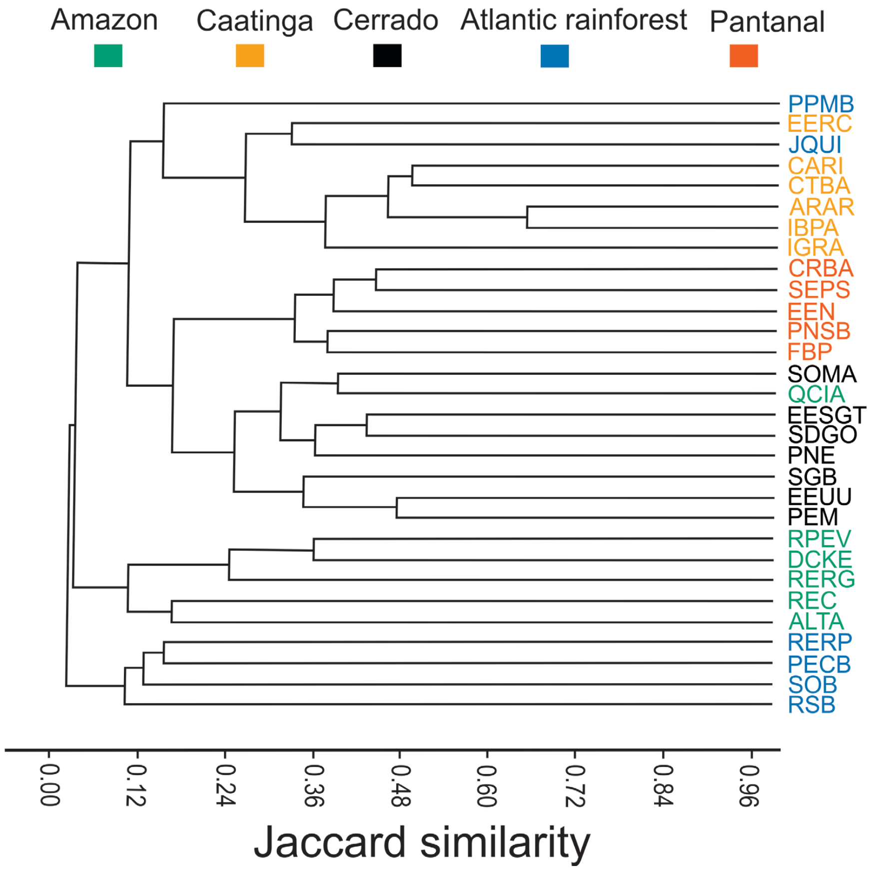

Figure 7. Similarity (Jaccard index and clustering method "UPGMA"; cophenetic correlation coefficient = 0.8841) among the anuran species composition of the Parque Estadual do Mirador (PEM), state of Maranhão, Northeastern Brazil, and 24 Brazilian locaties distributed in the following biomes: **Amazon** (*DCKE* - Reserva Ducke; *REC* - Reserva Experimental Catuaba; *ALTA* - Altamira; *RPEV* - Rio Preto da Eva; *RERG* - Reserva Extrativista do Rio Gregório; *QCIA* - Querência); **Caatinga** (*CARI* - Boa Vista e São João do Cariri; *IBPA* - Planalto da Ibiapaba; *ARAR* - Chapada do Araripe; *EERC* - Estação Ecológica Raso da Catarina; *IGRA* - Ilha Grande, Piauí; *PNC* - Parque Nacional do Catimbau); **Cerrado** (*SOMA* - Bacia do Rio Tocantins, Sudoeste do Maranhã; *SGB* - Serra do Gado Bravo; *PNE* - Parque Nacional das Emas; *EESGT* - Estação Ecológica Serra Geral do Tocantins; *EEUU* - Estação Ecológica Uruçuí-Una; *SDGO* - Bacia dos Rios Piracanjuba e Peixe, Sudeste de Goiás; *PEM* - Parque Estadual do Mirador); **Atlantic Forest** (*PPMB* - Área de Preservação Permanente Mata do Buraquinho; *RERP* - Reserva Rio das Pedras; *SOB* - Serra do Ouro Branco; *PECB* - Parque Estadual Carlos Botelho; *JQUI* - Jequié; *RSB* - Reserva de Serra Bonita); and **Pantanal** (*CRBA* - Corumbá; *SEPS* - Serras de Entorno do Pantanal Sul; *EEN* - Estação Ecológica Nhumirim; *PNSB* - Parque Nacional da Serra da Bodoquena; *FBP* - Fazenda Baía de Pedra.

the Caatinga biome). This pattern of species composition in the PEM is commonly observed in studies of anurans from the Cerrado biome (Uetanabaro et al. 2007, Brasileiro et al. 2008, Valdujo et al. 2009, 2011, Dal Vechio et al. 2013) and could be a result of its location in the region of Chapadas do Alto Itapecuru. This region receives the influence from elements of the Amazon and Caatinga biomes (e.g. vegetation, climate, hydrography, soil), which forms a mosaic of connected vegetation that enables a possible exchange of species between neighboring areas (Ganem 2007). In this case, PEM is located in a transition zone (humid vs. semiarid) and the spatial arrangement of habitat patches provides corridors for possible dispersal among the nearby wetlands (Maltchik et al. 2008).

Our research is one of the few studies to inventory the amphibians' species of south-central region of the state of Maranhão. It also provides important data about the amphibian communities of the PEM, which can contribute to implementation of management plan for the PEM and the for development of conservation measures of the amphibians' species in the northern part of the Brazilian Cerrado. Despite the observed species richness, we believed that the number of species is even greater, since the PEM has several not yet sampled environments and the methods adopted here do not include fossorial species registration or reproductive habits of the explosive type. Thus, there is a need to conduct future surveys to improve the knowledgement about the diversity and distribution of anurans in this important area in the Northeastern Brazil. In addition, further ecological studies are needed to understand the species richness pattern and the dynamics of PEM's amphibian populations, as these data are essential for the implementation of measures for species conservation. In fact, systematic studies on conservation planning are extremely urgent in the region, because of the threat imposed by agricultural expansion in the Cerrado biome that is replacing the natural floristic composition by large grain monoculture plantations, causing numerous environmental impacts and leading to the loss of regional biodiversity in the northern distribution of the Cerrado biome. Furthermore, the Parque Estadual do Mirador is an important bioregional corridor of preservation, which is crucial for the maintenancy of species diversity.

Supplementary material

The following online material is available for this article:

Appendix 1 – Voucher specimens

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Author contributions

Etielle Barroso de Andrade: contributed in data acquisition, analysis and interpretation of data, and writing of the paper.

Luiz Norberto Weber and José Roberto de Souza Almeida Leite: contributed in the conception and design of the paper and critical review adding intellectual content.

Conflicts of interest

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

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Inventory

Inventory of Ferns and Lycophytes of the RPPN Pedra D'Antas, Pernambuco state, northeastern Brazil

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Abstract: The Northeastern Atlantic Forest (NAF) has a historic of intense habitat loss and fragmentation. In this context, knowledge of the species from forest remnants is urgently needed and represents a key tool for future studies and conservation strategies. Here, we present the floristic inventory of ferns and lycophytes from the Private Natural Heritage Reserve - RPPN Pedra D'Antas, located in a NAF area. The study area associated with the RPPN Frei Caneca is the Serra do Urubu, which is a hotspot for the diversity of several biological groups in the NAF. We recorded 74 fern species, distributed in 17 families and 46 genera. The most diverse families were Pteridaceae (17 species), Polypodiaceae (14 spp.), Dryopteridaceae (nine spp.) and Thelypteridaceae (seven spp.). Lycophytes were represented by four species, two families and two genera. A new record for the Northeast of Brazil, *Serpocaulon hirsutulum*, is presented in this study. The area has a large number of species, some of which are restricted to the NAF (e.g. *Megalastrum umbrinum*). Based on the richness of the study area associated with another inventory held at the RPPN Frei Caneca (138 spp.), the Serra do Urubu is corroborated as a hot-spot for the diversity of ferns and lycophytes in the NAF.

Keywords: *Atlantic Forest, biodiversity, conservation, floristic survey, seedless vascular plants*

Inventário de Samambaias e Licófitas da RPPN Pedra D'Antas, estado de Pernambuco, nordeste do Brasil

Resumo: A Floresta Atlântica Nordestina (FAN) possui um histórico de intensa perda e fragmentação de hábitats. Neste contexto, o conhecimento das espécies a partir das áreas ainda remanescentes é urgentemente requerido, constituindo a ferramenta básica para estudos futuros e estratégias conservacionistas. Aqui, apresentamos o inventário de samambaias e licófitas a partir da Reserva Particular do Patrimônio Natural - RPPN Pedra D'Antas, situada em área da FAN. A área de estudo associada a RPPN Frei Caneca constitui a Serra do Urubu, conhecida como um hot-spots de diversidade para diversos grupos biológicos na FAN. Registramos 74 espécies de samambaias, distribuídas em 17 famílias e 46 gêneros. As famílias mais diversas foram Pteridaceae (17 espécies), Polypodiaceae (14 spp.), Dryopteridaceae (nove spp.) e Thelypteridaceae (sete spp.). As licófitas foram representadas por quarto espécies, duas famílias e dois gêneros. Apresentamos um novo registro para o Nordeste brasileiro, *Serpocaulon hirsutulum*. A área possui um número expressivo de espécies, algumas das quais com destruição restrita a FAN (e.g. *Megalastrum umbrinum*). A partir da riqueza da área de estudo associada a outro inventário realizado na RPPN Frei Caneca (138 spp.), corroboramos a Serra do Urubu como um hot-spot de diversidade para as samambaias e licófitas na FAN.

Palavras-chave: *Floresta Atlântica, biodiversidade, conservação, levantamento florístico, plantas vasculares sem sementes*

Introduction

The number of floristic inventories for the various ecosystems of the Brazilian territory has grown in recent decades, as evidenced by the Forzza et al. 2010. However, thorough knowledge of the high plant diversity occurring in the country, which is highly threatened by many anthropogenic pressures, still requires more efforts. Inventories are the means by which such knowledge is achieved (Magurran 2004). They allow recognizing the areas that hold highest richness as well as update the floras and biogeographical patterns of distribution of species (e.g. new records of species and/or species with restricted distribution), which represents key information for several biological studies and future conservation strategies (see Prado & Hirai 2011, Gasper et al. 2012), identifying forest remnants most important to conservation on a given geographic or politic scale.

The need for more inventories is stressed by the excessive loss of species caused by intense levels of degradation in Brazilian ecosystems. It is likely that some species may disappear even before registration at local, regional or national level. Particularly, the Northeastern Atlantic Forest (NAF) in Brazil (region north of the São Francisco River, covering the States of Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, and enclaves in Ceará State) has a notorious scenario of intense habitat loss and fragmentation resulting from a long historical period of disturbances. Furthermore, few protected areas have been established in this biogeographical unit, which is recognized by its high vulnerability and loss of species (Tabarelli et al. 2006, Ribeiro et al. 2009).

The Serra do Urubu comprises two protected areas: the Private Natural Heritage Reserve (Reserva Particular do Patrimônio Nacional – RPPN) Frei Caneca and the RPPN Pedra D'Antas, and represents one of the last largest forest NAF remnants, with approximately 1000 ha. This area has high species richness and endemism, being considered a hotspot for biodiversity of flowering plants in the NAF (Melo et al. 2016), and one of the most important areas for bird conservation in the Neotropics (SAVE Brasil 2016). Moreover, the RPPN Frei Caneca has the highest richness of ferns and lycophytes, the seedless vascular plant groups, ever recorded to a NAF (138 spp.) (Lopes 2003). Here, we present the inventory of the ferns and lycophytes flora of the RPPN Pedra D'Antas, where a first record of *Serpocaulon hirsutulum* for the northeastern Brazil is reported and the status of the Serra do Urubu as a hotspot for ferns diversity in NAF is confirmed.

Material and Methods

1. Study area

The RPPN Pedra D'Antas (8°42'14''S – 35°51'10''W, max. alt.: 800 m) is located in Lagoa dos Gatos (47.25% of the area), Jaqueira (6.72%) and São Benedito do Sul (46.03%) municipalities of the State of Pernambuco, northeastern Brazil. The area presents approximately 325 hectares of Open Ombrophylous Atlantic Forest in diverse stages of preservation (Figure 1). The relief of the area is hilly with flat tops, steep slopes and narrow valleys. The area has predominantly sandy-clay, yellowish red argisol soil (IBGE 1995). There are several perennial water bodies of the Una River Basin (CPRM 2005). The local climate is tropical moist as with hot and dry summer and rainy winter, according to Koppen classification (1948). The average annual rainfall is approximately 1,345 mm. The annual average temperature is 23.6°C, ranging from 18°C to 30°C (CPRM 2005).

Figure 1. Part of the RPPN Pedra D'Antas, a remnant of Open Ombrophilous Atlantic Forest, situated in state of Pernambuco, northeastern Brazil.

2. Inventory

Fifteen trips were carried out from October/2011 to January/2013 to the study area. On each trip, the forest fragment was explored through systematic walks, georeferencing points with GPS and giving priority to the preferential environments for establishment of ferns and lycophytes (e.g. forest edge and interior, areas near streams and other water bodies, ravines, valleys and slopes and rocky areas). Besides the systematic exploration of these environments, random walks were also conducted to cover the most of the forest fragment area. Collection and herborization of specimens followed the usual techniques for vascular plants (Mori et al. 1989). Voucher specimens were incorporated into the UFP herbarium collection. Identification was carried out with aid of specialized bibliography, consultation with specialists and comparison with herborized material from the UFP herbarium determined by specialists. The classification presented follows the Pteridophyte Phylogeny Group (PPG 2016). The names of the authors of the species were abbreviated according to the Flora do Brasil (2020).

Field notes at the collection site of specimens were taken, ranking the species in the following categories: terrestrial, rupicolous, hemiepiphyte and holoepiphyte (Salvo Garcia & Verdugo 1990). For comparison of species richness in the study area with other areas, we consulted the available inventories in the NAF. Comments about some taxa (distribution and taxonomic characteristics) were based on cited literature.

Results and Discussion

Seventy-four species of ferns (Polypodiospsida Class), representing 46 genera and 17 families were recorded in the RPPN Pedra D'Antas (Table 1). As for lycophytes (Lycopodiopsida Class), four species in two families and two genera were recorded (Table 1). The fern and lycophyte richness of the study area (79 spp.) can be considered high when contrasted to other NAF areas (Table 2). This number of species represents about 36% of the richness of ferns and lycophytes estimated for the state of Pernambuco (214 spp.) according to Prado et al. (2015). This emphasizes the relevance of preserving the area for the conservation of the studied groups.

The most representative families were Pteridaceae (17 species), Polypodiaceae (14 spp.), Dryopteridaceae (nine spp.) and Thelypteridaceae (seven spp.). These families account for approximately 64% of the sampled ferns. Among the sampled families, 26% are represented by only one species. The genus *Adiantum* L. presented the highest specific representativeness, nine species (Table 1).

The fern and lycophyte flora of the present area is mostly composed of species common to other NAF inventoried areas (e.g. Santiago & Barros 2003, Xavier & Barros 2003, Santiago et al. 2004, Xavier & Barros 2005, Pietrobom & Barros 2006, Pereira et al. 2011, Pereira et al. 2013). However, some taxa are not usually registered in NAF, such *as Megalastrum umbrinum* (Farias et al. 2015), which has been found only in the present area, as well *Elaphoglossum glabellum* and *Ctenitis glandulosa*, which had few records in this unit and were previously recorded in two other NAF areas.

In addition, *Serpocaulon hirsutulum* was found, representing a new record for the Northeast of Brazil. The species *S. hirsutulum* (see characteristics in Schwartsburd & Smith 2013) has distribution restricted to the states of Minas Gerais, São Paulo (southeast) and Pernambuco (northeast). In the latter, it is recorded in two areas (i.e. Serra do Urubu): the RPPN Frei Caneca (recently determined and unpublished material) and the present studied area.

Only one exotic species was recorded in the RPPN Pedra D'Antas: *Macrothelypteris torresiana*. This species is native from Madagascar, Japan, Indonesia, Thailand, Malaysia, Queenslândia (Robinson et al., 2010), and it is widely naturalized and often collected in Brazilian forests, particularly in the areas of the Atlantic Forest and Cerrado, according to Salino & Almeida (2015).

As for the types of habitat, terrestrial species (55%) were predominant. This result supports the majority of fern and lycophyte inventories in the NAF (e.g. Santiago & Barros 2003, Xavier & Barros 2003, Santiago et al. 2004, Xavier & Barros 2005, Pietrobom & Barros 2006, Pereira et al. 2011, Pereira et al. 2013). This pattern of predominance of terrestrial habitat can be explained by the diversity of favorable conditions promoted by this substrate, such as water availability, shade, as well as greater availability of nutrients (Sota 1971).

The high richness recorded here for the RPPN Pedra D'Antas and reported by Lopes (2003) for the RPPN Frei Caneca supports the concept this area as a hotspot for the fern and lycophyte diversity in the NAF. As a consequence, the importance of effective protection of the area is reinforced.

Table 1. Ferns (Polypodiopsida) and Lycophytes (Lycopodiopsida) recorded in Conservation Unit of the Northeastern Atlantic Forest - RPPN Pedra D'Antas, Pernambuco, Brazil, between October/2011 and January/2013.

Class/Family	Species	Habitats	Voucher Number	Collector Number
POLYPODIOPSIDA				
Anemiaceae	*Anemia hirta* (L.) Sw.	Rupicolous	UFP 81.287	R.P. Farias 121
	Anemia villosa Humb. & Bonpl. ex Willd.	Rupicolous	UFP 81.285	R.P. Farias 134
Aspleniaceae	*Asplenium auritum* Sw.	Holocorticicolous	UFP 81.278	R.P. Farias 90
	Asplenium formosum Willd.	Rupicolous / Holocorticicolous	UFP 81.279	R.P. Farias 115
	Asplenium salicifolium L.	Rupicolous	UFP 81.280	R.P. Farias 141
	Asplenium serratum L.	Rupicolous / Holocorticicolous	UFP 81.281	R.P. Farias 101
Blechnaceae	*Neoblechnum brasiliense* (Desv.) Gasper & V.A.O. Dittrich	Terricolous	UFP 81.338	R.P. Farias 100
	Blechnum occidentale L.	Terricolous	UFP 81.337	R.P. Farias 107
	Telmatoblechnum serrulatum (Rich.) Perrie, D.J. Ohlsen & Brownsey	Terricolous	UFP 81.336	R.P. Farias 120
	Salpichlaena volubilis (Kaulf.) J.Sm.	Hemicorticicolous	UFP 81.340	R.P. Farias 110
Cyatheaceae	*Cyathea abreviata* I.Fern.	Terricolous	UFP 81.371	R.P. Farias 25
	Cyathea microdonta (Desv.) Domin	Terricolous	UFP 81.372	R.P. Farias 181
	Cyathea praecincta (Kunze) Domin	Terricolous	UFP 81.363	R.P. Farias 125
Dennstaedtiaceae	*Pteridium arachnoideum* (Kaulf.) Maxon	Terricolous	UFP 81.320	R.P. Farias 168
Dryopteridaceae	*Ctenitis distans* (Brack.) Ching	Terricolous	UFP 81.117	R.P. Farias 155
	Ctenitis glandulosa R.S. Viveiros & Salino	Terricolous	UFP 81.119	R.P. Farias 157
	Cyclodium heterodon (Schrad.) T. Moore	Terricolous	UFP 81.317	R.P. Farias 34
	Cyclodium meniscioides (Willd.) C. Presl	Terricolous	UFP 81.341	R.P. Farias 94
	Elaphoglossum glabellum J. Sm.	Holocorticicolous	UFP 81.370	R.P. Farias 119
	Megalastrum umbrinum (C. Chr.) A.R. Sm & R.C. Moran	Terricolous	UFP 75.754	R.P. Farias 15
	Mickelia guianensis (Aubl.) R.C Moran, Sundue & Labiak	Hemicorticicolous	UFP 81.345	R.P. Farias 79
	Olfersia cervina (L.) Kunze	Terricolous	UFP 81.352	R.P. Farias 127
	Polybotrya osmundaceae Willd.	Hemicorticicolous	UFP 81.343	R.P. Farias 77
Gleicheniaceae	*Gleichenellla pectinata* (Willd.) Ching	Terricolous	UFP 81.369	R.P. Farias 88
Hymenophyllaceae	*Didymoglossum hymenoides* (Hedw.) Copel.	Rupicolous / Holocorticicolous	UFP 81.311	R.P. Farias 140
	Didymoglossum krausii (Hook. & Grev.) C. Presl	Rupicolous / Holocorticicolous	UFP 81.312	R.P. Farias 136
	Hymenophyllum polyanthos (Sw.) Sw.	Holocorticicolous	UFP 81.316	R.P. Farias 139
Lindsaeaceae	*Lindsaea lancea* (L.) Bedd.	Terricolous	UFP 81.342	R.P. Farias 83
Lomariopsidaceae	*Lomariopsis japurensis* (Mart.) J. Sm.	Hemicorticicolous	UFP 81.353	R.P. Farias 81
	Nephrolepis biserrata (Sw.) Schott	Rupicolous	UFP 81.354	R.P. Farias 126
Lygodiaceae	*Lygodium venustum* Sw.	Terricolous	UFP 81.277	R.P. Farias 122
	Lygodium volubile Sw.	Terricolous	UFP 81.276	R.P. Farias 114
Marattiaceae	*Danaea geniculata* Raddi	Terricolous	UFP 81.308	R.P. Farias 129
Polypodiaceae	*Campyloneurum nitidum* (Kaulf.) C. Presl	Rupicolous / Holocorticicolous	UFP 81.335	R.P. Farias 149
	Campyloneurum repens (Aubl.) C. Presl	Rupicolous / Holocorticicolous	UFP 81.355	R.P. Farias 82
	Pleopeltis desvauxii (Klotzsch) Salino	Rupicolous / Holocorticicolous	UFP 81.328	R.P. Farias 164
	Microgramma vacciniifolia (Langsd. & Fisch.) Copel.	Holocorticicolous	UFP 81.349	R.P. Farias 78
	Microgramma lycopodioides (L.) Copel	Holocorticicolous	UFP 81.346	R.P. Farias 72
	Pecluma pilosa (A.M. Evans) M. Kessler & A.R. Sm.	Holocorticicolous	UFP 81.333	R.P. Farias 178
	Phlebodium pseudoaureum (Cav.) Lellinger	Holocorticicolous	UFP 81.331	R.P. Farias 144
	Phlebodium decumanum (Willd.) J. Sm.	Holocorticicolous	UFP 81.332	R.P. Farias 143
	Pleopeltis astrolepis (Liebm.) E. Fourn.	Rupicolous / Holocorticicolous	UFP 81.358	R.P. Farias 95
	Pleopeltis furcata (L.) A.R. Sm.	Holocorticicolous	UFP 81.351	R.P. Farias 68
	Pleopeltis hirsutissima (Raddi) de la Sota	Rupicolous / Holocorticicolous	UFP 81.366	R.P. Farias 62
	Polypodium dulce Poir.	Rupicolous / Holocorticicolous	UFP 81.303	R.P. Farias 153
	Serpocaulon catharinae (Langsd. & Fisch.) A.R. Sm.	Holocorticicolous	UFP 81.305	R.P. Farias 151
	Serpocaulon hirsutulum (T. Moore) Schwartsb. & A.R. Sm	Terricolous	UFP 81.302	R.P. Farias 150

*Specimens with sterile material.

Table 1. Continued...

Class/Family	Species	Habitats	Voucher Number	Collector Number
Pteridaceae	*Adiantopsis radiata* (L.) Fée	Terricolous	UFP 81.244	R.P. Farias 105
	Adiantum abscissum Schrad.	Terricolous	UFP 81.245	R.P. Farias 54
	Adiantum dolosum Kunze	Terricolous	UFP 81.247	R.P. Farias 48
	Adiantum glaucescens Klotzsch	Terricolous	UFP 81.250	R.P. Farias 52
	Adiantum latifolium Lam.	Terricolous	UFP 81.252	R.P. Farias 43
	Adiantum obliquum Willd.	Terricolous	UFP 81.258	R.P. Farias 47
	Adiantum pulverulentum L.	Terricolous	UFP 81.259	R.P. Farias 50
	Adiantum terminatum Kunze ex Miq.	Terricolous	UFP 81.261	R.P. Farias 44
	Adiantum tetraphyllum Willd.	Terricolous	UFP 81.263	R.P. Farias 55
	Ananthacorus angustifolius (Sw.) Underw. & Maxon	Rupicolous	UFP 81.275	R.P. Farias 61
	Doryopteris sagittifolia J. Sm.	Rupicolous	UFP 81.270	R.P. Farias 97
	Doryopteris varians Sm.	Rupicolous	UFP 81.269	R.P. Farias 75
	Hemionitis palmata L.	Rupicolous	UFP 81.274	R.P. Farias 102
	Hemionitis tomentosa (Lam.) Raddi	Rupicolous	UFP 81.272	R.P. Farias 106
	Pityrogramma calomelanos (L.) Link	Terricolous	UFP 81.268	R.P. Farias 113
	Vittaria graminifolia Kaulf.	Holocorticícola	UFP 81.267	R.P. Farias 93
	Vittaria lineata (L.) Sm.	Rupicolous / Holocorticicolous	UFP 81.266	R.P. Farias 111
Saccolomataceae	*Saccoloma elegans* Kaulf.*	Terricolous		
Tectariaceae	*Triplophyllum dicksonioides* (Fée) Holttum	Terricolous	UFP 81.307	R.P. Farias 86
	Triplophyllum hirsutum (Holttum) J. Prado & R.C Moran	Terricolous	UFP 81.306	R.P. Farias 84
Thelypteridaceae	*Christellla hispidula* (Decne.) Holttum	Terricolous	UFP 81.321	R.P. Farias 174
	Cyclosorus interruptus (Willd.) H. Itô	Terricolous	UFP 81.360	R.P. Farias 132
	Goniopteris jamesonii (Hook.) Salino & T.E. Almeida	Terricolous	UFP 81.327	R.P Farias 177
	Macrothelypteris torresiana (Gaudich.) Ching*	Terricolous		
	Meniscium macrophyllum Kunze	Terricolous	UFP 81.326	R.P. Farias 142
	Meniscium serratum Cav.	Terricolous	UFP 81.359	R.P. Farias 131
	Steiropteris polypodioides (Raddi) Salino & T.E. Almeida	Terricolous	UFP 81.323	R.P. Farias 170
LYCOPODIOSPSIDA				
Lycopodiaceae	*Palhinhaea cernua* (L.) Vasc. & Franco	Terricolous	UFP 81.310	R.P. Farias 73
Selaginellaceae	*Selaginella muscosa* Spring	Terricolous	UFP 81.121	R.P. Farias 159
	Selaginella producta Baker	Terricolous	UFP 81.120	R.P. Farias 158
	Selaginella sulcata (Desv. ex. Poir.) Spring ex Mart.	Terricolous	UFP 81.122	R.P. Farias 160

*Specimens with sterile material.

Table 2. Ten inventories of Ferns (Polypodiopsida) and Lycophytes (Lycopodiopsida) performed Northeastern Atlantic Forest with greatest number of species recorded.

Inventories	Study Site/State	Number of species
Lopes (2003)	RPPN Frei Caneca/Pernambuco	138
Pereira et al. (2013)	ESEC Murici/Alagoas	107
Santiago et al. (2004)	Bonito/Pernambuco	91
Pietrobom & Barros (2003)	Mata do Estado/Pernambuco	90
Barros et al. (2006)	Serra Grande Mill/Alagoas	85
Pietrobom & Barros (2007)	Água Azul Mill/Pernambuco	83
This Study	**RPPN Pedra D'Antas/Pernambuco**	**79**
Pereira et al. (2007)	Gurjaú Ecological Forest/Pernambuco	75
Pietrobom & Barros (2006)	Maria Maior Forest/Alagoas	72
Xavier & Barros (2005)	João Vasconcelos Sobrinho Ecological Park/Pernambuco	66

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Author's Contribution

Rafael de Paiva Farias: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Ivo Abraão Araújo da Silva: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Anna Flora de Novaes Pereira: contribution to data analysis and interpretation, contribution to manuscript preparation.

Augusto César Pêssoa Santiago: contribution to data analysis and interpretation, contribution to manuscript preparation.

Iva Carneiro Leão Barros: Contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Identification Key

Identification key for fishes from coastal streams of the Atlantic forest of southeastern Brazil

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Abstract: In recent decades, current knowledge about fish from Neotropical streams has greatly increased, but is still deficient. Here we present an identification key for fishes from coastal freshwater streams from a large conservation area of Atlantic rainforest of southeastern Brazil, including 39 species. Considering that most of these species (61.2%) are endemic to the coastal streams, this identification key will be useful not only for the species recognition of the sampled area, but also for the surrounding coastal region.

Keywords: *taxonomy, endemic and endangered fishes, Neotropical streams, Juréia-Itatins Ecological Station.*

Chave de identificação para os peixes de riachos costeiros da Mata Atlântica, sudeste do Brasil

Resumo: Nas últimas décadas, o conhecimento acerca dos peixes de riachos neotropicais aumentou consideravelmente, mas apesar disso ainda é deficiente. Apresentamos aqui uma chave de identificação para as 39 espécies de peixes de uma grande unidade de conservação da Mata Atlântica do sudeste do Brasil. Considerando que a maioria destas espécies (61,2%) são endêmicas dos riachos costeiros, esta chave de identificação será útil não só para o reconhecimento de espécies da área amostrada, mas também da região costeira do entorno.

Palavras-chave: *taxonomia, peixes endêmicos e ameaçados, riachos neotropicais, Estação Ecológica Juréia-Itatins.*

Introduction

The Serra do Mar was formed from an uplift process during the Cretaceous, which originated a sequence of 1,000 km of mountains near the newly formed coast. Its current relief was shaped over millions of years by erosion and tectonic activities and currently covers the states of Rio de Janeiro to Santa Catarina (Oyakawa et al. 2006). The Atlantic forest is a complex biome that covers practically the entire Serra do Mar, and although human occupation has degraded 90% of its area, this biome harbors a significant part of the Brazilian biodiversity (Joly et al. 1999); 40% of the 2,000 vertebrate species are endemic (Oyakawa et al. 2006).

In this region, there are mountain streams with clear and fast waters due to the high slope of the relief, low temperatures and high concentration of dissolved oxygen, and the lowland streams that drains the less steep coastal plain forming meanders with black, slower, turbid and acidic waters with higher temperatures, lower dissolved oxygen concentration and sandy bottom (Por 2004). Due to the altitudinal gradient, coastal streams rise with waters typical of mountain streams that change when they reach the plains and with the proximity of the mouth in the sea (Gonçalves & Braga 2012).

These and other habitat characteristics influence the ichthyofauna of coastal streams (Abilhoa et al. 2011, Barrella et al. 2014, Gonçalves et al. 2015). The conservation of riparian forest is important since deforestation negatively impacts the survival of fish due to silting, increased sunlight incidence and decreased invertebrate fauna, among other impacts (Lorion & Kennedy 2009, Leite et al. 2015). This can lead to drastic consequences such as changes in reproductive and feeding behaviors of many species (Menezes et al. 2007, Ferreira et al. 2012, Lobón-Cerviá et al. 2016).

The Juréia-Itatins Ecological Station is a conservation unit on the south coast of the state of São Paulo with streams that protects several endemic species (and some endangered) of the Atlantic forest (Gonçalves & Braga 2013, Gonçalves et al. 2016, Gonçalves & Pérez-Mayorga 2016). Characiformes and Siluriformes are predominant, but other orders such as Cichliformes and Gobiiformes (*sensu* Betancur-R et al. 2017), Synbranchiformes, Cyprinodontiformes, and Gymnotiformes are also present (Gonçalves & Pérez-Mayorga 2016), as well as primarily marine families (Sabino & Silva 2004). Fish occupy different stream reaches according to their feeding habits and swimming capacity, varying between rapids, marginal backwaters or position in the water column (Sabino & Silva 2004). In the freshwater streams of of Juréia-Itatins Ecological Station, fish feed primarily on resources provided by riparian forest, such as terrestrial insects and vegetable debris (also consumed by immature forms of aquatic insects that will feed aquatic insectivorous fish), stressing the importance of legally protected areas in Atlantic forest (Gonçalves et al. 2013). The small size of most species of these coastal streams and the scarcity of keys can make it difficult to identify the fish of this region. The objective of this paper is to provide an identification key for fishes from coastal streams of Juréia-Itatins Ecological Station.

Material and Methods

The material used in this study was collected every three months between April 2009 and February 2010, and once in June 2013 (cf. Gonçalves & Braga 2012, 2013, Gonçalves & Pérez-Mayorga 2016) at the Juréia-Itatins reserve, an Atlantic rainforest pristine area with 79.240 ha on the south coast in the State of São Paulo, Brazil (24°18’, 24°32’ S and 47°00’, 47°30’ W). Average annual rainfall and temperature are 2,277 mm and 21.4 °C, respectively. A hot and rainy season occurs from October to April, and the less rainy season from May to September (Marques & Duleba 2004). Altitudes vary from sea level at alluvial plains to 1,240 m a.s.l. at steep mountains (Por 1986, Souza & Souza 2004). Due to this, local hydrography is influenced by the different vegetation types of dense ombrophilous forest found at different elevation: black waters (rich in humic substances with pH ca. 4) drains the alluvial dense ombrophilous forest and the lowland dense ombrophilous forest (also known as restinga forest), and clear waters (nutrient poor with pH ca. 6) drains the submontane dense ombrophilous forest and the montane dense ombrophilous forest (Por 1986, Por & Lopes 1994, Por 2004). Fish were sampled at 73 locations, using electrofishing, fishing nets, traps, and sieve (Gonçalves & Pérez-Mayorga 2016). Specimens were anesthetized with benzocaine, fixed in 10% formalin, and then kept in 70% ethanol until the analysis. Counts were taken on the left side of specimens as proposed by Fink & Weitzman (1974). The identification key provided was based on easily recognizable external morphological characters in most cases. The fishes classification followed Betancur-R et al. (2017). Voucher specimens (Table 1) are deposited in the fish collections of Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP/DZSJRP), Câmpus São José do Rio Preto, and Museu de Zoologia of Universidade de São Paulo (MZUSP), São Paulo, Brazil.

Table 1. The thirty-nine fish species registered in the coastal freshwater streams from Juréia-Itatins Ecological Station, a large conservation area of Atlantic rainforest of southeastern Brazil. (1) endemic to the Atlantic forest (Menezes et al. 2007), (2) endangered fish (State Decree Nº 60.133 07/02/2014), (-) voucher not available.

Order/Family	Species	Voucher
CHARACIFORMES		
Curimatidae	^{1}*Cyphocharax santacatarinae* (Fernández-Yépez, 1948)	DZSJRP 20732
Crenuchidae	^{1}*Characidium lanei* Travassos, 1967	DZSJRP 20733
	^{1}*Characidium pterostictum* Gomes, 1947	DZSJRP 20738
	^{1,2}*Characidium schubarti* Travassos, 1955	DZSJRP 13252
Characidae	^{1}*Astyanax ribeirae* Eigenmann, 1911	DZSJRP 13256
	^{1}*Deuterodon iguape* Eigenmann, 1907	DZSJRP 13240
	^{1}*Hollandichthys multifasciatus* (Eigenmann & Norris, 1900)	DZSJRP 13253
	^{1}*Hyphessobrycon griemi* Hoedeman, 1957	DZSJRP 13247
	^{1}*Hyphessobrycon boulengeri* (Eigenmann, 1907)	DZSJRP 13248
	^{1}*Mimagoniates microlepis* (Steindachner, 1877)	DZSJRP 13250
	^{1}*Oligosarcus hepsetus* (Cuvier, 1829)	DZSJRP 13251
Erythrinidae	*Hoplias* cf. *malabaricus* (Bloch, 1794)	DZSJRP 13238
SILURIFORMES		
Ariidae	*Genidens genidens* (Lacepède, 1803)	-
Callichthyidae	^{1}*Scleromystax barbatus* (Quoy & Gaimard, 1824)	DZSJRP 13242
	^{1,2}*Scleromystax macropterus* (Regan, 1913)	DZSJRP 13246
	^{1,2}*Scleromystax prionotos* (Nijssen & Isbrücker, 1980)	DZSJRP 20740
Loricariidae	^{1}*Kronichthys heylandi* (Boulenger, 1900)	DZSJRP 13262
	^{1}*Pseudotothyris obtusa* (Miranda Ribeiro, 1911)	DZSJRP 13245
	Rineloricaria sp.	DZSJRP 20729
	^{1}*Schizolecis guntheri* (Miranda Ribeiro, 1918)	DZSJRP 13239
Pseudopimelodidae	^{1}*Microglanis* cf. *parahybae* (Steindachner, 1880)	DZSJRP 20737
Heptapteridae	^{1}*Acentronichthys leptos* Eigenmann & Eigenmann, 1889	DZSJRP 13254
	^{1}*Pimelodella transitoria* Miranda Ribeiro, 1907	DZSJRP 13244
	Rhamdia aff. *quelen* (Quoy & Gaimard, 1824)	DZSJRP 20735
	^{1}*Rhamdioglanis transfasciatus* Miranda Ribeiro, 1908	DZSJRP 20728
GYMNOTIFORMES		
Gymnotidae	^{1}*Gymnotus pantherinus* (Steindachner, 1908)	DZSJRP 13255
CYPRINODONTIFORMES		
Aplocheilidae	^{1}*Atlantirivulus santensis* (Köhler, 1906)	DZSJRP 13257
Poeciliidae	*Phalloceros harpagos* Lucinda, 2008	DZSJRP 20739
	^{1}*Phalloceros reisi* Lucinda, 2008	DZSJRP 13236
	Poecilia vivipara Bloch & Schneider, 1801	DZSJRP 13237

Table 1. Continued...

Order/Family	Species	Voucher
SYNBRANCHIFORMES		
Synbranchidae	*Synbranchus* aff. *marmoratus* Bloch, 1795	DZSJRP 13241
INCERTAE SEDIS in CARANGARIA		
Centropomidae	*Centropomus parallelus* Poey, 1860	-
CICHLIFORMES		
Cichlidae	*Crenicichla* cf. *tingui* Kullander & Lucena, 2006	DZSJRP 20736
	Geophagus brasiliensis (Quoy & Gaimard, 1824)	DZSJRP 13234
GOBIIFORMES		
Eleotridae	*Dormitator maculatus* (Bloch, 1792)	DZSJRP 20731
	Eleotris pisonis (Gmelin, 1789)	MZUSP 110173
Gobiidae	*Awaous tajasica* (Lichtenstein, 1822)	DZSJRP 20734
	Bathygobius soporator (Valenciennes, 1837)	DZSJRP 20730
	Ctenogobius shufeldti (Jordan & Eigenmann, 1887)	MZUSP 110175

Identification key for fishes from coastal streams of the Atlantic forest of southeastern Brazil.

1. Fins present, at least pectorals and anal; two opercular openings 2

1'. Fins absent (or vestigial); a single opercular opening, ventral .... *Synbranchus* aff. *marmoratus* (Synbranchiformes)

2. Dorsal and caudal fins present; less than 100 branched anal fin rays 3

2'. Dorsal and caudal fins absent; more than 200 branched anal fin rays........... .... *Gymnotus pantherinus* (Gymnotiformes)

3. Body naked or covered with bony plates; barbels present (sometimes small) …….. 20 (Siluriformes)

3'. Body covered with scales; barbels absent... 4

4. Dorsal fin with 11 or less soft rays, *i.e.*, not transformed into spines 5

4'. Dorsal fin with more than 12 rays, the first normally rigid, transformed into spines……….. 32

5. Scales covering the head, dorsally; premaxilla protractile, *i.e.*, moving in front of the skull; small body size, maximum standard length less than 60 mm .... 6 (Cyprinodontiformes)

5'. Scales absent in the dorsal portion of the head; premaxilla non-protractile, *i.e.*, does not move in front of the skull; maximum standard length normally more than 70 mm .... 9 (Characiformes)

6. Anal fin of mature males with modified rays, *i.e.*, transformed into a gonopodium; dorsal fin at vertical through the mid-posterior portion in flank, its longer rays never extending to the beginning of caudal fin; flank scales with chromatophores at its edges, forming a reticulated pattern ....…... 7

6'. Anal fin of mature males with normal rays, not transformed into a gonopodium; dorsal fin posterior to the middle in flank, its larger rays extending to the beginning of caudal fin; flank scales with chromatophores uniformly distributed in their area .... *Atlantirivulus santensis* (Aplocheilidae)

7. No sexual color dimorphism, males showing the same color as females; pelvic fin of mature males anteriorly displaced, its origin located at vertical through pectoral fin origin; females with developed urogenital papilla, displaced after anal fin origin, and with spots vertically elongated in flank .... 8 (*Phalloceros*)

7'. Conspicuous sexual color dimorphism, males more colorful than females; pelvic fin of the mature males posteriorly displaced, its origin near the anal fin origin; females without developed urogenital papilla, and normally without spot in flank .... *Poecilia vivipara*

8. Female urogenital papilla approximately rectilinear along the mid-ventral line, between the anus and the base of first anal fin ray; border of the anal aperture separated from the first anal fin ray by the urogenital papilla .... *Phalloceros harpagos*

8'. Female urogenital papilla curved to the right, laterally; border of the anal aperture in contact with the first anal fin ray or close to it .... *Phalloceros reisi*

9. Jaw teeth present, even if small; humeral spot present or absent and/or spot in the caudal peduncle normally absent; when present restricted to the medial portion of the caudal peduncle.. 10

9'. Jaw teeth absent; humeral spot absent, but with large spot in the caudal peduncle, relatively rounded .... *Cyphocharax santacatarinae* (Curimatidae)

10. Adipose fin present; forked caudal fin 11

10'. Adipose fin absent; rounded caudal fin .... *Hoplias* cf. *malabaricus* (Erythrinidae)

11. Jaw teeth with three or more cusps; lateral line with less than 40 perforated scales; small mouth, its opening does not extend until the vertical that passes through the origin of the orbit 12

11'. Canine and conical jaw teeth; lateral line with more than 45 perforated scales; wide mouth opening, extending to the vertical through the origin of the orbit .... *Oligosarcus hepsetus* (Characidae)

12. Anal fin with less than 10 branched rays; dark band between snout and the orbit present; premaxilla with conical or tricuspid teeth .... 13 (*Characidium*)

12'. Anal fin with more than 10 rays; dark band between snout and the orbit absent; premaxillary teeth with four or more cusps 15

13. Dark longitudinal stripe on flank with relatively irregular borders, with spots projecting dorsally and/or ventrally 14

13'. Dark longitudinal stripe on flank with approximately straight edge, with small spots (not covering one scale size) below the dark longitudinal stripe ....…... *Characidium schubarti*

14. Caudal and adipose fins hyaline; dark spots below the dark longitudinal stripe not extending to pelvic fin origin .... *Characidium lanei*

14'. Caudal fin with dark spots; blackened adipose fin; dark spots below the dark longitudinal stripe extending to pelvic fin origin .... *Characidium pterostictum*

15. Lateral line complete 16

15'. Lateral line incomplete 17

16. Large mouth opening, extending to the vertical that passes through the nostrils origin, teeth of the inner row of the premaxillary with up to nine cusps; mature males without bony hooks in anal fin; 3-4 (mode 3) maxillary teeth.. *Deuterodon iguape*

16'. Small mouth opening, distinctly anterior to the vertical that passes through the nostrils origin, teeth of the inner row of the premaxillary with up to seven cusps; mature males with bony hooks in anal fin; 1-3 (mode 2) maxillary teeth... *Astyanax ribeirae*

17. Flank with a dark stripe or absent stripes; humeral blotch present 18

17'. Flank with several black stripes, usually forming a zigzag pattern; humeral blotch absent.. *Hollandichthys multifasciatus*

18. One humeral spot; spot in caudal peduncle present 19

18'. Two humeral spots; spot in caudal peduncle absent .... *Hyphessobrycon griemi*

19. Narrow longitudinal stripe on flank, covering less than one scale in longitudinal series; origin of the dorsal fin vertically passing before the origin of the anal fin; flank scales with reticulated pattern; anal fin with 15-22 branched rays .... *Hyphessobrycon boulengeri*

19'. Relatively wide longitudinal stripe on flank, covering two or more longitudinal series of scales; origin of the dorsal fin distinctly posterior to the vertical that passes before the origin of the anal fin; flank scales hyaline or with sparse chromatophores; anal fin with 26-31 branched rays .... *Mimagoniates microlepis*

20. Body covered with bony plates 21

20'. Body naked, *i.e.*, covered by thick skin 27

21. Body covered with two longitudinal rows of bone plates, plain (without lateral keels), subterminal mouth (not modified into a sucking disk) .... 22 (Callichthyidae)

21'. Body covered with four or more longitudinal rows of bone plates, normally with small lateral keels; inferior mouth, modified into a sucking disk .... 24 (Loricariidae)

22. Longitudinal stripe faded or absent on flank; blotches projecting dorsally and/or ventrally on flank; dark stripe below longitudinal stripe extending from the pelvic fin to the anal fin absent; uniformly colored head, with chromatophores uniformly scattered 23

22'. Median dark longitudinal stripe conspicuous on flank, followed below by a narrow dark stripe extending from the pelvic fin to the anal fin; blotches projecting ventrally on flank absent; head with dark coloration, and small golden spots .... *Scleromystax barbatus*

23. Three or four vertical dark blotches on the flank, caudal fin with dark narrow stripes .... *Scleromystax macropterus*

23'. Blotches on flank absent, with chromatophores uniformly scattered in the caudal fin .... *Scleromystax prionotos*

24. Adipose fin absent ……….. 25

24'. Adipose fin present... *Kronichthys heylandi*

25. Caudal peduncle rounded, without conspicuous lateral keels 26

25'. Caudal peduncle depressed, with two conspicuous lateral keels ....…..…..... *Rineloricaria* sp.

26. Pectoral girdle totally exposed, posterior portion of the supraoccipital with well-developed odontodes in adults .... *Pseudotothyris obtusa*

26'. Pectoral girdle covered by skin, exposed only laterally, posterior portion of the supraoccipital without odontodes in adults .... *Schizolecis guntheri*

27. Gill membranes are free to each other and to the isthmus; adipose fin origin anterior to anal fin origin 28

27'. Gill membranes joined to each other and to the isthmus; adipose fin origin at vertical or posterior to anal fin origin .... *Genidens genidens* (Ariidae)

28. Free orbital margin, *i.e.*, eyes not covered by skin; hyaline dorsal fin or with sparse chromatophores, evenly distributed; flank with longitudinal dark stripes .... 29 (Heptapteridae)

28'. Orbital margin not free, *i.e.*, orbital margin covered by skin; dorsal fin with conspicuous dark blotches; flank with vertical dark stripes .... *Microglanis* cf. *parahybae*

29. Dorsal fin origin distinctly ahead at vertical through pelvic fin origin; adipose fin not extending to caudal fin origin (free caudal peduncle dorsally) 30

29'. Dorsal fin origin proximately at vertical through pelvic fin origin; adipose fin elongate, extending to caudal fin origin (covering the caudal peduncle area) .... *Acentronichthys leptos*

30. Maxillary barbell short, not extending to anal fin origin; flank with longitudinally conspicuous dark stripe or dorsally dark stripes; adipose fin origin at vertical through posterior base of pelvic fin …........................... 31

30'. Maxillary barbell long, extending to anal fin origin; flank uniformly colored; adipose fin origin at vertical through middle base of pelvic fin.... .... *Rhamdia* aff. *quelen*

31. Long supraoccipital process, reaching the dorsal fin origin; longitudinal dark stripe extending at vertical through dorsal fin origin to caudal peduncle end; maxillary barbell long, surpassing the pelvic fin origin ....….. ....……………………...… *Pimelodella transitoria*

31'. Short supraoccipital process, not reaching the dorsal fin origin; longitudinal dark stripe absent; albeit with dorsal dark stripes; maxillary barbell short, not reaching the pelvic fin origin …..................................... ....*Rhamdioglanis transfasciatus*

32. Single dorsal fin, *i.e.*, undivided; lateral line divided into two portions: upper dorsal and inferior ventral ……......................... 33 (Cichliformes)

32'. Dorsal fin divided into two parts; single lateral line, undivided 34

33. Dark longitudinal stripe extending from eye to caudal peduncle; preopercular posterior margin serrated; rounded spot in the upper portion of caudal fin beginning; first gill arch without lobe in its upper portion .... *Crenicichla tingui*

33'. Humeral spot rounded in the medial portion in flank; preopercular posterior margin smooth; dark spot in caudal fin absent; first gill arch with developed lobe .... *Geophagus brasiliensis*

34. Caudal fin not bifurcated, *i.e*. emarginated, truncated, tapered or rounded; yellowish brownish flank, with dark blotches or concentrated chromatophores; small sized body, less than 60 cm in total length .... 35 (Gobiiformes)

34'. Caudal fin bifurcated; silver flank, without dark blotches, and blackened lateral line; mid-sized body - ca. 70 cm in total length .... *Centropomus parallelus* (Centropomidae - *Incertae Sedis* in Carangaria)

35. Terminal or slightly superior mouth; free pelvic fins or with attached bases, but not forming an adhesive disk .... 36 (Eleotridae)

35'. Slightly inferior mouth; pelvic fins attached by a membrane, forming an adhesive disk .... 37 (Gobiidae)

36. Dark stripe below the eye extending to the dentary; 25-35 scales along longitudinal series; preopercular spine absent .... *Dormitator maculatus*

36'. Dark stripe below the eye absent; more than 40 scales in a longitudinal series; preopercular spine present... *Eleotris pisonis*

37. Scales on flank beginning at the vertical through the end of the opercular bone; a pair of anterior interorbital pores present; two or three dark stripes on the nostril …... 38

37'. Scales in the superior portion of the head extending until at vertical through preopercule; normally a single anterorbital pore; dark stripes on the nostril absent .... *Bathygobius soporator*

38. Caudal fin truncated or slightly rounded; two dark stripes between the orbit and the nostril; gill rakers present in the upper portion of the first branchial arch ....….. *Awaous tajasica*

38'. Posteriorly tapered caudal fin; three dark stripes between the orbit and the nostril; gill rakers in the upper portion of the first branchial arch absent ....…..……..… *Ctenogobius shufeldti*

Results

Seven orders, 17 families, and 39 fish species were identified (Table 1). Siluriformes were the most representative order (five families and 13 species), followed by Characiformes (four families and 12 species), Gobiiformes (two families and five species), and Cyprinodontiformes (two families and four species). Gymnotiformes and Synbranchiformes were represented by one species, each one. Twenty-four species (61.5%) are endemic to the Atlantic forest, and three are endangered (Table 1). An identification key for fishes from coastal streams of Juréia-Itatins Ecological Station is provided.

Discussion

In recent decades, current knowledge about fish from Neotropical streams has increased but is still deficient, even in better-exploited regions as southeastern Brazil. Juréia-Itatins Ecological Station represents an important refuge for the conservation of stream fishes, especially *Characidium schubarti*, *Scleromystax macropterus,* and *S. prionotos* that are threatened with extinction in the state of São Paulo since 2014 (State Decree 60.133), as well as *Pseudocorynopoma heterandria*, *Hoplias lacerdae*, and *Brachyhypopomus jureiae*, sampled during other studies (Sabino & Silva 2004, Oyakawa et al. 2006). The main threat to species found in lowland streams such as *S. macropterus* is the deforestation of restinga (Gonçalves et al. 2016). Furthermore, the studied area has a great regional diversity of fishes, since the high diversity of aquatic environments (e.g. mountain streams with clear waters, lowland streams with black waters, and estuarine rivers with brackish waters), allows a greater diversity of species (Gonçalves & Braga 2012, Ferreira et al. 2014).

An identification key for Atlantic forest fish was published by Oyakawa et al. (2006), but only mentioned 16 of the 39 species reported by us in Juréia-Itatins Ecological Station streams. The present identification key will be useful for the recognition of species in the studied area, especially if used jointly with the color guide available online for free (Gonçalves 2014), which contains 38 photos of the Juréia-Itatins Ecological Station fish. Considering that most of these species are endemic to the coastal streams, this identification key will be useful not only for the species recognition of the sampled area, but also for the surrounding coastal region.

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Author Contributions

Cristina da Silva Gonçalves: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Fernando Rogério Carvalho: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

María Angélica Pérez Mayorga: contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation and revision.

Isadora Francesconi de Oliveira: contribution to data analysis and interpretation; contribution to manuscript preparation and revision.

Conflicts of interest

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

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The vascular flora and vegetation of Queimada Grande Island, São Paulo State, southeastern Brazil

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Abstract: Studies of the vegetation on islands off the coast of southeastern Brazil are still very scarce, despite their importance for assessing, managing, and conserving insular biodiversity. We present here a list of the vascular flora of Queimada Grande Island (QGI; 24°29′10″ S, 46°40′30″ W, 57 ha, 33.2 km from the coast) in southeastern Brazil and describe its phytophysiognomies. The island is covered mainly by Atlantic Forest (Dense Ombrophilous Forest), as well as with rock outcrop and anthropogenic vegetation with herbaceous-shrub phytophysiognomies. QGI showed relatively low species richness (S = 125) when compared to other Brazilian coastal islands. Herbaceous (52) and climbing species (31) predominated on QGI. The richest families were Fabaceae (11 species), Poaceae (9), and Apocynaceae, Asteraceae and Orchidaceae (8 species each). Most species (S = 112) are autochthonous from different phytophysiognomies of the southeastern Brazilian Atlantic Forest complex. Many species associated with anthropically disturbed areas (S = 26) can be found on QGI, including the invasive grass *Melinis minutiflora*. There was a slight predominance of zoochory (S = 50). We did not identify any species endemic to QGI. One of its species (*Cattleya intermedia*, Orchidaceae) is vulnerable at both national and regional levels, and another (*Barrosoa apiculata*, Asteraceae) is presumably extinct on the mainland in São Paulo State. The vascular flora of QGI originated from the mainland Atlantic Forest complex, following the pattern of other coastal islands in southeastern Brazil. The flora and vegetation of QGI reflect the combination of insular conditions, the small size of the island, habitat restriction, steep topography, incipient soils, and the past use of the area with the introduction of several foreign species. We recommend permanent monitoring of the vegetation cover of QGI and its management, in order to ensure the conservation of the local native biota.

Keywords*:* *Atlantic Forest; biodiversity conservation; coastal islands; dispersal syndromes; invasive species; protected areas.*

Flora vascular e vegetação da Ilha Queimada Grande, São Paulo, sudeste do Brasil

Resumo: Estudos sobre a vegetação de ilhas costeiras no sudeste do Brasil ainda são muito escassos, apesar de sua importância para a avaliação, manejo e conservação da biodiversidade insular. Nós apresentamos aqui uma lista da flora vascular da Ilha Queimada Grande (IQG; 24°29′10″ S, 46°40′30″ W, 57 ha, 33,2 km da costa), sudeste do Brasil, e descrevemos suas fitofisionomias. A ilha é recoberta principalmente por Floresta Atlântica (Floresta Ombrófila Densa), bem como por vegetação sobre afloramento rochoso e vegetação antrópica com fisionomias herbáceo-arbustivas. A IQG apresentou riqueza relativamente baixa (S = 125) comparada a de outras ilhas costeiras do Brasil. Espécies herbáceas (52) e trepadeiras (31) predominaram na IQG. As famílias mais ricas foram Fabaceae (11 espécies), Poaceae (9), Apocynaceae, Asteraceae e Orchidaceae (8 espécies cada). A maioria das espécies (S = 112) é autóctone de diferentes fitofisionomias do complexo da Floresta Atlântica do sudeste do Brasil. Muitas espécies associadas a áreas antropicamente alteradas (S = 26) são encontradas na IQG, incluindo a gramínea invasora *Melinis minutiflora*. Houve ligeira predominância de zoocoria (S = 50). Nós não identificamos espécies endêmicas para a IQG. Uma espécie (*Cattleya intermedia*, Orchidaceae) encontra-se vulnerável em nível nacional e estadual, e outra (*Barrosoa apiculata*, Asteraceae) está presumivelmente extinta no estado de São Paulo. A flora vascular da IQG originou-se no complexo da Floresta Atlântica continental, seguindo o padrão de outras ilhas costeiras do sudeste do Brasil. A flora e a vegetação da IQG refletem a combinação da condição insular, tamanho reduzido da ilha, restrição de habitat, topografia acidentada, solos incipientes e o uso pretérito da área com a introdução de várias espécies alóctones. Nós recomendamos o monitoramento permanente da vegetação da IQG e seu manejo, visando garantir a conservação da biota nativa local.

Palavras-chave*: Floresta Atlântica; conservação da biodiversidade; ilhas costeiras; síndromes de dispersão; espécies invasoras; áreas protegidas.*

Introduction

Islands have long attracted the attention of scientists and naturalists, and studies of their biota have produced important insights into the interactions of processes and patterns in biogeography (Lomolino 2000). The Equilibrium Theory of Island Biogeography (MacArthur & Wilson 1967), for example, has strongly influenced studies of ecology and conservation biology through its proposal that the number of species on a given island is the result of both immigration and extinction rates, and that these two opposing forces are closely related to the size and isolation of the island. According to Kreft et al. (2008), the richness of the vascular flora of an island is mainly determined by the island's size, followed by its degree of geographic isolation, current climatic conditions, and its topography and geology. Island biotas are seriously threatened by climate change, habitat loss and, especially, the introduction of invasive alien species (Kreft et al. 2008, Serafini et al. 2010).

Queimada Grande Island (QGI), located off the southern coast of São Paulo State in southeastern Brazil, has stimulated the interests of various researchers in recent decades. The golden lancehead pit viper, *Bothrops insularis* (Amaral, 1921), is endemic to the island and is critically endangered both nationally (Brasil 2014) and globally (Marques et al. 2004). Although several studies have been carried out on QGI focusing on the biology of this snake (e.g., Wüster et al. 2005, Martins et al. 2008, Marques et al. 2012, Guimarães et al. 2014), information about the island's vegetation cover is still extremely incipient (Martins et al. 2008, Bataus & Reis 2011). Data on its flora are quite rare in the literature, except for Campos & Mello-Filho (1966), who presented a study of the flora that referred to approximately twenty (mostly ruderal) species.

Studies of the vegetation on coastal islands in southeastern Brazil have been very scarce (e.g., Barros et al. 1991, Salino et al. 2005, Silva & Britez 2005, Ferreira et al. 2007, Callado et al. 2009, Bovini et al. 2013, 2014) in spite of their importance for assessing, managing, and conserving biodiversity. Small island plant inventories are even more scarce (Kemenes 2003, Ferreira et al. 2007, Bovini et al. 2014). These studies have frequently indicated that the floras of coastal islands are basically composed of mainland Atlantic Forest complex species (*sensu* Oliveira-Filho & Fontes 2000, Scarano 2002), and are related to their recent histories of isolation from the mainland.

We present here and analyze the terrestrial vascular flora of QGI, describe the phytophysiognomies and biodiversity present on the island, and present data useful for managing that area. This contribution was part of a research project conducted jointly by the Instituto de Pesquisas Jardim Botânico do Rio de Janeiro and the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) to map and characterize the vegetation cover of QGI and promote the conservation of that island.

Material and Methods

1. Study site

QGI (center point: 24°29′10″ S, 46°40′30″ W) is located 33.2 km from the southern coast of São Paulo State in southeastern Brazil (Figure 1). The island has no beaches or plateaus, with very steep slopes and cliffs in its southern

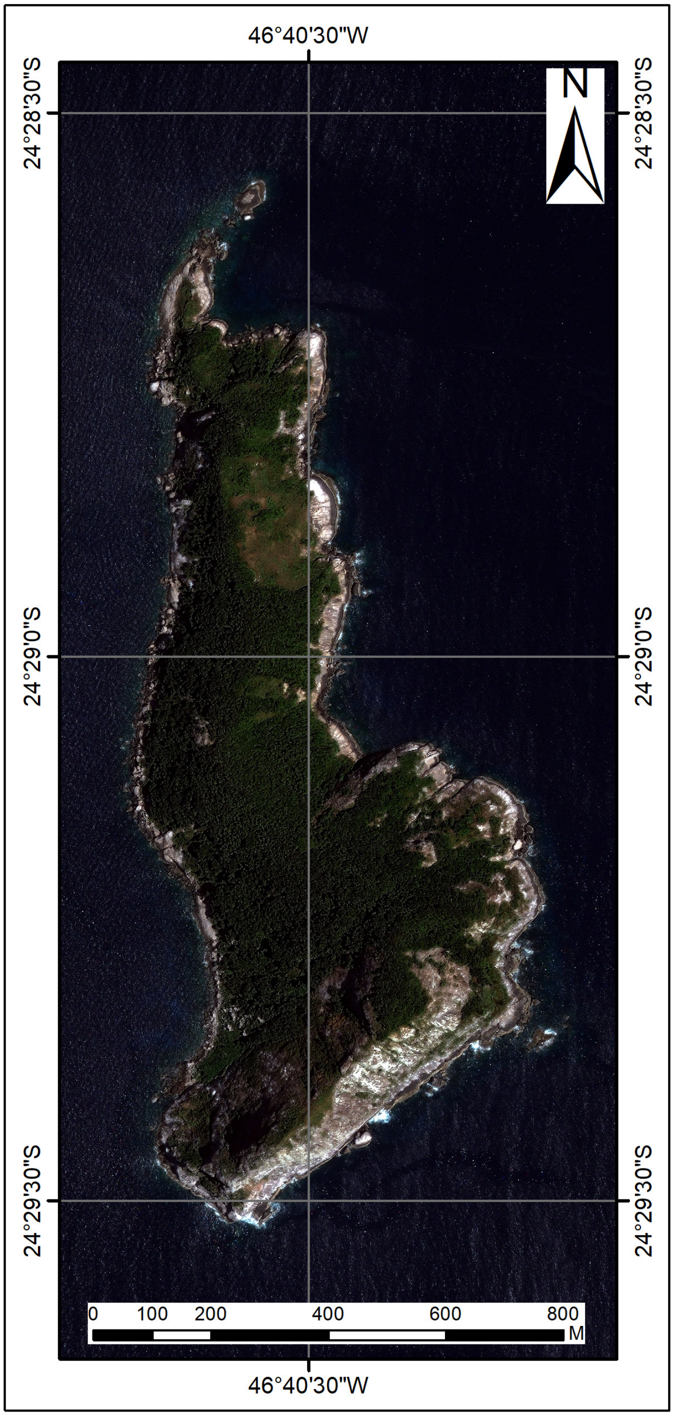

Figure 1. Location of Queimada Grande Island, São Paulo, southeastern Brazil. QuickBird image, December 31, 2013, Datum SIRGAS 2000.

portion (Figure 2) at elevations from 0–210 m above sea level (Bataus & Reis 2011). Its projected surface area is 57 ha (B.C. Kurtz et al., unpubl. data) and its soils are shallow, with many boulders and rocky outcrops (pers. obs.). Climate data specific for QGI are extremely scarce. According to Guimarães et al. (2014), the local climate is subtropical with two distinct seasons: rainy and warm (October–March) and dry and cold (April–September). Average monthly temperatures ranged from 18.3°C (August) to 27.2°C (March) in 2007–2008, and rainfall from 0.2 mm (July) to 135.2 mm (December) (Marques et al. 2012). The general climate for the coast where QGI is located is classified as Af (Tropical, rainforest), following the Köppen's system (Rolim et al. 2007). Itanhaém, for example, the closest city to QGI, has an average annual rainfall of 2030 mm (Cepagri 2017). The island is relatively distant from the Serra do Mar Range along the mainland coast, however, and should be less affected by orographic rains.

This small coastal island was connected by a land bridge to the mainland during the last glaciation (Wisconsin) maximum about 17,000 years BP, when sea levels were approximately 110 m lower than today (Tessler & Goya 2005). The last contact of QGI with the mainland occurred about 11,000 years ago (Marques et al. 2002) when rising sea levels during the Holocene isolated many coastal areas of different sizes and at varying distances from the coastline in southeastern Brazil.

QGI is covered mainly by Dense Ombrophilous Forest (IBGE 2012), with shorter trees than found on the mainland due to local environmental conditions. Additional phytophysiognomies include rock outcrop vegetation (see Meirelles et al. 1999), areas covered by anthropogenic vegetation (characterized by an herbaceous-shrub physiognomy and corresponding to the initial phases of secondary growth as described by IBGE 2012), and a small banana (*Musa paradisiaca*) plantation (see description below). The latter two formations are the result of past human use of the area.

In 1909, the Brazilian Navy built a lighthouse on QGI, and lighthouse keepers lived there until 1925. The lighthouse has been automated since then and periodically maintained (Bataus & Reis 2011). QGI was declared part of the Area of Relevant Ecological Interest (AREI) of the Queimada Pequena and Queimada Grande Islands in 1985, a Federal Conservation Area for Sustainable Use managed by ICMBio.

2. Data collection

The current floristic list was compiled from collections made on QGI between May/2014 and March/2015 (SISBIO N. 44050-1) and between March/1996 and September/1997, covering all of the seasons of the year. Additionally, samples from the *Herbário Virtual da Flora e dos Fungos* (INCT 2016) were included, using 'São Paulo' as the search keyword for the state, and 'Ilha Queimada Grande' for the locality. We also included three species that were not collected (found only sterile, or in places of difficult access), but identified in the field. The list was also complemented with information from Wanderley et al. (2001, 2002, 2003, 2005, 2007, 2009, 2012, 2016). The botanical material was identified by the authors and/or specialists. The descriptions of the local phytophysiognomies are based on observations made during field excursions.

3. Data analysis

Names and botanical families of all species followed the Flora do Brasil 2020 website (2016) (under construction), which also provided information about life-forms, substrates, and distributions. Species indicated as 'liana/scandent/vine' were treated here simply as climbers (see Morellato & Leitão-Filho 1996). The conservation statuses of the species at global and national levels followed IUCN (2017) and CNCFlora (2017), respectively. Additionally, we used São Paulo (2016) to confirm the

Figure 2. Aerial photograph of Queimada Grande Island, São Paulo, Brazil, showing its rugged topography and its different vegetation physiognomies. Note the area covered by anthropogenic vegetation (in the center of the island near the lighthouse). Photo by J.M. Rosa.

conservation statuses in São Paulo State. Based on the information about the collection sites found on herbarium labels, species were assigned to one or more of the following habitat classes: forest interior, forest edges (interfaces between forests and open areas), rock outcrop vegetation, anthropogenic vegetation, and banana plantation. By consulting the literature (Ichaso 1980, Pennington et al. 1981, Pennington 1990, Lorenzi 1998, 2000, Barroso et al. 1999, Bovini et al. 2001, Wanderley et al. 2001, 2002, 2003, 2005, 2007, 2009, 2012, 2016, Mansano et al. 2004, Passos & Oliveira 2004, Rodrigues et al. 2005, Reis 2006, Souza & Morim 2008, Ferreira 2009, Gomes-Costa & Alves 2012, Silva-Luz et al. 2012, Ferreira & Miotto 2013, Soares Neto et al. 2014), specialists, and through examinations of material deposited at the RB Herbarium, the species of angiosperms were classified into four major groups according to the morphological criteria of Pijl (1982): 1) anemochoric, with diaspores adapted to wind dispersal; 2) zoochoric, with diaspores adapted to animal dispersal; 3) hydrochoric, with diaspores adapted to water dispersal; and 4) autochoric, with diaspores displaying no apparent specific adaptation to the above dispersal agents, including barochoric species (gravity dispersal) and those with explosive dispersal. By consulting the same aforementioned sources, we also determined which species are autochthonous to ombrophilous forests and/or pioneer formations (*sensu* IBGE 2012): beach ridge vegetation (*restinga*; Lacerda et al. 1993) and rock outcrop vegetation (Meirelles et al. 1999), of the Atlantic Forest complex in southeastern Brazil, and which species are associated with anthropically disturbed areas (ruderal species; *sensu* Moro et al. 2012). Finally, we identified the invasive alien species in the Atlantic Forest complex that could be competing or displacing populations of native species and hampering vegetation regeneration on QGI, based on *Base de Dados Nacional de Espécies Exóticas Invasoras I3N Brasil* (Instituto Hórus 2017).

We compared the species richness at QGI with that of other coastal islands in eastern and southeastern Brazil by selecting sites with comprehensive published surveys and physiographies comparable to that of QGI.

Results

We identified 125 species of vascular plants on QGI, distributed among 115 genera and 57 families, including four pteridophytes and 121 angiosperms (Table 1). The richest families were Fabaceae (11 species), Poaceae (nine species), and Apocynaceae, Asteraceae and Orchidaceae (eight species each). Considering the maximum life-form expression of each species, there was a predominance of herbs (S = 52; 41.6% of the total) and climbers (S = 31; 24.8%). Shrubs and subshrubs totaled 25 species (20%); only 17 species (13.6%) show arboreal habits. Our results indicated a very low richness of epiphytes on the island, with strictly epiphytic species accounting for only 2.4% of the local flora (S = 3: *Acianthera saundersiana*, *Epidendrum densiflorum* and *Peperomia glabella*), although this number rises to 8% if hemiepiphytes (*Monstera praetermissa*) and facultative epiphytes (i.e., those plants capable of living as rupiculous or terrestrial individuals) with six species (including *Rumohra adiantiformis*, *Aechmea caudata* and *Cattleya intermedia*) are included. QGI showed a relatively low number of species when compared to other coastal islands of eastern and southeastern Brazil (Table 2).

The vast majority of the species (S = 112; 89.6%) are autochthonous from ombrophilous forests and/or pioneer formations (*restinga* and rock outcrop vegetation) within the Atlantic Forest complex of southeastern Brazil; 13 (10.4%) species are allochthonous from those formations (e.g., *Tetragonia tetragonoides*, *Crotalaria laeta*, *Musa paradisiaca* and *Rivina humilis*). Additionally, several species (26) were indicated in the literature (or by specialists) as characteristic of anthropically disturbed areas (e.g., *Asclepias curassavica*, *Bidens pilosa*, *Ipomoea cairica*, *Merremia dissecta*, *Desmodium incanum*, *Sida rhombifolia*, *Digitaria insularis* and *Paspalum virgatum*), with two invasive alien species in the Atlantic Forest complex (*Oeceoclades maculata* and *Melinis minutiflora*). Forty species were identified in the local Dense Ombrophilous Forest, and 20 on rock outcrop vegetation. Forty-five species were found in anthropogenic vegetation (including banana plantations) and 37 along forest edges. Some species occurred in more than one habitat. Information concerning the habitats of 7 species was not available (Table 1).

Dense Ombrophilous Forest currently occupies about 50% of the island area, especially at higher altitude sites and those showing less human interference (Figure 2). Canopy height rarely exceeds 10 m, and species typical of mainland secondary forest formations occur there (such as *Guapira opposita*, *Myrsine guianensis* and *Gallesia integrifolia*). Some forest areas are dominated by the palm tree *Syagrus romanzoffiana*. The herbaceous layer is rich in Acanthaceae, Orchidaceae, and Araceae. Epiphytes are relatively rare, occurring only sporadically (*Lepismium cruciforme* and *Aechmea caudata*).

The rock outcrop vegetation type with an herbaceous or shrub phytophysiognomy occurs on steep slopes or on island peaks, associated with patches of incipient soil accumulation, forming vegetation clumps on the otherwise bare rocky matrix (Figure 3). Closer to the sea, under the influence of salt spray, the rock outcrop vegetation is composed mainly of small shrubs and fleshy herbs, especially *Begonia subvillosa*, *Cereus fernambucensis*, and the allochthonous species *Tetragonia tetragonoides*.

Anthropogenic vegetation, characterized by an herbaceous-shrub physiognomy, represents the early stages of ecological succession in areas originally covered by the Dense Ombrophilous Forest (Figure 2). These areas were formally occupied by rustic houses and small farms (see below), and grasses and other species associated with anthropically disturbed areas predominate there (including *Bidens pilosa*, *Solidago chilensis*, *Lepidium bonariense*, *Ipomoea cairica*, *Eleusine indica* and *Melinis minutiflora*). The anthropogenic vegetation type also includes a small abandoned banana plantation.

Considering only angiosperms, there was a slight predominance of zoochory (S = 50; 41.3%). Thirty-seven species (30.6%) are anemochoric and 33 (27.3%) autochoric, with four hydrochoric species (3.3%). These numbers include some species of Poaceae with more than one dispersal syndrome (Table 1). The predominant Dense Ombrophilous Forest showed a preponderance of zoochory (S = 21; 53.8% of the species surveyed in this vegetation), including species with fleshy fruits (e.g., *Eugenia* spp., *Guapira opposita*, *Myrsine guianensis*, *Rudgea minor* and *Sideroxylon obtusifolium*) or arilate seeds (e.g., *Trichilia casaretti* and *Cupania oblongifolia*) adapted to endozoochory. Zoochoric species, on the other hand, occurring in the anthropogenic vegetation and along forest edges showed different dispersal strategies, including structures that facilitate the adhesion of their diaspores to animals (or clothes) (e.g., *Cyathula prostrata*, *Bidens* spp., *Desmodium incanum* and *Sida* spp.).

This study did not detect any plant species endemic to QGI. Most of the species found on QGI have not yet been evaluated in terms of their conservation statuses at a national level, and only one species (*Cattleya intermedia*) is considered vulnerable (VU) at both national and regional levels. The only record of this orchid on the island is from 1922 (A. Gehrt s/n, SP 8146), and it may be presumed to be extinct on QGI, as it has not been found during recent expeditions. Additionally, *Barrosoa apiculata* is considered extinct (EX) in São Paulo State according to the official list of threatened species of São Paulo. The only known specimen of this species was collected on the island in 1920 (A. Gehrt s/n, SP 4535) and our collecting efforts failed to find any additional individuals. Although *Trichilia casaretti* has been classified as vulnerable (VU) at a global level, its status needs updating according to IUCN (2017).

Table 1. List of the vascular flora of Queimada Grande Island, São Paulo, Brazil. Species marked with an '*' are indicated in the literature as characteristic of anthropically disturbed areas, and species marked with a '†' are invasive in the Atlantic Forest complex in southeastern Brazil. Substrate: Epi (epiphytic); Hem (hemiepiphytes); Rup (rupiculous); Ter (terrestrial). Origin: Auto (autochthonous); Allo (allochthonous) from the Atlantic Forest complex in southeastern Brazil. Dispersal syndrome: Anemo (anemochoric); Auto (autochoric); Hydro (hydrochoric); Zoo (zoochoric). Conservation statuses at global [square brackets], national (no brackets), and regional {curly brackets} levels: NE (Not Evaluated); LC (Least Concern); VU (Vulnerable); EX (Presumably Extinct). Herbaria: ESA (Escola Superior de Agricultura Luiz de Queiroz); NY (The New York Botanical Garden); RB (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro); SP (Herbário do Estado Maria Eneyda P. Kaufmann Fidalgo); SPF (Universidade de São Paulo); UEC (Universidade Estadual de Campinas).

Family	Species	Life-form	Substrate	Origin	Habitat	Syndrome	Status	Voucher
PTERIDOPHYTES								
Aspleniaceae	*Asplenium claussenii* Hieron.	Herb	Rup, Ter	Auto	Forest	—	NE	V.C. Souza 11067: RB 629051
Blechnaceae	*Blechnum polypodioides* Raddi	Herb	Ter	Auto	No information	—	NE	A. Gehrt 4545: NY 814169
Dryopteridaceae	*Rumohra adiantiformis* (G. Forst.) Ching	Herb	Epi, Rup, Ter	Auto	Rock outcrop vegetation	—	NE	V.C. Souza 11037: RB 611809
Polypodiaceae	*Serpocaulon triseriale* (Sw.) A.R. Sm.	Herb	Rup, Ter	Auto	Forest edge	—	NE	V.C. Souza 11054: RB 627263
ANGIOSPERMS								
Acanthaceae	*Dicliptera mucronifolia* Nees	Subshrub	Ter	Auto	Forest	Auto	NE	G.O. Joaquim Jr. 16: RB 627046
	Justicia kleinii Wassh. & L.B. Sm.	Herb	Ter	Auto	Forest	Auto	LC	A.M. Magalhães 65: RB 636590
	Pseuderanthemum heterophyllum (Nees) Radlk.	Herb	Ter	Auto	Forest	Auto	NE	G.O. Joaquim Jr. 39: ESA 33144
	Ruellia brevifolia (Pohl) C. Ezcurra	Subshrub	Ter	Auto	Forest edge	Auto	NE	G.O. Joaquim Jr. 123: RB 627016
Aizoaceae	*Sesuvium portulacastrum* (L.) L.	Herb	Rup, Ter	Auto	Rock outcrop vegetation	Auto	NE	V.C. Souza 11045: ESA 27428
	Tetragonia tetragonoides (Pall.) Kuntze	Herb	Ter	Allo	Rock outcrop vegetation	Hydro	NE	V.C. Souza 11032: ESA 31994
Amaranthaceae	*Alternanthera brasiliana* (L.) Kuntze	Subshrub	Ter	Auto	Anthropogenic vegetation	Auto	NE	A. Gehrt s/n: SP 4565
	Cyathula prostrata Blume*	Subshrub	Ter	Auto	Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 38: RB 636575
	Dysphania ambrosioides (L.) Mosyakin & Clemants	Subshrub	Ter	Allo	Anthropogenic vegetation	Zoo	NE	A. Gehrt s/n: SP 4564
Amaryllidaceae	*Hippeastrum reticulatum* Herb.	Herb	Ter	Auto	Forest	Auto	NE	A.M. Magalhães 66: RB 636591
Apocynaceae	*Asclepias curassavica* L.*	Herb	Ter	Auto	Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 51: RB 636582
	Aspidosperma australe Müll. Arg.	Tree	Ter	Auto	Forest	Anemo	LC	A.M. Magalhães 96: RB 636611
	Forsteronia thyrsoidea (Vell.) Müll. Arg.	Climber	Ter	Auto	Forest edge	Anemo	NE	A. Gehrt s/n: RB 119773
	Gonolobus rostratus (Vahl) R. Br. ex Shult.	Climber	Ter	Auto	Forest edge, Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 99: RB 636613
	Jobinia connivens (Hook. & Arn.) Malme	Climber	Ter	Auto	Forest edge	Anemo	NE	G.O. Joaquim Jr. 127: ESA 34562
	Marsdenia macrophylla (Humb. & Bonpl. ex Schult.) E. Fourn.	Climber	Ter	Auto	Forest edge	Anemo	NE	A. Gehrt s/n: SP 28663
	Orthosia urceolata E. Fourn.	Climber	Ter	Auto	Forest edge	Anemo	NE	G.O. Joaquim Jr. 115: ESA 34577
	Temnadenia odorifera (Vell.) J.F. Morales	Climber	Ter	Auto	Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 74: RB 637080
Araceae	*Anthurium parasiticum* (Vell.) Stellfeld	Herb	Ter	Auto	Forest	Zoo	LC	V.C. Souza 11091: RB 427676
	Monstera praetermissa E.G. Gonç. & Temponi	Herb	Hem	Auto	Forest	Zoo	NE	V.C. Souza 11041: ESA 27427
	Xanthosoma sp.	Herb		Auto	Rock outcrop vegetation, Anthropogenic vegetation	Zoo		Not collected

Table 1. Continued...

Family	Species	Life-form	Substrate	Origin	Habitat	Syndrome	Status	Voucher
Arecaceae	*Syagrus romanzoffiana* (Cham.) Glassman	Palm Tree	Ter	Auto	Forest	Zoo	LC	Not collected
Asteraceae	*Austroeupatorium inulaefolium* (Kunth) R.M. King & H. Rob.	Shrub, Subshrub	Rup, Ter	Auto	Forest edge, Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 21: RB 637078
	Barrosoa apiculata (Gardner) R.M. King & H. Rob.	Herb	Ter	Auto	Rock outcrop vegetation	Anemo	LC {EX}	A. Gehrt s/n: SP 4535
	Bidens pilosa L.*	Herb	Ter	Allo	Anthropogenic vegetation	Zoo	NE	V.C. Souza 11015: ESA 26128
	Bidens segetum Mart. ex Colla	Climber, Subshrub	Ter	Auto	Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 39: RB 636576
	Chromolaena odorata (L.) R.M. King & H. Rob.*	Shrub	Ter	Auto	Forest edge, Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 24: RB 636569
	Cyrtocymura scorpioides (Lam.) H. Rob.	Subshrub	Ter	Auto	Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 59: RB 636585
	Mikania micrantha Kunth*	Climber	Ter	Auto	Anthropogenic vegetation	Anemo	NE	A.M. Magalhães 44: RB 636579
	Solidago chilensis Meyen*	Subshrub	Ter	Auto	Anthropogenic vegetation	Anemo	NE	V.C. Souza 11069: ESA 26144
Basellaceae	*Anredera cordifolia* (Ten.) Steenis	Climber	Ter	Auto	Forest edge	Auto	NE	V.C. Souza 11021: ESA 26119
Begoniaceae	*Begonia fernandocostae* Irmsch.	Subshrub	Ter	Auto	No information	Anemo	NE	A. Amaral & J. Domingues 26a: SP 3946
	Begonia subvillosa Klotzsch	Herb	Rup, Ter	Auto	Forest, Rock outcrop vegetation	Anemo	NE	V.C. Souza 11011: ESA 26125
Bignoniaceae	*Dolichandra quadrivalvis* (Jacq.) L.G. Lohmann	Climber	Ter	Auto	Forest	Anemo	NE	A. Gehrt s/n: SP 4556
Boraginaceae	*Tournefortia membranacea* (Gardner) DC.	Shrub, Climber, Subshrub	Ter	Auto	Forest edge	Zoo	NE	V.C. Souza 11090: ESA 26155
	Varronia curassavica Jacq.	Shrub	Ter	Auto	Forest edge, Anthropogenic vegetation	Zoo	NE	D.F. Bertani s/n: RB 552595
Brassicaceae	*Lepidium bonariense* L.*	Herb	Ter	Auto	Anthropogenic vegetation	Zoo	NE	G.O. Joaquim Jr. 14: ESA 33163
Bromeliaceae	*Aechmea caudata* Lindm.	Herb	Epi, Rup, Ter	Auto	Forest	Zoo	LC	V.C. Souza 11022: RB 471967
Cactaceae	*Cereus fernambucensis* Lem.	Shrub, Subshrub	Rup, Ter	Auto	Rock outcrop vegetation	Zoo	NE [LC]	A. Gehrt s/n: SP 4574
	Coleocephalocereus fluminensis (Miq.) Backeb.	Shrub	Rup	Auto	Rock outcrop vegetation	Zoo	NE [LC]	A.M. Magalhães 94: RB 636609
	Lepismium cruciforme (Vell.) Miq.	Herb	Epi, Rup	Auto	Forest	Zoo	LC [LC]	V.C. Souza 11060: ESA 26136
	Pereskia aculeata Mill.	Climber	Rup, Ter	Auto	Forest, Forest edge	Zoo	LC [LC]	A.M. Magalhães 76: RB 636598
Campanulaceae	*Hippobroma longiflora* (L.) G. Don*	Herb, Shrub	Ter	Auto	Anthropogenic vegetation	Auto	NE	A.M. Magalhães 62: RB 636588
Cannabaceae	*Trema micrantha* (L.) Blume	Shrub, Tree	Ter	Auto	Forest	Zoo	NE	V.C. Souza 11058: ESA 26153
Cannaceae	*Canna indica* L.	Herb	Ter	Auto	Forest edge, Anthropogenic vegetation	Auto	NE	A.M. Magalhães 63: RB 636589
Capparaceae	*Cynophalla flexuosa* (L.) J. Presl	Shrub	Ter	Auto	Forest	Zoo	NE	V.C. Souza 11068: ESA 26143
Celastraceae	*Hippocratea volubilis* L.	Climber	Ter	Auto	Forest edge	Anemo	NE	V.C. Souza 11030: ESA 27433
Cleomaceae	*Cleome rosea* Vahl ex DC.	Herb, Subshrub	Rup, Ter	Auto	Rock outcrop vegetation	Zoo	NE	V.C. Souza 11012: ESA 26126

Table 1. Continued...

Family	Species	Life-form	Substrate	Origin	Habitat	Syndrome	Status	Voucher
	Hemiscola aculeata (L.) Raf.*	Herb	Rup, Ter	Auto	Forest edge	Zoo	NE	A. Gehrt s/n: SPF 100854
Commelinaceae	*Commelina erecta* L.*	Herb	Rup, Ter	Auto	Forest edge, Rock outcrop vegetation	Auto	NE [LC]	A.M. Magalhães 95: RB 636610
	Gibasis geniculata (Jacq.) Rohweder	Herb	Rup, Ter	Auto	Forest edge	Auto	NE	V.C. Souza 11092: ESA 26157
	Tradescantia fluminensis Vell.	Herb	Epi, Rup, Ter	Auto	Forest	Auto	NE	V.C. Souza 11093: ESA 26158
Convolvulaceae	*Ipomoea cairica* (L.) Sweet*	Climber	Ter	Auto	Anthropogenic vegetation	Anemo	NE	G.O. Joaquim Jr. 113: ESA 34579
	Ipomoea tiliacea (Willd.) Choisy*	Climber	Ter	Auto	Forest edge	Anemo	NE	V.C. Souza 11066: ESA 70940
	Ipomoea triloba L.*	Climber	Ter	Allo	Anthropogenic vegetation	Auto	NE	G.O. Joaquim Jr. 129: ESA 87102
	Jacquemontia ferruginea Choisy	Climber	Ter	Auto	Forest edge, Anthropogenic vegetation	Auto	NE	A.M. Magalhães 68: RB 636593
	Merremia dissecta (Jacq.) Hallier f.*	Climber	Ter	Auto	Forest edge, Anthropogenic vegetation	Auto	NE	A.M. Magalhães 85: RB 636601
Cucurbitaceae	*Melothria pendula* L.*	Climber	Ter	Auto	Forest edge	Zoo	NE	A.M. Magalhães 64: RB 637079
Cyperaceae	*Cyperus ligularis* L.	Herb	Rup, Ter	Auto	Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 78: RB 636599
Dioscoreaceae	*Dioscorea fodinarum* Kunth	Climber	Ter	Auto	Forest edge	Anemo	NE	V.C. Souza 11086: ESA 27114
Ebenaceae	*Diospyros inconstans* Jacq.	Tree	Ter	Auto	Forest, Forest edge	Zoo	LC	V.C. Souza 11010: ESA 26134
Erythroxylaceae	*Erythroxylum cuspidifolium* Mart.	Shrub, Tree	Ter	Auto	Forest	Zoo	NE	V.C. Souza 11078: ESA 27107
Euphorbiaceae	*Algernonia riedelii* (Müll. Arg.) G.L. Webster	Tree	Ter	Auto	Forest	Auto	NE	V.C. Souza 11087: ESA 27115
	Euphorbia insulana Vell.	Herb	Ter	Auto	Anthropogenic vegetation	Auto	NE	V.C. Souza 11061: ESA 26137
	Tragia volubilis L.	Climber	Epi, Ter	Auto	Forest edge	Auto	NE	A. Amaral & D. Lemos s/n: SP 3887
Fabaceae	*Canavalia rosea* (Sw.) DC.	Herb, Climber	Ter	Auto	Rock outcrop vegetation	Hydro	NE	V.C. Souza 11031: ESA 27434
	Centrosema virginianum (L.) Benth.	Climber	Ter	Auto	Anthropogenic vegetation	Auto	NE	G.O. Joaquim Jr. 122: RB 587987
	Chaetocalyx brasiliensis (Vogel) Benth.	Climber	Ter	Auto	Rock outcrop vegetation	Auto	NE	A. Gehrt s/n: UEC 84324
	Condylostylis candida (Vell.) A. Delgado	Climber	Ter	Auto	Forest edge	Auto	NE	V.C. Souza 11096: ESA 26161
	Crotalaria laeta Mart. ex Benth.	Subshrub	Ter	Allo	Anthropogenic vegetation	Auto	NE	A.M. Magalhães 75: RB 636597
	Crotalaria vitellina Ker Gawl.	Shrub, Subshrub	Ter	Auto	Forest edge, Rock outcrop vegetation	Auto	NE	F.T. Farah 30: RB 593080
	Desmodium incanum (Sw.) DC.*	Subshrub	Ter	Allo	Forest edge, Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 46: RB 636580
	Senegalia tenuifolia (L.) Britton & Rose	Shrub, Climber	Ter	Auto	Forest, Forest edge, Anthropogenic vegetation	Auto	NE	A.M. Magalhães 69: RB 636594
	Senna neglecta (Vogel) H.S. Irwin & Barneby*	Shrub	Rup, Ter	Auto	Anthropogenic vegetation	Auto	NE	V.C. Souza 11085: RB 591824
	Sigmoidotropis speciosa (Kunth) A. Delgado	Climber	Ter	Auto	Rock outcrop vegetation	Auto	NE	A. Gehrt s/n: SP 4553
	Zollernia ilicifolia (Brongn.) Vogel	Shrub, Tree	Ter	Auto	Forest	Zoo	NE	A. Gehrt s/n: SP 4559
Iridaceae	*Neomarica imbricata* (Hand.-Mazz.) Sprague	Herb	Ter	Auto	Forest	Auto	NE	A. Gehrt s/n: SP 4659

Table 1. Continued...

Family	Species	Life-form	Substrate	Origin	Habitat	Syndrome	Status	Voucher
Lamiaceae	*Mesosphaerum sidifolium* (L'Hérit.) Harley & J.F.B. Pastore	Herb, Subshrub	Ter	Auto	Forest edge	Zoo	NE	V.C. Souza 11065: ESA 26141
Malvaceae	*Sida planicaulis* Cav.*	Shrub, Subshrub	Ter	Auto	Forest edge, Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 40: RB 636577
	Sida rhombifolia L.*	Herb	Ter	Auto	Forest edge, Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 52: RB 636583
Marantaceae	*Maranta divaricata* Roscoe	Herb	Ter	Auto	Forest	Auto	NE	A.M. Magalhães 97: RB 636612
Meliaceae	*Trichilia casaretti* C. DC.	Tree	Ter	Auto	Forest	Zoo	LC [VU]	D.F. Bertani QT-96: RB 552254
Musaceae	*Musa paradisiaca* L.	Herb	Ter	Allo	Banana plantation	Sterile	NE	Not collected
Myrtaceae	*Eugenia astringens* Cambess.	Tree	Ter	Auto	Forest	Zoo	NE	D.F. Bertani QP-80: RB 552194
	Eugenia sulcata Spring ex Mart.	Tree	Ter	Auto	Forest	Zoo	NE	A. Gehrt s/n: SP 4528
Nyctaginaceae	*Guapira opposita* (Vell.) Reitz	Shrub, Tree	Ter	Auto	Forest, Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 60: RB 636586
Orchidaceae	*Acianthera saundersiana* (Rchb. f.) Pridgeon & M.W. Chase	Herb	Epi	Auto	No information	Anemo	NE [LC]	A. Gehrt s/n: SP 5452
	Cattleya intermedia Grah.	Herb	Epi, Rup	Auto	No information	Anemo	VU {VU}	A. Gehrt s/n: SP 8146
	Cyclopogon bicolor (Ker-Gaw.) Schltr.	Herb	Ter	Auto	Forest	Anemo	NE	G.O. Joaquim Jr. 23: ESA 33124
	Eltroplectris calcarata (Sw.) Garay & Sweet	Herb	Ter	Auto	Forest	Anemo	LC	G.O. Joaquim Jr. 24: ESA 33125
	Epidendrum densiflorum Hook.	Herb	Epi	Auto	Forest	Anemo	NE	A. Gehrt s/n: SP 4621
	Epidendrum fulgens Brongn.	Herb	Rup, Ter	Auto	Rock outcrop vegetation, Anthropogenic vegetation	Anemo	NE	A. Amaral s/n: SP 3967
	Mesadenella cuspidata (Lindl.) Garay	Herb	Ter	Auto	Forest	Anemo	NE	V.C. Souza 11042: ESA 27425
	Oeceoclades maculata (Lindl.) Lindl.†	Herb	Ter	Allo	Forest	Anemo	NE [LC]	V.C. Souza 11040: ESA 27423
Passifloraceae	*Passiflora suberosa* L.*	Climber	Ter	Auto	Forest edge	Zoo	NE	V.C. Souza 11025: RB 482678
Phytolaccaceae	*Gallesia integrifolia* (Spreng.) Harms	Tree	Ter	Auto	Forest	Anemo	NE	A.M. Magalhães 50: RB 636581
	Rivina humilis L.	Herb	Ter	Allo	Forest, Forest edge, Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 67: RB 636592
Piperaceae	*Peperomia glabella* (Sw.) A. Dietr.	Herb	Epi	Auto	No information	Zoo	NE	L.E. Mello Filho 1979: NY 558881
Plantaginaceae	*Scoparia dulcis* L.	Herb, Subshrub	Ter	Auto	Rock outcrop vegetation	Anemo	NE	V.C. Souza 11084: ESA 27112
Plumbaginaceae	*Plumbago scandens* L.	Subshrub	Ter	Auto	Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 36: RB 636574
Poaceae	*Digitaria ciliaris* (Retz.) Koeler*	Herb	Ter	Allo	Anthropogenic vegetation	Anemo, Zoo	NE	A.M. Magalhães 93: RB 636608
	Digitaria insularis (L.) Fedde*	Herb	Ter	Allo	Anthropogenic vegetation	Anemo, Zoo	NE	A.M. Magalhães 90: RB 636605
	Eleusine indica (L.) Gaertn.*	Herb	Ter	Allo	Anthropogenic vegetation	Zoo	NE [LC]	A.M. Magalhães 87: RB 636603
	Lasiacis ligulata Hitchc. & Chase	Herb	Ter	Auto	Forest	Zoo	NE	V.C. Souza 11062: ESA 26138
	Melinis minutiflora P. Beauv.*†	Herb	Ter	Allo	Anthropogenic vegetation	Anemo	NE	F.T. Farah 9: ESA 39563

Table 1. Continued...

Family	Species	Life-form	Substrate	Origin	Habitat	Syndrome	Status	Voucher
	Paspalum distichum L.	Herb	Ter	Auto	Anthropogenic vegetation	Hydro, Zoo	NE [LC]	G.O. Joaquim Jr. 44: ESA 33136
	Paspalum virgatum L.*	Herb	Ter	Auto	Anthropogenic vegetation	Auto	NE	A.M. Magalhães 91: RB 636606
	Sporobolus virginicus (L.) Kunth	Herb	Ter	Auto	Rock outcrop vegetation	Anemo	NE	A.M. Magalhães 92: RB 636607
	Stenotaphrum secundatum (Walter) Kuntze	Herb	Ter	Auto	Rock outcrop vegetation, Anthropogenic vegetation	Hydro, Zoo	NE	A.M. Magalhães 86: RB 636602
Polygonaceae	*Ruprechtia laurifolia* (Cham. & Schltdl.) A.C. Meyer	Tree, Climber	Ter	Auto	No information	Anemo	NE	A. Gehrt s/n: UEC 80555
Portulacaceae	*Portulaca oleracea* L.	Herb	Ter	Auto	Rock outcrop vegetation	Auto	NE	A.M. Magalhães 73: RB 636596
	Talinum paniculatum (Jacq.) Gaertn.	Herb	Rup, Ter	Auto	Rock outcrop vegetation	Auto	NE	A.M. Magalhães 100: RB 636614
Primulaceae	*Myrsine guianensis* (Aubl.) Kuntze	Shrub, Tree	Ter	Auto	Forest	Zoo	NE	D.F. Bertani QT-2: RB 560747
Rubiaceae	*Chiococca alba* (L.) Hitchc.	Shrub	Ter	Auto	Forest edge, Anthropogenic vegetation	Zoo	NE [LC]	A.M. Magalhães 61: RB 636587
	Rudgea minor (Cham.) Standl.	Shrub, Tree	Ter	Auto	Forest	Zoo	LC	A.M. Magalhães 71: RB 636595
Sapindaceae	*Cardiospermum halicacabum* L.*	Herb, Climber	Ter	Auto	Anthropogenic vegetation	Zoo	NE	A.M. Magalhães 102: RB 636616
	Cupania oblongifolia Mart.	Tree	Ter	Auto	Forest	Zoo	NE	D.F. Bertani QT-23: RB 551934
	Urvillea triphylla (Vell.) Radlk.	Climber	Ter	Auto	Forest edge	Anemo	NE	V.C. Souza 11075: ESA 27104
Sapotaceae	*Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn.	Shrub, Tree	Ter	Auto	Forest	Zoo	LC	A.M. Magalhães s/n: RB 637081
Urticaceae	*Pilea pubescens* Liebm.	Herb	Ter	Auto	No information	Auto	NE	A.R. Duarte 56: ESA 49651
Vitaceae	*Cissus verticillata* (L.) Nicolson & C.E. Jarvis	Climber	Ter	Auto	Forest edge	Zoo	NE	A.M. Magalhães 101: RB 636615

Figure 3. Rock outcrop vegetation on a steep cliff, with a large population of *Coleocephalocereus fluminensis*, Queimada Grande Island, São Paulo, southeastern Brazil. Photo by A.M. Magalhães.

Table 2. Number of plant species on islands off the coast of eastern and southeastern Brazil.

Site/Code	Coordinates	Phytophysiognomies	Approximate area (ha)/ Maximum altitude (m)	Distance to mainland (km)	Number of species	Reference
Queimada Grande Island, Peruíbe, São Paulo (QGI)	24°29′10″ S, 46°40′30″ W (center point)	Dense Ombrophilous Forest, rock outcrop vegetation, anthropogenic vegetation	57/210	33.2	125 (vascular plants)	This study
Abrolhos Marine National Park, Caravelas, Bahia (ABR)*	17°57′35″-17°58′56″ S, 38°41′27″- 38°42′56″ W	Rock outcrop vegetation, *restinga*	77/36	65	40 (angiosperms)	Kemenes (2003)
Franceses Island, Itapemirim, Espírito Santo (FRA)	20°55′36″ S, 40°45′15″ W (center point)	Dense Ombrophilous Forest, rock outcrop vegetation, *restinga*, mangrove swamps, anthropogenic vegetation	16/36	3.5	123 (angiosperms)	Ferreira et al. (2007)
Cagarras Islands Natural Monument, Rio de Janeiro, Rio de Janeiro (CAG)**	23°01′30″-23°04′32″ S, 43°11′23″-43°12′32″ W	Dense Ombrophilous Forest, rock outcrop vegetation, anthropogenic vegetation	79/240	3.8-8.6	169 (vascular plants)	Bovini et al. (2014)
Grande Island, Angra dos Reis, Rio de Janeiro (GRA)	23°04′30″-23°13′40″ S, 44°05′26″-44°22′43″ W	Dense Ombrophilous Forest, rock outcrop vegetation, *restinga*, mangrove swamps, anthropogenic vegetation	19,300/1,011	3.1	795 (vascular plants)	Callado et al. (2009)
Cardoso Island, Cananéia, São Paulo (CAR)	25°03′05″-25°18′18″ S, 47°53′48″-48°05′42″ W	Dense Ombrophilous Forest, rock outcrop vegetation, *restinga*, mangrove swamps, anthropogenic vegetation	22,500/840	<1	985 (angiosperms)	Barros et al. (1991)
Mel Island, Paranaguá, Paraná (MEL)	25°29′00″-25°34′32″ S, 48°17′15″-48°23′16″ W	Dense Ombrophilous Forest, rock outcrop vegetation, *restinga*, mangrove swamps, anthropogenic vegetation	2,894/148	2.8	~504 (vascular plants)	Kersten & Silva (2005); Kozera & Rodrigues (2005); Marques & Oliveira (2005); Salino et al. (2005)

* The survey included the five islands that make up ABR: Guarita, Redonda, Santa Barbara, Siriba and Sueste.

** The survey included the four largest islands at CAG: Cagarra, Comprida, Palmas and Redonda.

Discussion

The small islands (<100 ha) along the eastern and southeastern coast of Brazil have very non-diverse floras (Kemenes 2003, Ferreira et al. 2007, Bovini et al. 2014) related to their insular conditions, sizes, habitat restrictions, steep topographies, incipient soils, and use histories (see Lomolino 2000, Kreft et al. 2008). Species richness on QGI is lower than that on CAG (Bovini et al. 2014) and close to that on FRA (an island of only 16 ha; Ferreira et al. 2007) – which is apparently related to the distance of QGI from the coast, making the arrival and establishment of propagules more difficult. The very low richness on ABR (Kemenes 2003) can also be related to its greater distance from the coast.

Large islands generally have richer floras due to their great environmental heterogeneity (Barros et al. 1991, Kersten & Silva 2005, Kozera & Rodrigues 2005, Marques & Oliveira 2005, Salino et al. 2005, Callado et al. 2009). These islands can be considered insular samples of the mainland Atlantic Forest complex, and they harbor many mainland plant communities, i.e., altitudinal gradients of dense ombrophilous forests and pioneer formations: *restinga*, rock outcrop vegetation, and mangrove swamps.

The vascular flora of QGI originated from the mainland Atlantic Forest complex, and the vast majority of its species (including most species associated with anthropically disturbed areas) are autochthonous to that southeastern Brazilian complex, occurring frequently in dense ombrophilous forests or *restinga*. This pattern was also reported for FRA (Ferreira et al. 2007) and CAG (Bovini et al. 2014). Some species are widespread on those three sets of islands (e.g., *Sesuvium portulacastrum*, *Temnadenia odorifera*, *Syagrus romanzoffiana*, *Cyrtocymura scorpioides*, *Cereus fernambucensis*, *Ipomoea cairica*, *Maranta divaricata*, *Guapira opposita*, *Sporobolus virginicus*, *Talinum paniculatum* and *Chiococca alba*).

The Dense Ombrophilous Forest on QGI showed a very low richness of arboreal species (S = 17), contrasting with high richness often found in continental remnants of this formation (e.g., Scudeller et al. 2001). The island forest also showed an unusual oligarchic structure, with a predominance of *Guapira opposita*, *Rudgea minor*, and *Aspidosperma australe* (B.C. Kurtz et al., unpubl. data). QGI is essentially a small rocky outcrop with few areas with deep soils, which limits the growth, distribution, and diversity of tree species. This edaphic aspect could partly explain the low species richness of this life form and its distinct original physiognomy in contrast to continent forests or those of other large islands (e.g., Barros et al. 1991).

The tree species on QGI are mostly zoochoric, producing small fleshy fruits or arilate seeds consumed by the island's avifauna (e.g., Pineschi 1990, Lorenzi 1998, 2000, Passos & Oliveira 2004). Due to the absence of frugivorous mammals on QGI (Marques et al. 2002), birds are apparently the main local dispersers of those diaspores, and several species are known to feed on the fruits of *Guapira opposita*, *Myrsine guianensis*, and *Syagrus romanzoffiana*, which fructify during long periods of time and represent an important food resource for the local avifauna (Montanhini 2010). Two omnivorous passerines that visit the island at different times of the year are the main dietary items of the endemic and critically endangered pit viper *Bothrops insularis* (Marques et al. 2012).

The historical use of the island, especially during the period when the lighthouse was manually operated (1909–1925), strongly influenced the current vegetation cover of QGI and its flora. According to information obtained from the Brazilian Navy, the original Dense Ombrophilous Forest was reduced to construct the lighthouse and its support buildings, to establish a subsistence farm and, possibly, to create animal pasture. These changes were most likely made by cutting and burning the original vegetation (the latter was apparently a common practice that apparently gave rise to the island's name – 'Big Burn Island'; Bataus & Reis 2011). Thus, there were intentional and unintentional introductions of several foreign plant species to QGI. This set of species seems to be well-established now on the island, competing with the original vegetation and preventing its full recovery. Although the largest area of anthropogenic vegetation (located near the lighthouse) has not expanded over the last 10 years, its natural recovery appears to be extremely slow (B.C. Kurtz et al., unpubl. data). Additionally, many of the species recorded by A. Gehrt in the early 1920's were not encountered during our more recent expeditions.

The flora of QGI can be further analyzed by considering the composition of its life forms. The number of tree species (S = 17) corresponds to roughly half the number of climbers (S = 31), a group that is widely considered an indicator of disturbance in tropical forests (Gerwing 2001; Laurance et al. 2001). The herbaceous flora (S = 52) is the richest among the various life forms on QGI, with several species occurring exclusively in the anthropogenic vegetation. Additionally, although the mainland Atlantic Forest shows very high vascular hemiepiphytic/epiphytic species richness (2,256 species or 15.4% of its vascular flora; Freitas et al. 2016), our surveys showed a low richness of this group of plants on the island, making it plausible to argue that the past uses of QGI were responsible for major changes in its already impoverished insular flora.

Of the two species considered invasive in the Atlantic Forest complex (Instituto Hórus 2017), molasses grass (*Melinis minutiflora*; 'capim gordura') is abundant in the anthropogenic vegetation sites on QGI. It has been observed that massive occurrences of this grass are related to a number of factors that prevent the regeneration of natural environments – such as changes in nutrient cycles, light availability, soil microclimate, and wind velocity. Also, high infestations of molasses grass directly interfere with natural regeneration by creating a thick layer of plant material that lends considerable competitive advantage over other species of the lower stratum, including seedlings and saplings (Barger et al. 2003; Martins et al. 2004). *Oeceoclades maculata*, on the other hand, does not currently appear to behave as invasive species on QGI.

The introduction and establishment of allochthonous species is not a problem exclusive to QGI. Many small islands distributed along the southeastern coast of Brazil are partially covered by alien species (pers. obs.). The islands that make up CAG (Bovini et al. 2014), for example, are partially covered by *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs, a grass of African origin, and 13% of the surface of FRA (Ferreira et al. 2007) is occupied by *Leucaena leucocephala* (Lam.) de Wit, an arboreal American legume. These two alien species have great invasive potential in the Atlantic Forest complex (Instituto Hórus 2017), and the introduction of invasive alien species has been highlighted as one of the main threats to island biota around the world (Kreft et al. 2008, Serafini et al. 2010).

As such, we recommend permanent and routine monitoring of the vegetation cover of QGI, using both high-resolution satellite images and field studies, to evaluate changes in areas covered by anthropogenic vegetation. Managers should consider controlling alien species, especially the invasive grass *Melinis minutiflora*, and planting seedlings of native species identified in this study. These activities will be essential to the *in situ* conservation of the critically endangered *Bothrops insularis*.

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Author Contributions

Bruno Coutinho Kurtz: substantial contribution in the concept and design of the study; contribution to data collection, analysis, interpretation, and manuscript preparation.

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Adriana Melo Magalhães: contribution to data collection, analysis, and interpretation.

Juliana de Paula-Souza: substantial contribution in the concept and design of the study; contribution to data collection, analysis, interpretation, and manuscript preparation.

Alexandre Romariz Duarte: contribution to data collection.

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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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Confirmation of the northermost population of the endangered butterfly *Heliconius nattereri* C. Felder & R. Felder, 1865 (Lepidoptera: Nymphalidae)

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Abstract: *Heliconius nattereri* C. Felder & R. Felder, 1865 is an enigmatic and rarely collected species. Because of severe habitat reduction, the species is currently listed as endangered. We here report the confirmation of a northernmost population. This collection and further observations reported here add two more known localites to the distribution of the species. Two of the three confirmed locations are in protected areas. We worry that the northern population may be at great risk because it is not formally protected. We urge conservation efforts to preserve the local forests where the species is found.

Keywords: *endangered species, conservation, Atlantic Forest, range map*

Confirmação de uma população no limite norte da borboleta ameaçada *Heliconius nattereri* C. Felder & R. Felder, 1865 (Lepidoptera: Nymphalidae).

Resumo: *Heliconius nattereri* C. Felder & R. Felder, 1865 é uma espécies enigmática e raramente coletada. Em função de severa perda de habitat, a espécie encontra-se atualmente listada como ameaçada. Neste trabalho, relatamos a confirmação do registro de uma população no limite norte da distribuição da espécie. Nossas coletas e outras observações aqui relatadas adicionam dois novos locais para a distribuição da espécie. Duas das três localizações confirmadas encontram-se em áreas protegidas. Nos preocupa saber que a população aqui relatada pode estar em risco por falta de proteção formal. Esforços de conservação das florestas que abrigam esta espécie são urgentemente necessários.

Palavras-chave: *espécies ameaçadas, conservação, Mata Atlântica, mapa de distribuição*

The species *Heliconius nattereri* C. Felder & R. Felder, 1865 has inspired much interest and curiosity among butterfly enthusiasts and evolutionary biologists (Emsley 1965, Brown 1972). It is one of the rarest species in the iconic *Heliconius* Kluk, 1870 genus, and less than two dozen specimens had been collected until Keith Brown's rediscovery of this obscure species provided essential information regarding its basic biology (Brown 1970, 1972). It is one of the few sexually dimorphic *Heliconius*; females participate in the tiger mimicry ring, while the males display a yellow and black pattern (Brown 1972).

Heliconius nattereri has a very limited geographical range, from the states of Espírio Santo to Bahia, Brazil, a range that is less than 1000 km in length (Figure 1). Unfortunately this is also one of the most disturbed areas of the Atlantic Forest in Brazil. Populations occurs mostly on mountains above 600 m in Eastern Brazil, normally at low densities. Because of its limited geographical range and habitat use (dense woods), human pressure and deforestation have contributed to severe habitat loss, placing the species in the endangered category (Ministério do Meio Ambiente 2014). This means that many populations may have disappeared because of habitat reduction and deterioration caused by forest cutting, and presumably by competition from other Heliconiini that invade modified habitats (Brown 1972, Brown & Freitas 2008). While it is encouraging to know that some populations are protected because they occur in federal reserves, such as the population in the Biological Reserve Augusto Ruschi at Santa Teresa, Espírito Santo, studied by Brown in 1972, it is nonetheless fundamental that the status of the remaining populations is assessed and the search for additional populations is needed. The National Action Plan for the Conservation of Endangered Species of Brazilian Lepidopterans (Freitas & Marini-Filho 2011) has listed this as a critical action. To fulfill this need, our team has been carrying a long-term project with the aim of assessing current genetic diversity in remnant populations of *H. nattereri* and verifying the occurrence of the species in potential areas of its geographic range. We here report the confirmation of the occurrence of a population in the northern edge of its geographical distribution (Figure 1).

We have visited remnant forests in Amargosa, Bahia (39°36' S, 13°01'W) and neighboring districts, including Serra da Jibóia, in Elísio Medrado and Santa Teresinha municipalities (39°30'S, 12°48'W), following tips from Professor Olaf Mielke (Universidade Federal do Paraná), who collected the species in Amargosa in 1997 and 2002. During a short visit in July 2013 to Amargosa municipality, we confirmed the occurrence of the host plant (*Passiflora contracta* Vitta, 2004), although no butterflies were seen. In April 2016, two of us (MB & ML) spent 54 hours (six days) in the same locality and visually confirmed the presence of males and females of *H. nattereri*. In April 2017, we returned to the same location

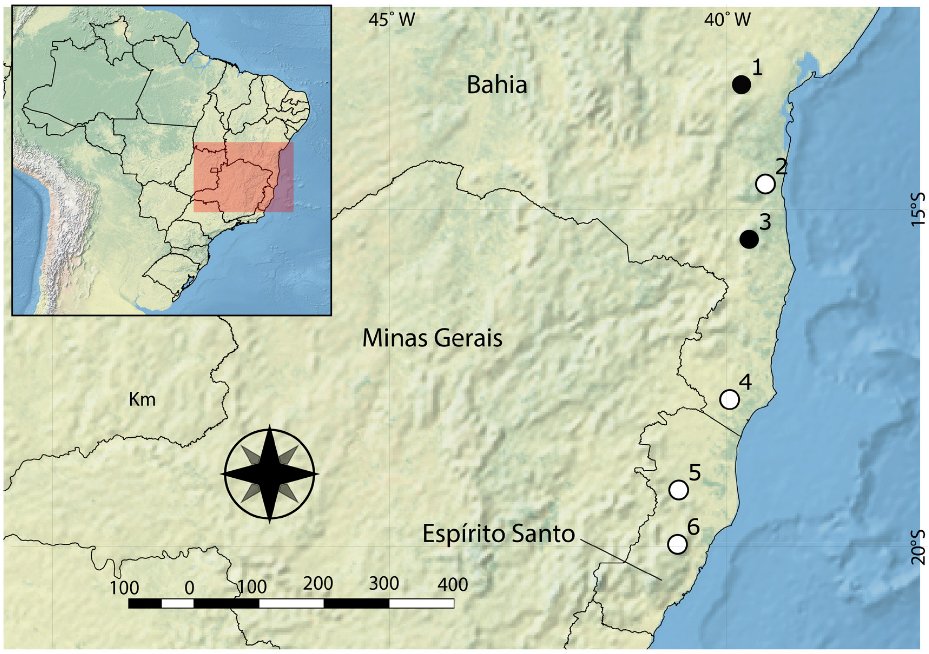

Figure 1. *Heliconius nattereri* range map in Eastern Brazil. Open circles are points derived from data in Brown (1979) and black circles represent our data. 1-4 Bahia. 1: Amargosa, 2: Uruçuca (uncertain), 3: Camacan, 4: Posto da Mata (uncertain), 5-6 Espírito Santo. 5: Colatina (uncertain), 6: Santa Teresa.

and after five days of intensive search (7-9 hours per day) we successfully collected one male (Figure 2) while it was feeding on *Gurania* (Schltdl.) Cogn., 1875 flowers (Cucurbitaceae) about 10 m high (Figure 3). We also saw other 3 individuals flying. We thus have continued collecting evidence for the occurrence of the species in this area, this being the northernmost population reported so far. Our searches were made along trails near the entrance to the Reserva do Timbó, and consisted of systematically walking along the trails and actively searching for the butterfly. We also stopped at flower sources (*Gurania*) and waited for visitors to appear. There was not a systematic attempt to control for search or collection effort. We chose to collect in April as a way to avoid collecting during the colder period of June and July, when our first visit was made and very few butterflies were observed in activity.

Besides the observation of *H. nattereri* likely larval host plants (*P. contracta*) we also observed at least six other *Passiflora* species, five of which are shown in Figure 3, together with photos of the butterfly habitat. The following *Heliconius* were collected in the woods at or near the site of *H. nattereri*: *H. erato* (L., 1758), *H. melpomene* (L., 1758), *H. sara* (Fabricius, 1793), *H. ethilla* (Godart, 1819), and *H. numata* (Cramer, 1780). Canopy flying *Philaethria* Billberg, 1820 was also observed but not collected; the same with *Dryas iulia* (Fabricius, 1775). We did not endeavour to collected in lower lands where other species were more abundant, such as *Agraulis* Boisduval & Le Conte, [1835] and some *Euides* Hübner, 1816. It was extremely difficult to find *H. nattereri* despite intensive search along trails and awaiting at feeding areas (*Gurania* vines), probably because of its habitat of flying high in the canopy. We could not confirm any female sightings in 2017, although some tiger pattern *Heliconius* were seen. Our present collection only yielded *H. numata* and *H. ethilla* from this color pattern.

Our finding opens the possibility of finding other undiscovered populations nearby and further to the south, although habitat destruction may make this search fruitless. We are also able to report that the butterfly was seen flying in 2016 at Serra Bonita Reserve in Camacan, Bahia, located 250 km away to the South, approximately midway between the Amargosa and Santa Teresa populations. Sightings in Camacan have also been reported by other researchers (K. S. Brown & A. V. L. Freitas, personal communication). Yet, a recent range map (Rosser et al. 2012) does not list these two locations, although Amargosa is shown in the Brazil's Red List Book (Machado et al. 2008). Our new range map (Figure 1) corrects these omissions, including all known localities of the species, and indicates uncertain points that have not been independently confirmed.

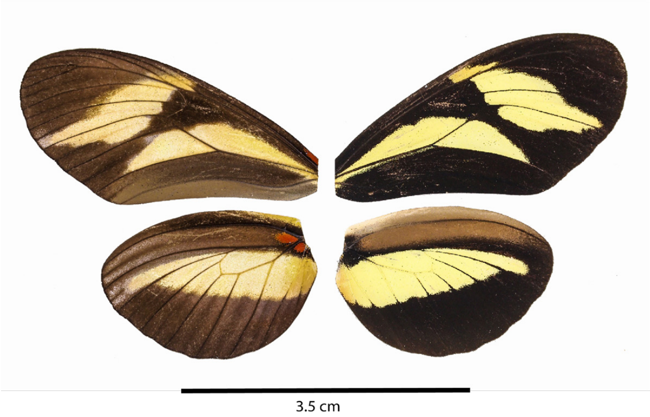

Figure 2. *Heliconius nattereri* (male) ventral (left) and dorsal (right) sides. Collected on 7.IV.2017, Mata do Timbó, Amargosa, BA.

Given its rarity, and the seasonal occurrence and cryptic habits of the species, it will take much effort to check the existence of other remaining populations. Given the general destruction of native habitats, this could be a potentially isolated population with little or no gene flow with the other known remaining populations. Our preliminary results using whole genome sequencing of the southern population from Santa Teresa, Espírito Santo, suggests little genetic variation within the population (Massardo et al., unpublished results). The presumed lack of genetic variation should be

Figure 3. Habitat of *Heliconius nattereri* and plants in Amargosa, Bahia, Brazil. A *Passiflora jiboiaensis*; B *P. misera;* C *P. alata;* D *P. mansoi;* E *P. contracta*; F View of forest on mountain tops, habitat for *H. nattereri*. G *Gurania sp* pollen plant for *Heliconius;* H Forest clearing at forest edge in Mata do Timbó.

of great concern because the mountain range where this new population is reported is still threatened by illegal logging, even though a municipal reserve has been created in 2011 (Amargosa 2011). Early efforts in building a visitor center for the mountain range are derelict. Thus, urgent action is needed to protect these unique montane ecosystems, not only because they contain populations of the endangered *Heliconius nattereri*, but they are also home to other endemic butterfly species, such as the newly described *Euptychia atlantica* Nakahara & Freitas, 2017 (Nakahara et al. 2017), and the endangered amphibian *Proceratophrys sanctaritae* Cruz & Napoli, 2010 (Cruz & Napoli 2010) and the aptly named *Phasmahyla timbo* Cruz, Napoli & Fonseca 2008 (Cruz et al. 2008). Thus, the region not only is home to a very diverse butterfly fauna (Vasconcelos 2013, Nakahara et al. 2017), but is also a site of interest for conservation of amphibians in the Brazilian northeast (Camardelli & Napoli 2012). We hope this report will provide support for more effective conservation actions of such important remnants. In particular, we would like to see long term mark recapture studies in the area so that the viability of this population is better assessed. Based on the repeated collections of individuals in the area, and the presence of both adult and larval hostplants, we expect that there will be a resident population there. Nevertheless, we would like to see data that not only supports this assessment, but also provides guidance for the conservation of the forest remnants.

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Author Contributions

M Z Cardoso: substantial contribution in the concept and design of the study, data collection, and manuscript preparation

M R Brito: substantial contribution in data collection, and manuscript preparation,

M B Lion: substantial contribution in the concept and design of the study, data collection, and manuscript preparation

Conflicts of interest

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

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Museum collections indicate bird defaunation in a biodiversity hotspot

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Abstract: Ipanema National Forest, southeastern Brazil, once contained 340 bird species. Forest cover suffered for centuries from log exploitation and, as a result, most of the remaining forests are now an impoverished subset of the original vegetation. We show how the bird community changed over time by comparing historical and recent records. Currently, 228 species can be recorded, for a compilation of 410 species, of which 359 are documented. Some 89 forest species with historical records failed to be detected in recent surveys. Of the 72 Atlantic Forest or Cerrado endemic species, no more than 29 (40%) are still found. The bird community changed from one which used to be related to coastline rain forests to another, which relates more to drier semideciduous forests of the interior.

Keywords: *Atlantic Forest, Cerrado, hierarchical cluster analysis, multivariate analysis, semideciduous forests.*

Coleções de museus indicam defaunação de aves em um hotspot de biodiversidade

Resumo: A Floresta Nacional de Ipanema, sudeste do Brasil, já abrigou 340 espécies de aves. Sua cobertura florestal sofreu por séculos com a exploração de madeira e, desse modo, a maior parte da vegetação remanescente é uma sub-representação daquela original. Neste artigo é demonstrado como a comunidade de aves foi modificada com o passar do tempo por meio da comparação entre registros históricos e recentes. Atualmente, 228 espécies podem ser registradas, para um total de 410 espécies, das quais 359 possuem documentação. Das espécies registradas historicamente, 89 não foram mais detectadas. Das 72 espécies endêmicas da Mata Atlântica ou do Cerrado, apenas 29 (40%) ainda podem ser encontradas. A comunidade de aves, outrora similar à de florestas ombrófilas costeiras, atualmente é mais relaciona à comunidade de matas semideciduais mais secas do interior.

Palavras-chave: *Análise hierárquica de cluster, Análise multivariada, Cerrado, Floresta Semidecidual, Mata Atlântica.*

Introduction

Defaunation, i.e., the process of losing animal species, has been accelerated in the last five centuries, a fact by which some authors have contended that earth is experiencing a “sixth extinction wave” (Barnosky et al. 2011, Pimm et al. 2014, Ceballos et al. 2015, 2017). Besides extinctions, abundance of vertebrates is sharply declining, leading to functional extinction of several species (Butchart et al. 2010, Ceballos et al. 2017). Among different causes of this phenomenon (e.g. hunting, pollution, impacts from invasive species and climate change), habitat loss is one of the main drivers, especially in tropical regions, where deforestation rates are high (Dirzo et al. 2014, Johnson et al 2017). Among the different vertebrate groups suffering species loss and population decline, birds, which suffer population decline worldwide (especially accentuated in tropical regions, Ceballos et al. 2017), play important roles in ecosystems, such as pollination, pest control and seed dispersal (Whelan et al. 2008). In addition to the loss of important services, the local extinction and population decline of birds can cause changes in evolutionary processes (Galetti et al. 2013).

Although one can infer bird species loss of a given degraded area by comparing its community to nearby areas, where species composition is assumed original, few studies have documented the disappearance of species over time at a same locality (but see Blake & Loiselle 2015, 2016). Curtis & Robinson (2015) resurveyed sites in Oregon from a 60-year-old historic dataset, comparing bird communities of the same sites between this time interval. In Neotropical regions, due to rare long-term data or earlier surveys, researchers have tried to assess extinction rates by comparing the composition of pre- and post-fragmentation bird assemblages (Willis 1974, 1979, Leck 1979, Bierregaard & Lovejoy 1989). Few authors compared censuses separated by long time intervals. These are from little-surveyed sites at time lags of: 200 years in eastern Amazonia (Moura et al. 2014), 167 years in southwestern Amazonia (Silveira & D'horta, 2002), 130 years in eastern Brazil (Christiansen & Pitter 1997) and 100 years in Singapore (Sodhi et al. 2005). There are also examples of less than a century separating surveys, such as Barro Colorado Island, Panama (85 years, Robinson 1999), San Antonio, Colombia (80 years, Kattan et al. 1994), and the Viçosa region, Brazil (70 years, Ribon et al. 2003).

At Ipanema National Forest (hereafter INF), interior São Paulo state, almost 200 years of continuous and severe deforestation led to conspicuous habitat change. Many naturalists visited this iron foundry, including Johann Baptist von Spix and Karl Friedrich Philip von Martius (Spix & Martius 1824), Friedrich Sellow and Ignaz Franz Werner Maria von Olfers (Stresemann 1948), Baron Georg Heinrich von Langsdorff, Peter Wilhelm Lund and Johannes Reinhardt and Johann Christian Mikan (Mikan 1820, Reinhardt 1870, Pinto 1979, Krabbe 2007). No other naturalist, however, contributed more to the knowledge of this locality than Johann Natterer, whose zoological work has no parallel in the history of scientific expeditions in Brazil (Pinto 1979). Thus, the site's well-documented (as museum specimens collected by naturalists) original avifauna provides a singular opportunity to evaluate how habitat loss has changed this Atlantic Forest bird community over time.

We compiled all bird records from the INF. We searched for both historical (museum specimens acquired during the nineteenth century naturalists' expeditions) and recent records, which included field data we have been gathering since 1986 and publications. We specifically addressed the question whether recent bird species composition in a highly surveyed biodiversity hotspot differed from that of ~200 years ago by comparing species recorded in the 1800s to species recorded in recent surveys. We used similarity indices and cluster analysis comparing species richness at the INF to other well-surveyed rain and semideciduous forests wishing to determine to which of these forests the INF used to be more closely related. We then conducted these same analyses only with the species we recently recorded at the INF to determine whether species richness changed over time. We hypothesized species richness changed considerably from one closely related to rain forests to another, which is more similar to semidecidual forests.

Material and Methods

1. Study area

INF (23°26' S, 47°36' W) is located in the state of São Paulo, southeastern Brazil, within the municipalities of Araçoiaba da Serra, Capela do Alto and Iperó, ca 120 km west of the city of São Paulo (Figure 1). The region is predominantly inserted within the Atlantic Forest domain with some Cerrado enclaves. These two domains are biodiversity hotspots that, despite harboring several endemic and threatened species have suffered from intense habitat loss (Ribeiro et al. 2009, Strassburg et al. 2017). INF is drained by the Sapucaí River to the west and Ipanema River to the east, with elevations ranging from 550-970 m asl. According to Spix & Martius (1824:52), during the 1800s the vegetation cover was "almost everywhere covered with thick woods (…) The forests, which stand more luxuriant and thicker in the hollow than in the higher parts, are uncommonly rich in the most various kinds of wood". Currently, it encompasses ~ 5,000 ha, of which 75% are covered by semideciduous (90% of all forests) and rain forests in several successional stages (~ 2,800 ha), secondary growth and savannas, including ecotones between semideciduous forests and Cerrado *sensu lato* (~ 540 ha), water reservoirs and dams (~ 250 ha) and native reforestations (~ 220 ha, Radambrasil [1983]). The current vegetation is composed of secondary forests due to the extensive exploitation of tree species in the last two centuries to fuel the iron foundry furnaces (Saint-Hilaire 1976). Climate is considered as Cfa in Koeppen's classification, with defined wet (September-February) and dry (March-August) seasons. Annual mean rainfall is 1,400 mm, whereas mean annual temperature is 20.4 °C, with a minimum of 11.6 C° in July and reaching 29.8 C° in February (MMA 2003). The land cover surrounding forests comprise water reservoirs and dams, regrowth, recovery of degraded areas, settlements of rural workers, the administrative headquarters and preserved historical sites, such as ruins of the foundry (MMA 2003).

The vegetation at INF suffered from tree exploitation since the beginning of the iron foundry (Saint-Hilaire 1976). Intensive deforestation began during the nineteenth century, when metal craft was transformed into a metallurgical industry at the cost of large-scale exploitation of mines at the Araçoiaba Mountain between 1811-1833 (Menon 1992). Due to Brazilian governmental orders, as much as 2,764 tons of charcoal fueled auto-ovens during the Brazil-Paraguay war in 1878 (Dupré 1885). Between 1926 and 1943, the Araçoiaba Mountain suffered from apatite extraction; during the 1950s the dominant activity was the production of cement, still produced today. It was only in May 1992, when the area was legally declared a National Forest (which still allows the selective use of natural resources according to Brazilian law), that deforestation stopped within the INF and ~ 350 hectares were destined for fast growing *Eucalyptus* plantations (~ 200 ha) and reforestations with native species (~ 150 ha, MMA 2003).

2. Bird records

2.1. Historical survey

At that time naturalists provided few details on bird collection data. Species collected by Natterer are museum specimens that are predominantly deposited at the Naturhistorisches Museum in Vienna, Austria. Refer to Appendix I.

2.2. Recent survey

We surveyed forest birds using point counts (Bibby et al. 2000) ever three months from May 2001-February 2002 and from August 2009-May 2013. We determined three unlimited-radius 10-min point counts at least 200 m apart on two existing trails (north and south) inside mature forest within the INF, which we visited on four consecutive days following the same sequence of points. Therefore, each campaign consisted of 12 point counts (if inclement weather did not impede us from surveying). Although there are representative portions of the forest block outside the limit of the INF, all point counts lay within this protected area. We also used eight mist nets (12 m x 3 m x 32 mm) inside forests to record secretive species during 3 consecutive days on the 2001 and 2002 campaigns. Nets touched bottom and remained open from 06:00-17:00 h. We checked nets at 1-hr intervals and released captured birds after identification. We performed non-systematic surveys with transect counts (Bibby et al. 2000) on

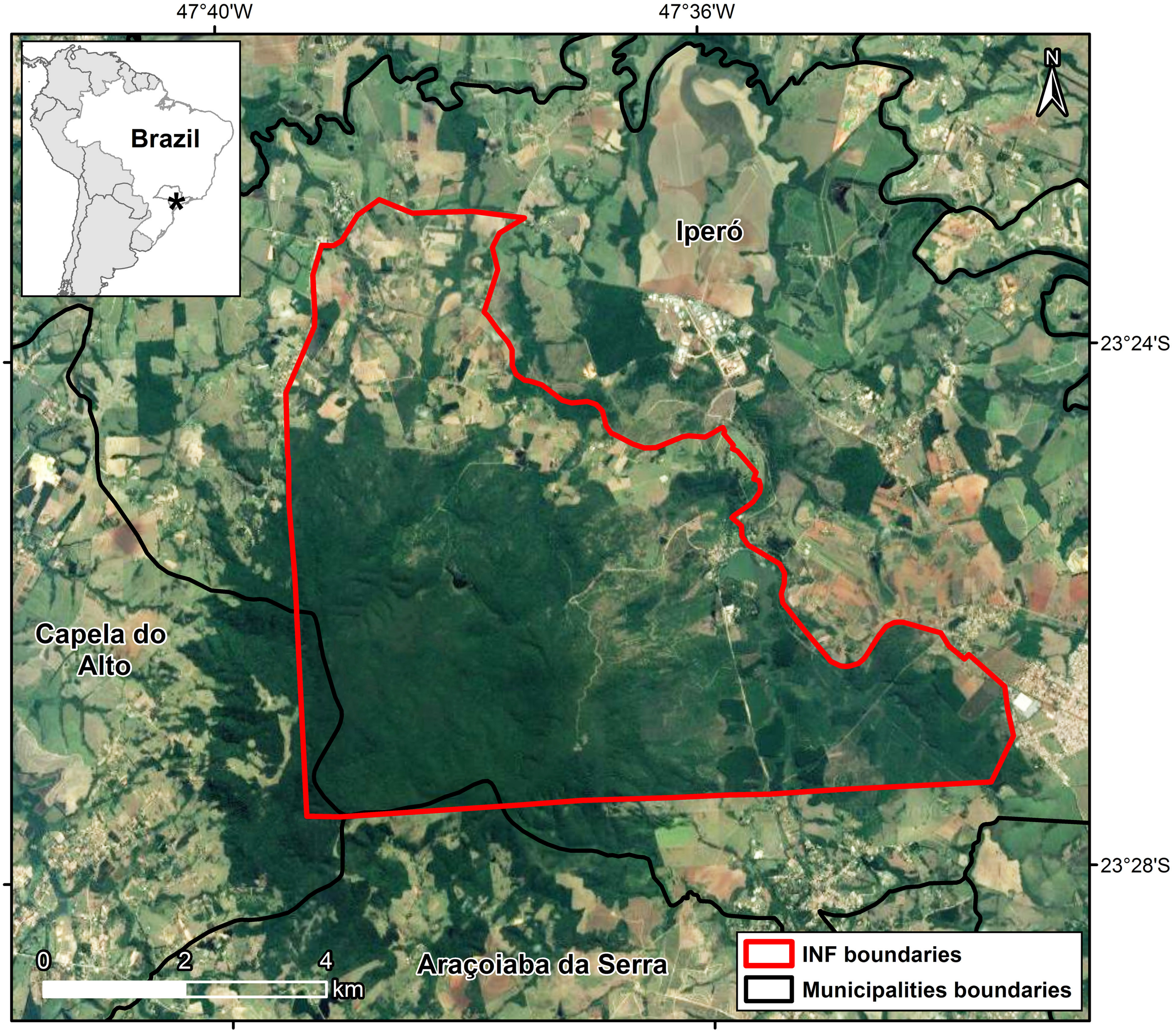

Figure 1. Location of the Ipanema National Forest (limited by a continuous line) within three municipalities in the state of São Paulo, southeastern Brazil.

26 July 2009, 14 November 2010, 21-22 July 2012 and 01 May 2013. To detect border and canopy species or flying-over birds (Robinson 1999) we further considered qualitative records conducted during *ad libitum* observations during the mornings and while moving between point counting. We walked randomly through different types of habitats, such as reed beds, marshes, pastures, *capoeiras* (early successional forests) and forest edges during mornings and afternoons (15:00-19:00 h). We surveyed different successional stages of vegetation, such as early succession, 5- to 10-year-old regrowth and mature secondary forests. We detected birds 15 min before sunrise with the aid of binoculars and/or by vocalizations for both the point count method and random observations, accumulating 624 point counts (104 h), approximately 540 h of random observations and 600 mist-netting hours, more than any recent bird censuses (Table 1).

To complement our results, we searched for birds recorded within the INF in Web of Science (https://www.webofknowledge.com) and Google Scholar (http://scholar.google.com.br) and included a few recent records from colleagues (Appendix II). In addition, we searched for "Floresta Nacional de Ipanema" archived digital vouchers on the internet on Wiki Aves (www.wikiaves.com.br) and on the global avian sound library Xeno-canto (www.xeno-canto.org). We did not consider misidentifications (records of species which do not conform to expected range) of published records. These included the record of counterparts from different biogeographic regions and the absence of the species which would occur in southern Brazil: Rusty-margined Flycatcher *Myiozetetes cayanensis*, Pied Water-Tyrant *Fluvicola pica*, and Cinereous Becard *Pachyramphus rufus* certainly correspond to the Social Flycatcher *M. similis*, Black-backed Water-Tyrant *F. albiventer* and the White-winged Becard *P. polychopterus*, respectively. Both the Blue-back Grosbeak *Cyanoloxia cyanoides* and the Red-breasted Blackbird *Sturnella militaris* were mistaken for their southern counterparts, the Ultramarine Grosbeak *C. brissonii* and the White-browed Blackbird

Table 1. Source of historical bird records and detailed information on recent bird records at Ipanema National Forest, São Paulo, southeastern Brazil. For question marks, refer to Results. An asterisk indicates three misidentified species.

Source	Date	Time spent	Number of species
J. B. Spix and K. P. F. Martius	Jan 1818	–	13?
J. C. Mikan	1816-1817	–	?
F. Sellow and I. F. W. M. Olfers	Dec 1819 - Jan 1820	–	4?
J. Natterer	Feb 1819 - Jul 1820 / Sep 1821 - Sep 1822	–	340
J. H. Langsdorff	1825	–	?
P. W. Lund and J. T. Reinhardt	Mar 1834	–	7
C. Silva and L. B. Regalado	January 1995	–	1
L. B. Regalado	1991–1996	128 h	39
Y. Oniki & E. O. Willis	1986	2 h	45
C. Silva	1993–2003	350 h	186*
Present authors	1986–2015	1,244 h	228

Table 2. Characteristics of seven localities with which the Ipanema National Forest was compared. Boraceia Biological Station (BBS), Barreiro Rico Farm (BRF), Carlos Botelho State Park (CBSP), Caetetus Ecological Station (CES), Intervales State Park (ISP), Lençóis Paulista (LP), Serra do Mar State Park (SMSP). Evidences are museum specimens (S), photographs (P) and sound recordings (R).

	Habitat	Elevation (m)	Area (ha)	Surveys (yr)	Evidence	Species richness
BBS	Ombrophylous forest	800	96	1945-2010	S,P,R	323
BRF	Semidecidous forest	450-586	1451	1957-2002	S,P,R	359
CBSP	Ombrophylous forest	20-1000	37,644	2006-2009	S,P,R	331
CES	Semidecidous forest	500-680	2,180	1976-2006	P,R	293
ISP	Ombrophylous forest	60-1010	38,000	1988-2015	S,P,R	338
LP	Semidecidous forest	570	1,600	1989-2002	P,R	300
SMSP	Ombrophylous forest	0-1200	300,000	1898-2012	S,P,R	417

S. superciliaris. For the most part, museum specimens identified by Pelzeln (1868) collected by nineteenth century naturalists accounted for our "historical records". Taxonomic arrangements follow the Brazilian Committee of Ornithological Records (Piacentini et al. 2015). Atlantic Forest endemic species are according to Parker et al. (1996) and Cerrado endemic species follow Silva & Bates (2002).

3. Analysis

To investigate to which protected forests in São Paulo the INF were most similar regarding its historic bird species richness (presence/absence) we performed a hierarchical cluster analysis using Euclidean distance within the R environment (R Development Core Team 2015). We then ran this analysis with the current species richness of the INF. We chose Ward´s minimum variance criterion as the objective function, defining which clusters merge at each step (Ward-Jr 1963). We also calculated Jaccard Dissimilarity Index (package vegan, function vegdist()), in which case lower values suggest higher similarities. We decided to compare INF with sufficiently well surveyed protected forests at least 250 km away. These included well surveyed (1) semideciduous forest with Cerrado enclaves in Lençóis Paulista (Donatelli et al. 2004), (2) semideciduous forests without Cerrado enclaves in Barreiro Rico Farm (Magalhães 1999) and Caetetus Ecological Station (Cavarzere et al. 2009) and (3) rain forests to the east of the INF – Boraceia Biological Station (Cavarzere et al. 2010), Serra do Mar State Park (Simpson et al. 2012) and to the west of the INF – Intervales State Park, (Vielliard & Silva 2001) and Carlos Botelho State Park (Antunes et al. 2013; Table 2; Figure 2).

Results

1. Recent records

Compared to the 340 bird species INF once harbored, we recorded 228 species of 21 orders and 51 families. The compilation of all records from the INF resulted in a list of 410 species of 23 orders and 68 families, of which 359 are documented in the form of museum vouchers, photographs or recordings. No novel records came from e-vouchers, which contained 25 species on the Wiki Aves, and nine species on the Xeno-canto databases until 24/02/2017. The only species we failed to detect whose recent recording is available at those sites is the Rufous-breasted Leaftosser *Sclerurus scansor* (Ménétriès 1835). Of the 69 Atlantic Forest endemic species, 40 have no recent records; of the three Cerrado endemic species, two have no recent records; five, of all species, are under threat categories. One is threatened at the global level and one is critically endangered in Brazil (Table 3).

We show a cluster analysis of the bird community similarity in Figure 3. There are two distinct groups composed of (1) rain forests (Serra do Mar State Park, Boraceia Biological Station, Intervales and Carlos Botelho State Parks) and (2) semideciduous forests (Barreiro Rico Farm, Caetetus Ecological Station and Lençóis Paulista). When accounting for historical bird records, INF (INF1 in Figure 3) is most similar to the first rain forests cluster. However, when we incorporated only recent bird community composition (INF2 in Figure 3), INF nested within semideciduous forests. Dissimilarity indexes showed the same pattern (Table 4).

Discussion

1. Changes in species richness

Overall, 410 species of birds are reported for the INF, but 89 forest species and another 16 typical Cerrado/wet grassland species may no longer occur there. Of the 202 species recorded both during the nineteenth century and recent surveys, most resident species are widespread in the Atlantic Forest (Del Hoyo et al. 1992-2011). Approximately 60% of the bird community of the INF is still found. Of this, 87 (43%) are forest species. However, another 72 (36%) forest species collected by Natterer seem absent, i.e. we did not detect 45% of forest species over the last 29 years.

Our multivariate analyses suggest modifications resulting from severe forest loss as INF changed its bird species richness from one closely related to rain forests running parallel to the coastline to another, which resembles drier semideciduous forests of the interior. The savannization of moist forests in Colombia has been previously suggested due to deforestation and land practices (Cavelier et al. 1998). This suggestion linked human induced-fire damaging soils dating as far as Pre-Columbian times. The 200 years span shown in this paper may prove to drive similar results in the Atlantic Forest vegetation.

1.1. Vegetation

Part of the remarkable historical species richness is also due to a Cerrado (tropical savanna) enclave, which increased substantially the diversity of the bird community with typical Cerrado species, such as

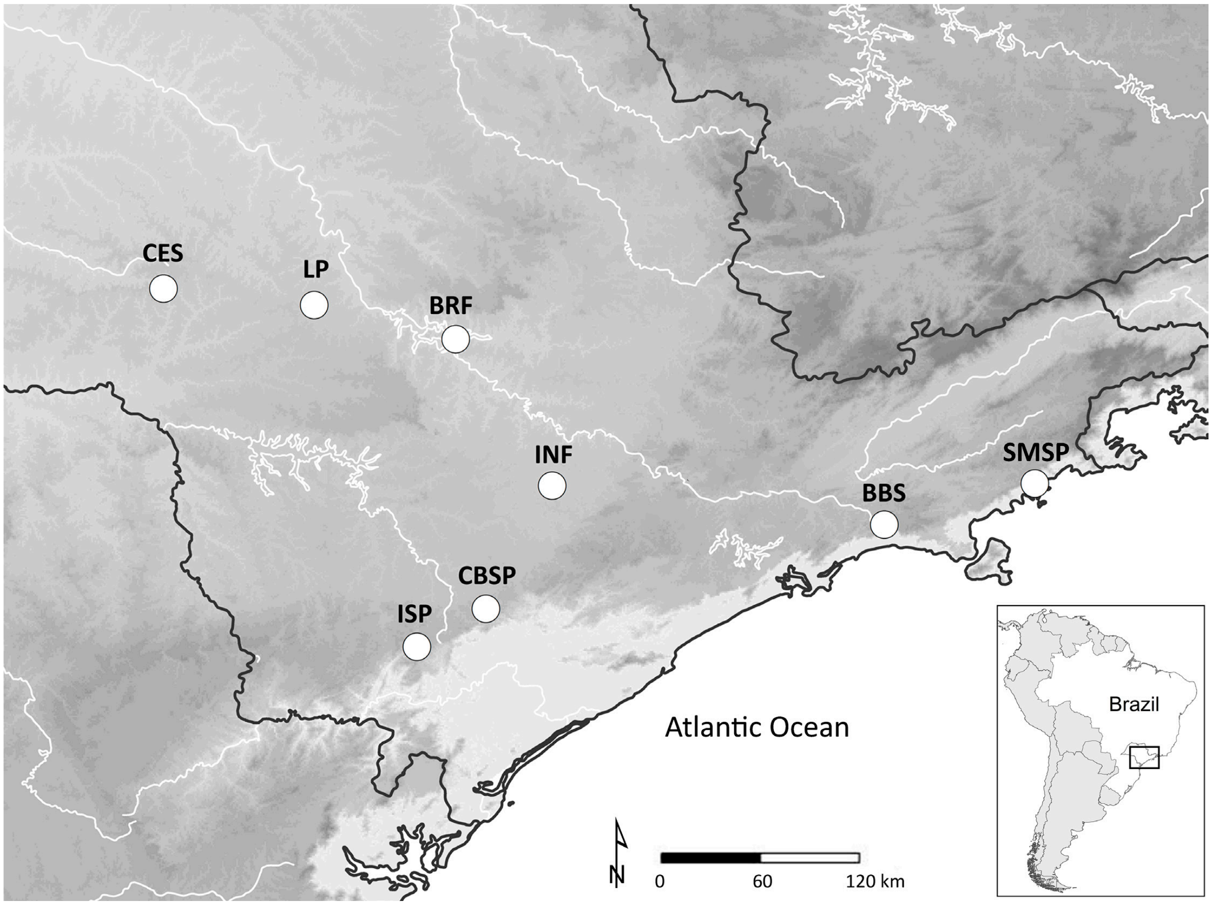

Figure 2. Locations between which we compared bird species richness. BBS – Boraceia Biological Station, CES – Caetetus Ecological Station, BRF – Bacury Farm, INF – Ipanema National Forest, LP – Lençóis Paulista, CBSP – Carlos Botelho State Park, ISP – Intervales State Park, SMSP – Serra do Mar State Park.

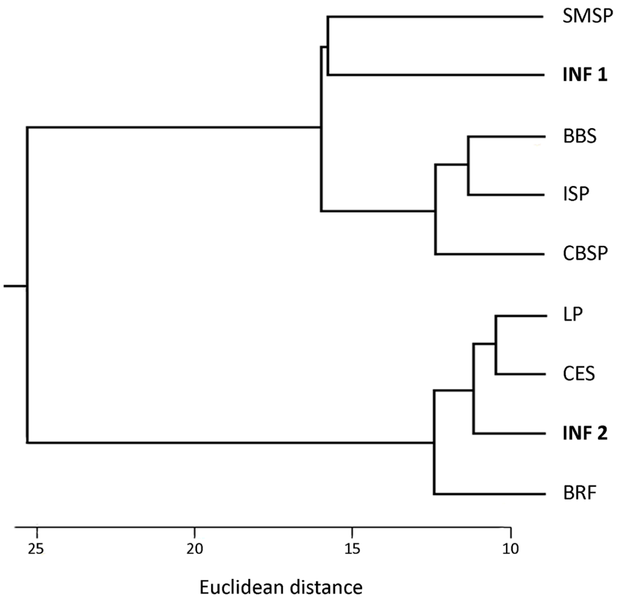

Figure 3. Cluster analysis of bird species richness similarity between eight Atlantic Forest remnants in São Paulo, southeastern Brazil. BBS – Boraceia Biological Station, CES – Caetetus Ecological Station, BRF – Bacury Farm, INF – Ipanema National Forest, LP – Lençóis Paulista, CBSP – Carlos Botelho State Park, ISP – Intervales State Park, SMSP – Serra do Mar State Park. INF 1 refers to historical species richness, whereas INF 2 refers to recent species richness.

Geositta poeciloptera (Wied 1830), *Alectrurus tricolor* (Vieillot 1816), *Cistothorus platensis* (Latham 1790) and *Coryphaspiza melanotis* (Temminck 1822). Although this enclave still exists, these species seem no longer present. Currently, no more than 29 Atlantic Forest and one Cerrado endemic species are detected in this area.

1.2. Fragmentation

The INF, where pristine forests once contained as much as 340 species of birds, currently has ca. 50% of forest birds and several (69) invaders, i.e. species that did not occur at the INF and benefited from several years of deforestation (Table 3). Most species demanding high-quality forests or that are sensitive to human presence seem absent. These include *Tinamus solitarius* (Vieillot 1819), *Aburria jacutinga* (Spix 1825), *Spizaetus ornatus* (Daudin 1800), *Triclaria malachitacea* (Spix 1824), *Trogon* spp., *Ramphastos* spp. and *Pteroglossus aracari* (Linnaeus 1758), Helmeted Woodpecker *Celeus galeatus* (Temminck 1822), Short-tailed Antthrush *Chamaeza campanisona* (Lichtenstein 1823), Antpittas *Grallaria varia* (Boddaert 1783) and *Hylopezus nattereri* (Pinto 1937). Ipanema is the type locality of *Phylloscartes eximius* (Temminck 1822), a species without recent records. It is a sensitive mid-story flycatcher which requires riverine forests and occurs at low densities (Tonetti & Pizo 2016). The species richness decline witnessed in the Psittaciformes and Trogoniformes is quite remarkable. Currently, one species of forest-dwelling parrot *Pionus maximiliani* (Kuhl 1820) is infrequent, and no *Trogon* sp. species remains. Because these species play essential ecological roles

Table 3. List of bird species (indicating those we consider forest dwellers) recorded within the Ipanema National Forest, São Paulo, southeastern Brazil. Sources: 1 (Pelzeln 1868), 2 (Reinhardt 1870), 3 (Hellmayr 1906), 4 (Stresemann 1948), 5 (Krabbe 2007), 6 (Silva & Regalado 1998), 7 (Regalado 1999), 8 (Willis & Oniki 2003), 9 (Silva 2010), 10 (present authors), WA (Wiki Aves), XC (Xeno-canto). Atlantic Forest (AF) and Cerrado (CE) endemic species are according to Parker et al. (1996) and Silva & Bates (2002), respectively. Evidences acquired in this study are aural (A) or visual (V) undocumented records, or photographed (P), audio-recorded (R) or mist-netted (MN). Skins collected during the nineteenth century (sources 1-5) represent museum vouchers. A question mark denotes a probable misidentification or typographic error (see Lopes et al. 2017). Threat status: Critically Endangered (CR), Endangered (EN), Vulnerable (VU). IUCN = globally threatened taxa; BR = taxa threatened in Brazil; SP = taxa threatened is São Paulo. Invader species are indicated as $^{\text{inv}}$ on the recent records column.

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Tinamiformes						
Tinamidae						
Tinamus solitarius	1		AF			Forest
Crypturellus obsoletus	1					Forest
Crypturellus parvirostris	1	8, 10		A		
Crypturellus tataupa	1	10		A		Forest
Rhynchotus rufescens	1,3					
Nothura maculosa	1	8, 10		P		
Anseriformes						
Anhimidae						
Anhima cornuta	1					
Anatidae						
Dendrocygna viduata		10$^{\text{inv}}$		V		
Cairina moschata	1	10		P		
Amazonetta brasiliensis	1	9, 10		V		
Nomonyx dominicus	1					
Galliformes						
Cracidae						
Penelope superciliaris	1	9, 10		P		Forest
Aburria jacutinga	1		AF		EN$^{\text{BR}}$	Forest
Odontophoridae						
Odontophorus capueira	1		AF			Forest
Podicipediformes						
Podicipedidae						
Tachybaptus dominicus	1, 3	10		R,P		
Podilymbus podiceps	1	9, 10		V		
Suliformes						
Phalacrocoracidae						
Nannopterum brasilianus	1	9, 10		V		
Anhingidae						
Anhinga anhinga		9, 10$^{\text{inv}}$		V		
Pelecaniformes						
Ardeidae						
Tigrisoma lineatum	1	10		V		
Ixobrychus exilis	1	8				
Nycticorax nycticorax	1	9, 10		V		
Butorides striata	1	9, 10		V		
Bubulcus ibis		9, 10$^{\text{inv}}$		V		
Ardea cocoi	1	9, 10		V		
Ardea alba	1	9, 10		V		
Syrigma sibilatrix		9, 10$^{\text{inv}}$		V		
Pilherodius pileatus	1					
Egretta thula	1	9, 10		V		
Egretta caerulea	1					
Threskiornithidae						
Plegadis chihi	1					
Mesembrinibis cayennensis	1	10		V		
Platalea ajaja	1					
Cathartiformes						
Cathartidae						
Cathartes aura	1	9, 10		P		
Coragyps atratus	1	8,9,10		P		
Sarcoramphus papa		6,9,10$^{\text{inv}}$		P	EN$^{\text{SP}}$	

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Accipitriformes						
Accipitridae						
Leptodon cayanensis	1	10		P		Forest
Elanoides forficatus	1					
Elanus leucurus		8,9,10^{inv}		P		
Harpagus diodon	1	10		V		Forest
Accipiter poliogaster	1					
Accipiter superciliosus	1					
Ictinia plumbea	1	9, 10		P		
Rostrhamus sociabilis		9, 10^{inv}		V		
Heterospizias meridionalis	1	9, 10		V		
Urubitinga urubitinga		10^{inv}		V		
Urubitinga coronata		9^{inv}		V	EN^{IUCN}, EN^{BR},CR^{SP}	
Rupornis magnirostris	1	9, 10		P		
Geranoaetus albicaudatus	1	9, 10		V		
Pseudastur polionotus	1		AF			Forest
Buteo brachyurus		9^{inv}		V		
Spizaetus tyrannus	1, 3					Forest
Spizaetus melanoleucus	1					
Spizaetus ornatus	1					Forest
Gruiformes						
Aramidae						
Aramus guarauna		9, 10^{inv}		V		
Rallidae						
Aramides cajaneus		9, 10^{inv}		V		
Aramides saracura	1	10	AF	P		Forest
Laterallus melanophaius		10^{inv}		A		
Mustelirallus albicollis	1	9				
Pardirallus nigricans	1	9, 10		A		
Gallinula galeata	1	8,9,10		P		
Porphyrio martinicus	1	9				
Charadriiformes						
Charadriidae						
Vanellus chilensis	1	8,9,10		A,V		
Pluvialis dominica	1					
Recurvirostridae						
Himantopus melanurus	1					
Scolopacidae						
Gallinago paraguaiae	1					
Gallinago undulata	1					
Numenius borealis	1					
Actitis macularius	1					
Tringa solitaria	1					
Tringa flavipes	1					
Calidris alba	1					
Calidris fuscicollis	1					
Calidris melanotos	1					
Calidris subruficollis	1					
Jacanidae						
Jacana jacana	1	8,9,10		A,V		
Laridae						
Chroicocephalus maculipennis	1					
Sternidae						
Sternula superciliaris	1					
Rynchopidae						
Rynchops niger	1					

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Columbiformes						
Columbidae						
Columbina talpacoti		8,9,10^{inv}		A,V		
Columbina squammata		9, 10^{inv}		A,V		
Claravis pretiosa	1					Forest
Claravis geoffroyi	1		AF		CR^{BR}	Forest
Columba livia		9^{inv}		V		
Patagioenas picazuro		9, 10^{inv}		P		
Patagioenas cayennensis	1	9, 10		A,V		Forest
Zenaida auriculata	1	8,9,10		A,V		
Leptotila verreauxi	1	9, 10		MN,P		Forest
Leptotila rufaxilla	1	9, 10		A		Forest
Geotrygon violacea	1					Forest
Geotrygon montana	1	10		A		Forest
Cuculiformes						
Cuculidae						
Piaya cayana	1	9, 10		P		
Coccyzus melacoryphus	1					
Coccyzus euleri		10^{inv}		P		
Crotophaga ani	1	8,9,10		P		
Guira guira	1	8,9,10		A,V		
Tapera naevia	1	9, 10		R,P		Forest
Dromococcyx phasianellus	1					Forest
Dromococcyx pavoninus		10^{inv}		A		
Strigiformes						
Tytonidae						
Tyto furcata	1	10		V		
Strigidae						
Megascops choliba	1	9, 10		A		Forest
Pulsatrix koeniswaldiana	1	10	AF	A		Forest
Strix hylophila	1		AF			Forest
Strix virgata	1					Forest
Glaucidium brasilianum	1					
Athene cunicularia		8,9,10^{inv}		A,V		
Asio clamator	1					
Asio flammeus	1					
Caprimulgiformes						
Nyctibiidae						
Nyctibius aethereus	1				EN^{BR}	Forest
Nyctibius griseus	1	9, 10		A		Forest
Caprimulgidae						
Nyctiphrynus ocellatus	1					Forest
Antrostomus rufus		9, 10^{inv}		R		
Antrostomus sericocaudatus	1					Forest
Lurocalis semitorquatus	1, 4	10		A,V		Forest
Nyctidromus albicollis	1	9, 10		A,V		
Hydropsalis parvula	1	10		A,V		
Hydropsalis anomala	1					
Hydropsalis maculicaudus	1					
Hydropsalis torquata	1	9, 10		P		
Hydropsalis forcipata	1		AF			
Podager nacunda	1, 4	10		V		
Chordeiles minor	1					

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Apodiformes						
Apodidae						
Cypseloides fumigatus	1					
Cypseloides senex	1					
Streptoprocne zonaris	1	9, 10		V		
Streptoprocne biscutata	1					
Chaetura meridionalis	1	8,9,10		A,V		
Trochilidae						
Phaethornis squalidus	1		AF			
Phaethornis pretrei		9, 10^{inv}		A,V		
Phaethornis eurynome	1	9, 10	AF	A,V		Forest
Campylopterus largipennis	1?					
Eupetomena macroura		9, 10^{inv}		P		
Aphantochroa cirrochloris	1					Forest
Florisuga fusca	1	9, 10		A,V		
Colibri serrirostris	1	8, 10		P		
Anthracothorax nigricollis	1	9				
Lophornis magnificus	1					Forest
Lophornis chalybeus	1, 3					Forest
Chlorostilbon lucidus	1	9, 10		P		
Thalurania glaucopis	1	9, 10	AF	MN,P		Forest
Leucochloris albicollis	1	9, 10		P		Forest
Amazilia versicolor	1	10		A,V		Forest
Amazilia lactea		9, 10^{inv}		A,V		
Heliodoxa rubricauda	1		AF			Forest
Heliothryx auritus	1					
Heliomaster squamosus	1	10		P		
Calliphlox amethystina	1					
Trogoniformes						
Trogonidae						
Trogon surrucura	1					Forest
Trogon rufus	1					Forest
Coraciiformes						
Alcedinidae						
Megaceryle torquata	1	9, 10		A,V		
Chloroceryle amazona	1	8,9,10		V		
Chloroceryle americana	1	9, 10		V		
Momotidae						
Baryphthengus ruficapillus	1	10		A,V		Forest
Galbuliformes						
Galbulidae						
Jacamaralcyon tridactyla	1, 3		AF			
Bucconidae						
Notharchus swainsoni	1		AF			Forest
Nystalus chacuru	1	9				
Malacoptila striata	1	9, 10		MN,P		Forest
Nonnula rubecula	1					Forest
Piciformes						
Ramphastidae						
Ramphastos toco	1	9, 10		V		
Ramphastos dicolorus	1		AF			Forest
Pteroglossus aracari	1					Forest

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Picidae						
Picumnus cirratus	1	9, 10		A,V		Forest
Picumnus temminckii	1	9, 10	AF	A,V		Forest
Melanerpes candidus	1	8,9,10		A,V		
Melanerpes flavifrons	1					Forest
Veniliornis spilogaster	1	9, 10		A,V		Forest
Piculus aurulentus	1		AF			Forest
Colaptes melanochloros	1	9, 10		R		
Colaptes campestris	1	8,9,10		A,V		
Celeus galeatus	1		AF		EN^{BR}	Forest
Celeus flavescens	1	9, 10		A,V		Forest
Dryocopus lineatus	1	9, 10		P		
Campephilus robustus	1		AF			Forest
Cariamiformes						
Cariamidae						
Cariama cristata		9, 10^{inv}		A,V		
Falconiformes						
Falconidae						
Ibycter americanus	1					
Caracara plancus	1	9, 10		P		
Milvago chimachima	1, 3	9, 10		V		
Herpetotheres cachinnans	9	10		A		
Micrastur ruficollis	1					Forest
Micrastur semitorquatus		10^{inv}		A		
Falco sparverius	1	9, 10		V		
Falco femoralis	1	9, 10		V		
Psittaciformes						
Psittacidae						
Primolius maracana	1					Forest
Psittacara leucophthalmus	1	10		A,V		
Aratinga auricapillus	1	10		R		
Pyrrhura frontalis	1					Forest
Forpus xanthopterygius	1	9, 10		A,V		
Brotogeris tirica		10^{inv}	AF	A,V		
Pionopsitta pileata	1		AF			Forest
Pionus maximiliani	1	9, 10		A,V		Forest
Amazona vinacea	1, 3		AF		VU^{BR}	Forest
Amazona aestiva		10^{inv}		A,V		
Triclaria malachitacea	1		AF			Forest
Passeriformes						
Thamnophilidae						
Rhopias gularis	1					Forest
Dysithamnus mentalis		9, 10^{inv}		A,MN,V		
Herpsilochmus rufimarginatus	1					Forest
Thamnophilus doliatus		10^{inv}		P		
Thamnophilus ruficapillus	1	10		R		
Thamnophilus caerulescens	1	7,9,10		A,MN, V		Forest
Hypoedaleus guttatus	1, 3		AF			Forest
Batara cinerea	1					Forest
Mackenziaena leachii	1		AF			Forest
Mackenziaena severa	1	7,9,10	AF	A,MN,V		Forest
Biatas nigropectus	1		AF			Forest
Myrmoderus squamosus	1	10	AF	A		Forest
Pyriglena leucoptera	1	7,9,10	AF	A,MN,V		Forest
Drymophila ferruginea	1		AF			Forest
Drymophila ochropyga	1		AF			Forest
Drymophila malura	1	7,9,10	AF	R		Forest

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Conopophagidae						
Conopophaga lineata	1	7,9,10		A,MN,V		Forest
Grallariidae						
Grallaria varia	1					Forest
Hylopezus nattereri	1		AF			Forest
Rhinocryptidae						
Eleoscytalopus indigoticus	1	9, 10	AF	A		Forest
Formicariidae						
Chamaeza campanisona	1					Forest
Scleruridae						
Sclerurus scansor	1	WA,XC	AF	R		Forest
Geositta poeciloptera	1		CE		EN$^{\text{BR}}$	
Dendrocolaptidae						
Sittasomus griseicapillus	1	7,9,10		A,MN,V		Forest
Xiphorhynchus fuscus	1	7,9,10		A,V		Forest
Campylorhamphus falcularius	1	7,9,10	AF	A,V		Forest
Lepidocolaptes angustirostris		10$^{\text{inv}}$		V		
Lepidocolaptes falcinellus	1	10	AF	V		Forest
Dendrocolaptes platyrostris	1	7,9,10		A		Forest
Xiphocolaptes albicollis	1					
Furnariidae						
Xenops minutus	1					Forest
Xenops rutilans	1	7, 10		A,V		Forest
Furnarius rufus		8,9,10$^{\text{inv}}$		A,V		
Lochmias nematura	1	7,9,10		A,MN		Forest
Automolus leucophthalmus	1	7,9,10		A,MN		Forest
Anabazenops fuscus	1		AF			Forest
Anabacerthia amaurotis	1		AF			Forest
Anabacerthia lichtensteini	1		AF			Forest
Philydor atricapillus	1	8	AF			Forest
Philydor rufum	1					Forest
Heliobletus contaminatus	1	7, 9	AF			Forest
Syndactyla rufosuperciliata	1	9, 10		A,MN,V		Forest
Phacellodomus ferrugineigula		8, 10$^{\text{inv}}$	AF	P		
Certhiaxis cinnamomeus	1	9, 10		A		
Synallaxis ruficapilla	1	7,9,10	AF	A,MN,V		Forest
Synallaxis cinerascens	1		AF			Forest
Synallaxis frontalis		9, 10$^{\text{inv}}$		MN,P		
Synallaxis albescens		9$^{\text{inv}}$		A		
Synallaxis spixi	1	9, 10		A		
Cranioleuca pallida	1	7,9,10	AF	A		Forest
Pipridae						
Neopelma chrysolophum	1		AF			Forest
Manacus manacus	1					Forest
Ilicura militaris	1					Forest
Chiroxiphia caudata	1	7,9,10	AF	A,MN,V		Forest
Tityridae						
Myiobius atricaudus	1					Forest
Schiffornis virescens	1, 5	10	AF	A		Forest
Laniisoma elegans	1	M. Cohn-Haft and A. Whittaker		R	VU$^{\text{SP}}$	Forest
Tityra inquisitor	1	9				Forest
Tityra cayana	1	7, 9				Forest
Pachyramphus viridis	1	10		A		Forest
Pachyramphus castaneus	1					Forest
Pachyramphus polychopterus		9, 10$^{\text{inv}}$		A,V		
Pachyramphus validus	1	9, 10		A,V		

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Cotingidae						
Pyroderus scutatus	1	7			VU$^{\mathrm{SP}}$	Forest
Lipaugus lanioides	1		AF			Forest
Procnias nudicollis	1	9	AF		VU$^{\mathrm{SP}}$	Forest
Pipritidae						
Piprites chloris	1					Forest
Platyrinchidae						
Platyrinchus mystaceus	1, 5	7,9,10		A,MN,V		Forest
Rhynchocyclidae						
Mionectes rufiventris	1	7,9,10		A,V		Forest
Leptopogon amaurocephalus	1	7,9,10		A,MN,V		Forest
Corythopis delalandi	1	10		A,MN,V		Forest
Phylloscartes eximius	1		AF			Forest
Phylloscartes ventralis	1	10		R		Forest
Tolmomyias sulphurescens	1	7,9,10		A,V		Forest
Todirostrum poliocephalum		9, 10$^{\mathrm{inv}}$	AF	A,V		
Todirostrum cinereum		9, 10$^{\mathrm{inv}}$		A,V		
Poecilotriccus plumbeiceps	1	7,9,10		A		Forest
Myiornis auricularis	1	10	AF	P		Forest
Hemitriccus diops	1		AF			Forest
Hemitriccus orbitatus	1	10	AF	A		Forest
Hemitriccus nidipendulus	1		AF			Forest
Tyrannidae						
Hirundinea ferruginea	1	8,9,10		P		
Euscarthmus meloryphus	1					
Tyranniscus burmeisteri	1					Forest
Camptostoma obsoletum	1	9, 10		A,V		
Elaenia flavogaster	1	8, 10		A,V		
Elaenia spectabilis		10$^{\mathrm{inv}}$		P		
Elaenia parvirostris		10$^{\mathrm{inv}}$		P		
Elaenia mesoleuca	1	10		A,V		
Elaenia chiriquensis	1					
Elaenia obscura	1	10		A,V		
Myiopagis caniceps	1					Forest
Myiopagis viridicata	1	9, 10		A,V		Forest
Capsiempis flaveola	1	10		P		Forest
Phaeomyias murina	1					
Phyllomyias virescens	1	10	AF	R		Forest
Phyllomyias fasciatus	1	10		A,V		Forest
Culicivora caudacuta	1					
Serpophaga nigricans	1					
Serpophaga subcristata	1			A,V		
Attila rufus	1	7, 10	AF	A,V		Forest
Legatus leucophaius	1					
Myiarchus swainsoni	1	7,9,10		P		Forest
Myiarchus ferox		9, 10$^{\mathrm{inv}}$		P		
Myiarchus tyrannulus	1	10		A,V		
Sirystes sibilator	1	10		A		Forest
Pitangus sulphuratus	1	8,9,10		A,V		
Machetornis rixosa		8,9,10$^{\mathrm{inv}}$		A,V		
Myiodynastes maculatus	1	7,9,10		A,MN,V		
Megarynchus pitangua	1, 2	9, 10		A,V		
Myiozetetes similis	1	9, 10		A,V		
Tyrannus melancholicus	1, 5	8,9,10		A,V		
Tyrannus savana	1	8,9,10		P		
Empidonomus varius	1	9, 10		P		
Conopias trivirgatus	1					Forest

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Colonia colonus	1, 5	9, 10		A,V		
Myiophobus fasciatus	1	9, 10		A,V		
Pyrocephalus rubinus	1	9				
Fluvicola albiventer		9^{inv}		A,V		
Fluvicola nengeta		9, 10^{inv}		A,V		
Arundinicola leucocephala		8,9^{inv}		V		
Gubernetes yetapa		8,9,10^{inv}		V		
Alectrurus tricolor	1				VU^{BR}	
Cnemotriccus fuscatus	1					Forest
Lathrotriccus euleri	1	7,9,10		P		Forest
Contopus cinereus	1	10		A,V		
Knipolegus cyanirostris	1	10		V		
Knipolegus lophotes		9, 10^{inv}		V		
Satrapa icterophrys	1					
Xolmis cinereus	1	9, 10		V		
Xolmis velatus		8, 10^{inv}		V		
Muscipipra vetula	1, 3	10	AF	V		
Vireonidae						
Cyclarhis gujanensis	1	9, 10		A,V		
Hylophilus amaurocephalus		10^{inv}		A,V		
Hylophilus poicilotis	1	10	AF	A,V		Forest
Vireo olivaceus	1	7,9,10		A,V		
Corvidae						
Cyanocorax cristatellus	1	10	CE	A,V		
Hirundinidae						
Pygochelidon cyanoleuca	1	8,9,10		A,V		
Alopochelidon fucata	1					
Stelgidopteryx ruficollis	1	8,9,10		A,V		
Progne tapera		9, 10^{inv}		V		
Progne chalybea	1	8,9,10		A,V		
Tachycineta albiventer	1					
Tachycineta leucorrhoa	1	9				
Troglodytidae						
Troglodytes musculus	1	9, 10		A,V		
Cistothorus platensis	1					
Donacobiidae						
Donacobius atricapilla		8,9,10^{inv}		P		
Turdidae						
Turdus leucomelas	1	9, 10		A,MN,V		
Turdus rufiventris	1	9, 10		A,MN,V		
Turdus amaurochalinus	1	9, 10		A,V		
Turdus subalaris		10^{inv}		A		
Turdus albicollis	1	7,9,10		A,MN,V		Forest
Mimidae						
Mimus saturninus	1	8,9,10		A,V		
Motacillidae						
Anthus lutescens		9^{inv}		A,V		
Passerellidae						
Zonotrichia capensis	1	8,9,10		A,V		
Ammodramus humeralis	1	8,9,10		A,V		
Arremon semitorquatus	1	7, 10	AF	A,V		Forest
Parulidae						
Setophaga pitiayumi	1	7,9,10		A,V		Forest
Geothlypis aequinoctialis	1, 5	8,9,10		A,V		
Basileuterus culicivorus	1	7,9,10		A,MN,V		Forest
Myiothlypis flaveola		9, 10^{inv}		MN,P		
Myiothlypis leucoblephara	1	7,9,10		A,MN,V		Forest
Myiothlypis rivularis	1					

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Icteridae						
Psarocolius decumanus	1	10		A,V		Forest
Icterus pyrrhopterus		10^inv^		A,V		
Gnorimopsar chopi	1					
Agelasticus cyanopus		8, 10^inv^		P		
Chrysomus ruficapillus		9, 10^inv^		P		
Pseudoleistes guirahuro		9, 10^inv^		A,V		
Molothrus oryzivorus	1					
Molothrus bonariensis	1	9, 10		A,V		
Sturnella superciliaris		8,9^inv^		A,V		
Thraupidae						
Pipraeidea melanonota	1	10		V		Forest
Cissopis leverianus	1					Forest
Schistochlamys ruficapillus	1					
Tangara seledon	1		AF			Forest
Tangara cyanoventris	1		AF			Forest
Tangara sayaca	1	8,9,10		A,V		
Tangara palmarum		9, 10^inv^		A,V		
Tangara ornata	1		AF			
Tangara cayana	1	9, 10		A,V		
Nemosia pileata		10^inv^		A,V		
Conirostrum speciosum		9, 10^inv^		R		
Sicalis flaveola	1	10		A,V		
Haplospiza unicolor	1		AF			Forest
Hemithraupis ruficapilla	1	7, 10	AF	P		Forest
Volatinia jacarina	1	8,9,10		A,V		
Trichothraupis melanops	1	7,9,10		A,MN,V		Forest
Coryphospingus cucullatus	1	9, 10		A,V		
Tachyphonus coronatus	1	7,9,10		MN,P		Forest
Ramphocelus carbo		10^inv^		P		
Tersina viridis	1	10		A,V		
Dacnis nigripes	1		AF			Forest
Dacnis cayana	1	9, 10		A,V		
Coereba flaveola	1	9, 10		A,V		
Sporophila lineola		9, 10^inv^		A,V		
Sporophila caerulescens	1, 5	8,9,10		P		
Sporophila leucoptera		10^inv^		A,V		
Sporophila angolensis	1	10		A,V		
Coryphaspiza melanotis	1		CE		EN^BR^	
Emberizoides herbicola	1	10		A,V		
Saltator similis	1	7,8,9,10		A,V		Forest
Saltator fuliginosus	1	10	AF	A,V		Forest
Thlypopsis sordida		9, 10^inv^		A,MN,V		
Pyrrhocoma ruficeps	1	7,9,10		V		Forest
Cardinalidae						
Piranga flava	1	9				
Habia rubica	1	7,9,10		A,V		Forest
Cyanoloxia glaucocaerulea		10^inv^		P		
Cyanoloxia brissonii	1	9, 10		V		
Fringillidae						
Spinus magellanicus	1	9, 10		V		
Euphonia chlorotica	1	9, 10		A,V		
Euphonia violacea	1					
Euphonia chalybea	1		AF			
Euphonia cyanocephala	1	10		V		Forest
Euphonia pectoralis	1		AF			
Chlorophonia cyanea	1					

Table 3. Continued...

Taxon	Record		Endemism	Evidence	Threat status	Habitat
	Historic	Recent				
Estrildidae						
Estrilda astrild		9^inv		A,V		
Passeridae						
Passer domesticus		8,9,10^int		A,V		

Table 4. Jaccard Dissimilarity Indices comparing bird species richness between eight protected forests in São Paulo, southern Brazil. INF 1 refers to historical species richness, whereas INF 2 refers to recent species richness. Ipanema National Forest (INF), Intervales State Park (ISP), Carlos Botelho State Park (CBSP), Caetetus Ecological Station (CES), Lençóis Paulista (LP), Serra do Mar State Park (SMSP), Boraceia Biological Station (BBS), Barreiro Rico Farm (BRF).

	ISP	CBSP	INF 1	INF 2	CES	LP	SMSP	BBS
CBSP	0.400							
INF 1	0.453	0.587						
INF 2	0.583	0.633	0.559					
CES	0.551	0.615	0.526	0.417				
LP	0.639	0.689	0.578	0.399	0.363			
SMSP	0.397	0.548	0.511	0.603	0.572	0.653		
BBS	0.320	0.416	0.470	0.581	0.571	0.647	0.462	
BRF	0.595	0.626	0.626	0.511	0.458	0.511	0.654	0.622

such as seed dispersal (Galetti et al. 2013), maintenance of the original vegetation structure may be seriously compromised at INF in the long term.

Pre- and post-isolation comparisons of tropical forests show the percentage of extinct species varies according to the time lag between isolation. It varies from 49% in a 4-ha fragment in Singapore over 100 years (Sodhi et al. 2005) to ca 30% after 50-90 years in Colombian forests (Kattan et al. 1994, Renfijo 1999) and Barro Colorado Island, Panama (Robinson 1999). For the Atlantic Forest, Aleixo & Vielliard (1995) noted that 54% of the forest avifauna of an isolated 251 ha southeastern Brazilian remnant was extinct after a 15-year interval. Authors who compared historical and recent data show a slightly better scenario. Christiansen & Pitter (1997) showed that almost 17% of all forest species were extinct from fragments in Lagoa Santa, southeastern Brazil (original data compiled from Krabbe [2007]), after 130 years. Willis & Oniki (2002), Ribon et al. (2003) and Silveira (2009) reported similar percentages (20.4-30%) of forest bird extinctions for Santa Teresa and Santo André, southeastern Brazil, after 50, 70 and 106 years separating deforestation time-lags, respectively.

1.3. Climate change

It is expected that habitat loss will act synergistically with climate change in the next decades, being major threats to biodiversity and human wellbeing (Pecl et al. 2017). Climate change has been suggested to reduce survival rates of Neotropical bird species (Blake & Loiselle 2015) and to reduce species richness and their current ranges both in the Atlantic Forest (Anciães & Peterson 2006) and Cerrado (Marini et al. 2009), as well as in several other regions in the planet (Şekercioğlu et al. 2012). Although the effects of climate change on species richness composition between periods must be considered, due to lack of detailed surveying methods during the 1800s, we cannot account for climatic changes on bird communities.

2. Changes in specific species

2.1. Extinctions

Although local extinction is quite probable, one cannot simply assume it (Scheffers et al. 2011) because three forest species collected by Natterer had no record until the second last survey we conducted at the INF in 2012. This is the case of a single recording of the Shrike-like Cotinga *Laniisoma elegans* (Thunberg 1823), and VC's aural and visual record of the Olivaceous Elaenia *Elaenia mesoleuca* (Deppe 1830). During our last visit, LFAF heard the Squamate Antbird *Myrmoderus squamosus* song, after a 190-year span. Robinson (1999) also reported similar re-encounters, although for a shorter (~25 years) time span.

It is also important to highlight one of the most important records at INF, the Eskimo Curlew *Numenius borealis* (Forster 1772). Once abundant, it has not been recorded with certainty since 1963, and none have been confirmed on wintering grounds since 1939 (BirdLife International 2015). The species exhibited an elliptical migratory route connecting its Arctic breeding grounds in North America to its wintering range in southern South America. Besides the specimen collected by Natterer at the INF, there are other specimens from the states of Mato Grosso and Amazonas, the only indications of its presence in Brazilian grounds (Straube 2008).

2.2. Recent records due to habitat change

It is intriguing that Natterer did not collect *Dysithamnus mentalis* (Temminck 1823) or *Todirostrum poliocephalum* (Wied 1831), both currently abundant at the INF. These forest edge species are quite common in early to medium successional stages of vegetation and are found, though more scantly, even in mature forests of the state as well. We suspect they must have been very rare hundreds of years ago, when pristine forests predominated the surroundings. A similar phenomenon was observed at Serra da Cantareira, where *Herpsilochmus rufimarginatus* (Temminck 1822) used to be rare and is suggested to be one of the most abundant species in a time span of only two decades (Tonetti et al. 2017). Another noteworthy absence during the nineteenth century is *Furnarius rufus* (Gmelin 1788), which probably began invading eastern parts of the country after large deforestation in western Brazil took place during the 1800s (Sick 1997).

In the case of some marsh birds, we assume there were no southern cattail *Typha domingencis* (Pers.) or bulrush *Scirpus californicus* (C. A. Mey) Steud marshes while Natterer stayed at the INF. Several typical marsh species such as *Laterallus melanophaius* (Vieillot 1819), *Phacellodomus ferrugineigula* (Pelzeln 1858), *Arundinicola leucocephala* (Linnaeus 1764), *Agelasticus cyanopus* (Vieillot 1819) and *Chrysomus ruficapillus* (Vieillot 1819) are missing from this period. Natterer probably would have collected these conspicuous species. Some species can help illustrate what kind of aquatic environments were present at the time. We suspect these were riverside vegetation because of the presence of *Anhima cornuta* (Linnaeus 1766) and *Serpophaga nigricans* (Vieillot 1817), while wet or flooded grasslands must have been visited, as suggested by the several Herons, Ibises and migratory Sandpipers.

3. Museum collections

Within the climax of both digital and molecular century, we take great pleasure in highlighting the importance of traditional museum skin collections. A special issue on museum collections effects on bird diversity and extinction did detail reasons for continued growth of collections (Collar et al. 2003). In addition, natural history collections were evidenced as sources of data that can complement past surveys. They also comprise samples that span the period of accelerated anthropogenic habitat destruction and climate warming, reflecting baseline conditions (Lister 2011). There could not be a comparison to be made without Natterer's committed bird collections and Pelzeln's (1868) publication, rendering our study a rare opportunity to evaluate how bird species richness differed within the same site almost 200 years apart.

Supplementary material

The following online material is available for this article:
Appendix I - Historical survey
Appendix II - Recent literature

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Author Contributions

Vagner Cavarzere: Substantial contribution in the concept and design of the study, contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Luís Fábio Silveira: Substantial contribution in the concept and design of the study, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Vinicius Rodrigues Tonetti: Substantial contribution in the concept and design of the study, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Pedro Ferreira Develey: Contribution to data analysis and interpretation, contribution to critical revision, adding intellectual content.

Flávio Kulaif Ubaid: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Luciano Bonatti Regalado: Contribution to data collection.

Luiz Fernando de Andrade Figueiredo: Contribution to data collection.

Conflicts of interest

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

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On the oviposition and egg masses of *Conus regius* (Neogastropoda: Conidae) from northeastern Brazil

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Abstract: Characteristics of the egg masses of *Conus regius* Gmelin, 1791 are described and figured for the first time for the coast of Brazil based on a female specimen found in the process of oviposition during the day in the state of Bahia, northeastern Brazil. Two clusters of egg masses were found in the subtidal zone of Itapuã beach attached to rocky substrate in a completely unprotected site. Oviposition likely began at least one day earlier, since the specimen had already affixed an entire egg mass and was ovipositing a second cluster at the time it was found. The egg masses were arranged in short, irregular rows of three to nine closely spaced capsules in parallel and facing the same direction. One egg mass cluster consisted of 34 capsules. *Conus regius* capsules are semi-transparent, vasiform in side view, higher than broad and have flattened to slightly convex sides with slight wrinkles constituted by transversal ridges. *Conus regius* is on the IUCN Red List of Threatened Species, but is still intensively collected in the study area and surrounding coastal environment by fishermen for the purposes of selling shells and as a food source.

Keywords*:* *Gastropoda, Conoidea, reproduction, ootheca, Bahia.*

Sobre a oviposição e massas de ovos de *Conus regius* (Neogastropoda: Conidae) no nordeste do Brasil

Resumo: Características de massas de ovos de *Conus regius* Gmelin, 1791 são descritas e figuradas pela primeira vez para a costa do Brasil com base em um espécime encontrado em processo de oviposição, durante o dia, no estado da Bahia, nordeste do Brasil. Dois aglomerados de massa de ovos foram achadas na zona subtidal da praia de Itapuã fixa a substrato rochoso em uma área completamente desprotegida. O processo de oviposição, provavelmente, começou um dia antes, uma vez que o espécime já tinha afixado uma massa de ovos e estava depositando um segundo aglomerado de ovos no momento em que foi encontrado. As massas de ovos apresentaram arranjos em fileiras curtas e irregulares contendo três a nove cápsulas estreitamente espaçadas, paralelas e posicionadas na mesma direção. A primeira massa de ovos observada conteve um total de 34 cápsulas. As cápsulas de *Conus regius* são semitransparentes, vasiformes em vista lateral (mais altas do que largas) e possuem parte lateral suavemente convexa a achatada com rugas suaves constituídas por elevações transversais. *Conus regius* está na Lista Vermelha de Espécies Ameaçadas da IUCN, mas ainda é intensamente coletada na área de estudo e ambientes costeiros circunvizinhos por pescadores por conta da venda das conchas e como fonte alimentar.

Palavras-chave*:* *Gastropoda, Conoidea, reprodução, ooteca, Bahia.*

Introduction

Conus Linnaeus, 1758 is a highly diversified genus of predatory marine neogastropods, with approximately 770 recent species worldwide (Bouchet & Gofas 2015). They live predominantly in shallow tropical habitats (Kohn 1966, Penchaszadeh 1984, Olivera et al. 1990, Kohn 1998, Duda et al. 2001, Terlau & Olivera 2004, Peters et al. 2013, Flores-Garza et al. 2014). Species of *Conus* are among the most well-known mollusks due to the beautiful color patterns of the typically heavy, obconical or biconical shells (Terlau & Olivera 2004, Kohn 1998). These species are also particularly dangerous due to a harpoon-like radular tooth that injects neurotoxins into prey (Olivera et al. 1990, Terlau & Olivera 2004, Braga et al. 2005, 2013, Haddad et al. 2006, 2009, Peters et al. 2013, Flores-Garza et al. 2014) and that can also injury and even death to humans (Olivera et al. 1990, Kohn 1998, Terlau & Olivera 2004, Haddad et al. 2006, 2009, Flores-Garza et al. 2014). There is economic importance in the extraction of compounds of medical and pharmacological interest (Olivera et al. 1990, Terlau & Olivera 2004, Braga et al. 2005, 2013, Gowd et al. 2005, Haddad et al. 2006, 2009, Peters et al. 2013) and as a food source for fishing communities on some Pacific islands (Terlau & Olivera 2004) and in northeastern Brazil.

Species of *Conus* that inhabit the intertidal to subtidal zones usually live in a heterogeneous habitat (Kohn 1959, 1998, Peters et al. 2013, Flores-Garza et al. 2014). They are quiescent when exposed to sunlight and typically seek shelter in shaded areas under algae, rocks and the sediment (Kohn 1998). Most species forage actively at night (Kohn 1998, Terlau & Olivera 2004, Flores-Garza et al. 2014) preying on a diversity of worms (e.g., polychaetes), hemichordates, echiurans, crustaceans, fishes and other mollusks (e.g., bivalves and gastropods) (Olivera et al. 1990, Kohn 1966, Cruz et al. 1978, Zehra & Perveen 1991, Kohn 1959, 1998, Duda et al. 2001, Terlau & Olivera 2004, Gowd et al. 2005, Haddad et al. 2006, 2009, Flores-Garza et al. 2014).

Conus are dioecious gastropods (Kohn 1961a, Flores-Garza et al. 2014). Reproductive aspects related to spawning, egg masses and larvae have been studied for a number of species worldwide (Lebour 1945, Ostergaard 1950, Natarajan 1957, Kohn 1961a,b, D'Asaro 1970a,b, Nybakken 1970, Bandel 1976, Cruz et al. 1978, Perron 1981a,b,c, Penchaszadeh 1984, Zehra & Perveen 1991). Females deposit dozens of large, usually flask-shaped egg capsules on or within different substrates (Perron 1981c, Zehra & Perveen 1991, Kohn 1998). Each egg capsule may contain a few to hundreds of embryos (Kohn 1998).

Conus fauna on the coast of Brazil includes about 20 species, five of which are endemic to the country (Rios 2009). *Conus regius* Gmelin, 1791 is a common, potentially dangerous and predominantly nocturnal species (Braga et al. 2005, 2013, Haddad et al. 2006, 2009, Rios 2009). It mainly lives on and under rocky, coral reefs and calcareous reef environments from Florida to Brazilian waters and actively feeds on worms, especially polychaetes (Bandel 1976, Braga et al. 2005, 2013, Lee 2009, Tunnell et al. 2010, Haddad et al. 2006, 2009, Rios 2009). This species may also be found half buried in the sand (Bandel 1976). Egg capsules from *C. regius* were succinctly described and poorly illustrated only by D'Asaro (1970a) and Bandel (1976) from south Florida – Bahamas and the caribbean sea of Colombia, respectively. *Conus regius* was once recognized as a very abundant shallow-water species, especially in northeastern Brazil (Haddad et al. 2009), but is currently on the IUCN Red List of Threatened Species (IUCN 2017).

To the best of our knowledge, so little information has been published about the reproductive biology of any Atlantic *Conus* species. Thus, the purpose of the study is to provide additional information on the oviposition and mainly egg capsules of *Conus regius* based on a specimen found in the subtidal zone in northeastern Brazil.

Material and Methods

1. Study Area

This study was conducted in the shallow subtidal zone of Itapuã beach (12°57'27"S, 38°21'21"W), which is located approximately 20 km from the city of Salvador in the state of Bahia, northeastern Brazil (Silva et al. 2009). The study site has a coastline of approximately 600 m (Silva et al. 2009) and reef platforms that are under the direct influence of wave impacts from the Atlantic Ocean (personal observation) (Figure 1).

The study area and surrounding coastal environment is a high-energy region characterized by a slightly steep subtidal zone composed of sandy, carbonate and rocky substrates as well as reef outcrops that are nearly exposed at low tide (Alves & Cerqueira 2000). The hard substrates undergo a strong weathering process in the region originating sediments composed of sand, quartz and carbonate (Bittencourt 1975). The environmental complexity of Itapuã and other beaches of Salvador offers a variety of micro-habitats and macro-habitats that favor the establishment of diverse communities of invertebrates (Alves & Cerqueira, 2000), especially mollusks, which constitute the second most diversified phylum in the world (Lima et al. 2017).

The area is under the influence of urbanized areas that input nutrients and domestic sewage. Moreover, fishing pressure on Itapuã beach has drastically reduced the populations of fishes, crustaceans and mollusks.

2. Field observations and laboratory procedures

This paper is based on observations of the oviposition and egg capsules of a specimen of *Conus regius* on 12 December 2016 at low tide in the subtidal zone of Itapuã beach. The female in oviposition was observed for approximately 25 minutes, was not handled during the oviposition process and was not collected to be deposited in a scientific collection. This species is well known in the literature and is threatened with extinction, especially at the study site, where it is captured for the purpose of selling the shell and as a food source. The rocky substrate with the first attached egg mass was manipulated for approximately four minutes to photograph it out of the water and for the removal of a few egg capsules. The egg capsules removed were preserved in 70% ethanol. The capsules were measured in the laboratory using the three dimensions described by Kohn (1961a) as well as an additional measure: ea – length of escape aperture, hp – height

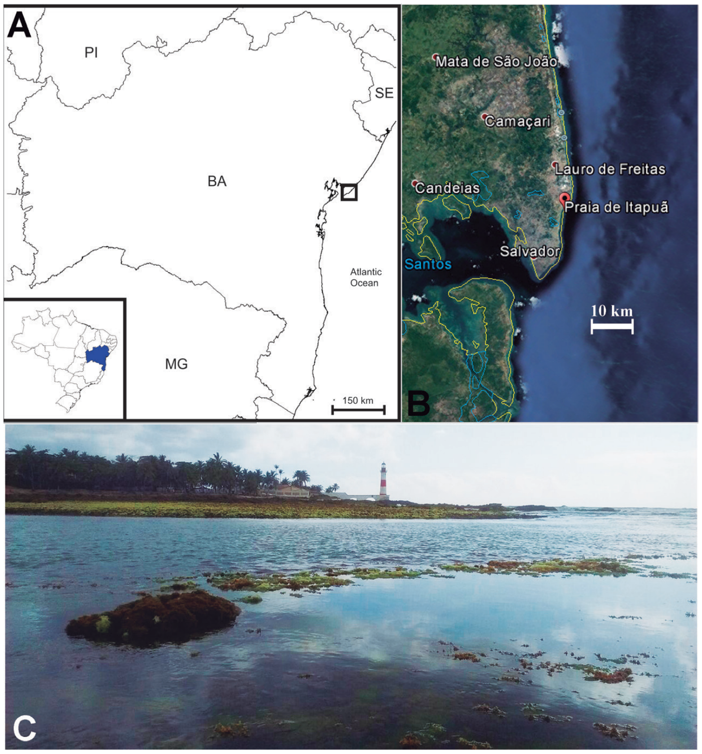

Figure 1. (**A**) Map of the Brazil (small square) highlighting the state of Bahia and the state of Bahia (large square) highlighting the location of the Itapuã beach (northeastern Brazil); (**B**) Map of stretch of the coast of Bahia showing the location of the Itapuã beach; (**C**) Photo of the area of observation and collection of spawning of *Conus regius* at Itapuã beach. Abbreviations: SE — Sergipe, BA — Bahia and MG — Minas Gerais.

of peduncle, mh – maximum height, and mw – maximum width. The total number of capsules in the egg mass was counted during manipulation of the substrate. Illustrations were also prepared from drawings.

The specimen of *Conus regius* shown here (Figure 2A) is deposited in the Coleção Zoológica do Laboratório de Zoologia, Universidade Federal de Campina Grande (UFCG_MOL 01), Centro de Formação de Professores, Cajazeiras, Paraíba, Brazil. The capsules are housed in the malacological collections of the Laboratório de Invertebrados Paulo Young, Universidade Federal da Paraíba (UFPB MOLL 3629: 03 capsules), João Pessoa, Paraíba; Museu de Zoologia, Universidade de São Paulo (MZSP 132042: 04 capsules), São Paulo, Brazil and UFCG_MOL 02: 06 capsules.

Results

1. Oviposition

A specimen of *Conus regius* (shell measuring approximately 54 mm in total length) was found ovipositing during sunny daylight (approximately 8:50 a.m.) in the subtidal zone of Itapuã beach (Figure 2). It was found

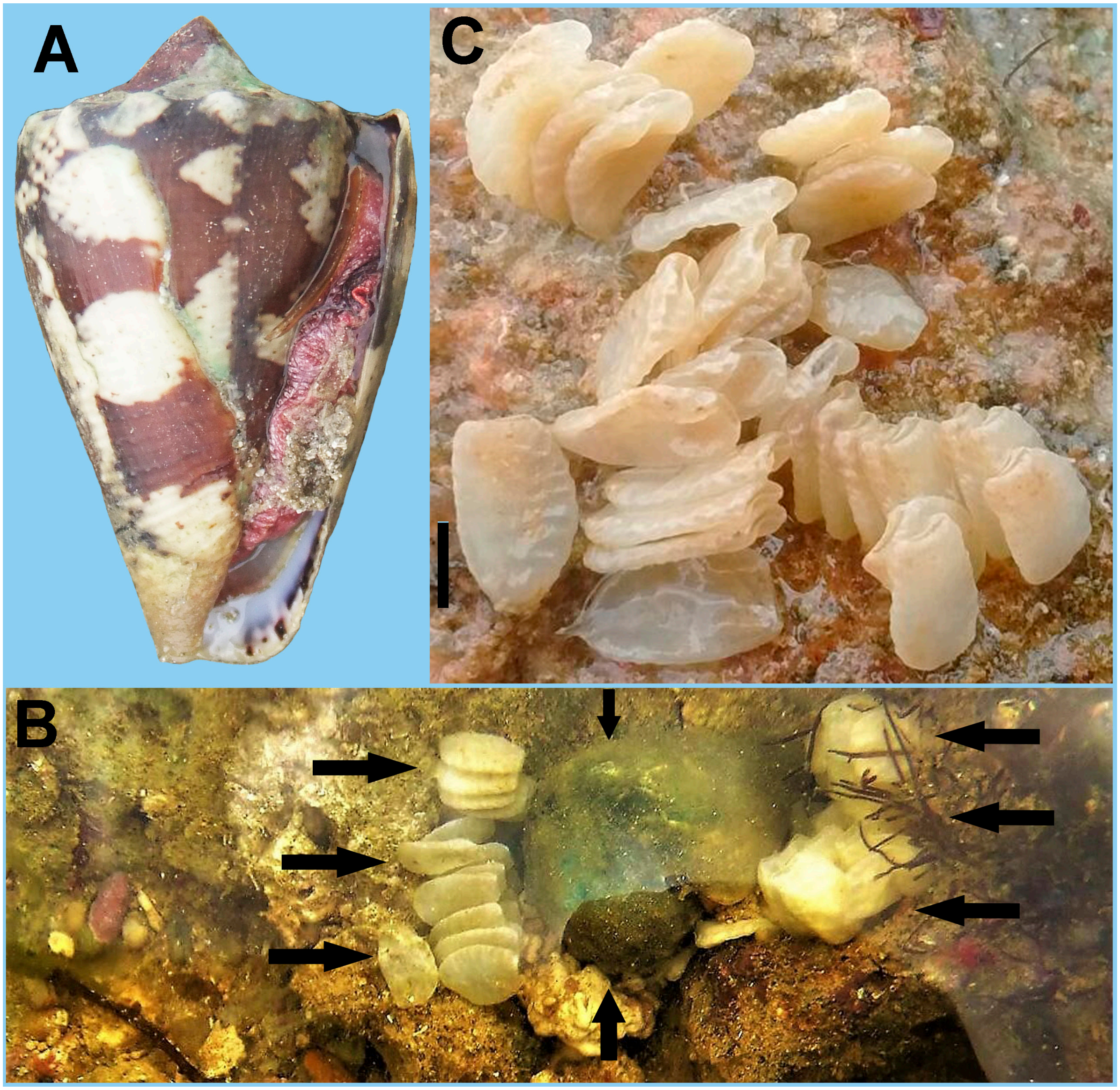

Figure 2. *Conus regius* from coast of Bahia: (**A**) Specimen collected from Morro de São (state of Bahia, northeastern Brazil – UFCG_MOL 01: 54 mm); (**B)** Female specimen in oviposition process on Itapuã beach (vertical arrows pointing to specimen; left horizontal arrows pointing to first egg mass; right horizontal arrows pointing to second egg mass being deposited); (**C**) Clusters of egg masses photographed out of water (same as Figure B indicated by left horizontal arrows; scale bar of nearest ootheca: 5 mm).

in a completely unprotected site with a mixed substrate of gravel, sand and small to medium-sized pieces of rocks at depth of approximately 30 cm. The oviposition process had likely begun at least one day earlier, since the specimen had already affixed an entire egg mass cluster to the substrate (Figure 2B – left horizontal arrows). It was observed ovipositing the second egg mass cluster at the time it was found (Figure 2B – vertical and right horizontal arrows). The second cluster was being affixed to the rocky substrate, which was partially covered with macroalgae filaments. No other spawn was found on the surrounding substrates. The specimen remained relatively motionless during the oviposition process, which was observed for approximately 25 minutes. It was not possible to photograph in detail and observe the entire extrusion process of the second egg mass cluster due to the rising tide.

2. Egg masses and capsules

Two clusters of egg masses of *Conus regius* were found on the substrate (Figure 2B – left and right horizontal arrows). One cluster had likely been produced by the specimen (Figure 2B – left horizontal arrows) and another was being extruded and affixed to the substrate at the time it was found (Figure 2B – horizontal and right horizontal arrows). Both clusters were well separated from each other (Figure 2) and arranged in short, irregular rows of three to nine closely spaced egg capsules exhibiting the same pattern (one alongside the other) and oriented in parallel facing the same direction, all attached at the underside of the piece (Figure 2C). In each cluster, immediately adjacent groups of rows of egg capsules were situated at various angles (Figure 2C). The first egg mass cluster consisted of about 34 capsules (Figure 2B – left horizontal arrows) and was carefully removed from the water to be better studied and photographed (Figure 2C). Most of the capsules were empty (without embryos) in this cluster (Figure 2C). The second egg mass cluster in oviposition contained about 18 capsules (Figure 2B – vertical and right horizontal arrows). This cluster under construction was not handled or studied in detail so as not to interrupt the oviposition process. A minimum of 52 egg capsules in both egg masses were likely laid by the female. The clusters of egg masses were affixed to the substrate without any overlapping or confluence of the pieces of the capsules.

Conus regius has thin, semi-transparent, vasiform (side outline) capsules (Figure 2C). Such capsules are much higher (mh – about 12 to 14 mm) than broad (mw – about 7 to 8.5 mm) (Table 1) with flattened to slightly convex sides that are slightly and unevenly wrinkled by few, low and rather well-spaced transversal ridges running from apical region to peduncle (Figures 2C and 3). Capsules with typically convex lateral edges and ridges (Figures 2C and, 3). Each capsule rises vertically from a short stalk with slight skew to one side (Figures 2C and 3). Capsules composed of two equal membranous halves along axis interrupted only by escape aperture (Figures 2C and 3). Capsular apex usually sigmoid shaped (Figures 2C and 3). Escape aperture very narrow and elongated (slit shaped: ea – about 3.9 to 5.5 mm), slightly concave extending along capsular apex (Figures 2C and 3). Peduncles short (hp – about 2 to 3 mm) (Table 1), narrow to enlarge, usually positioned in central part of capsular base (Figures 2C and 3). Basal membranes discrete and fused between peduncles (Figures 2C and 3). Empty capsules of opaque white (semi-transparent) color. Full capsules (with embryos) are light pinkish brown. Most of the capsules in the egg masses photographed were apparently empty (Figure 2C).

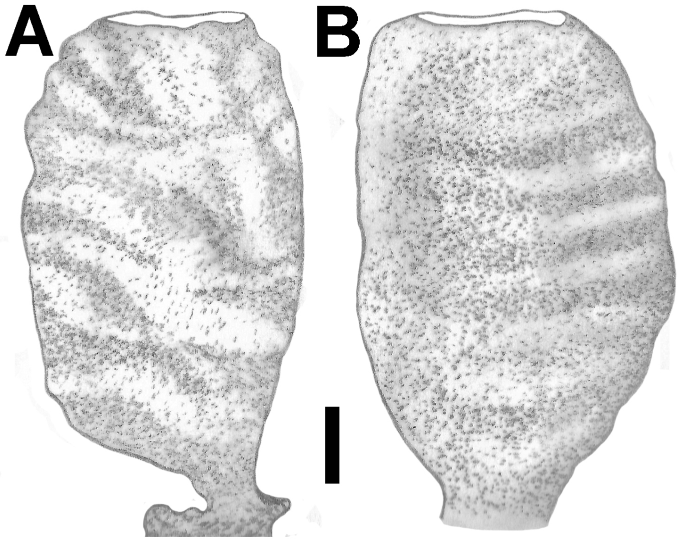

Figure 3. Two egg capsules of *Conus regius* from the coast of Bahia: **A**. Ootheca well wrinkled with narrow peduncle positioned in subcentral region of capsular base and part of basal membrane; **B**. Ootheca moderately wrinkled with wide peduncle positioned in central region of capsular base. Scale bar: 2 mm.

Table 1. Linear measurements (mm) of capsules of *Conus regius* found in the subtidal zone of Itapuã beach.

	Conus regius			
	N	**R**	**M**	**SD**
ea	11	3.90–5.50	4.62	0.56
hp	10	2.00–3.00	2.40	0.49
mh	11	12.0–14.0	12.7	0.75
mw	12	7.00–8.50	7.89	0.41

Discussion

Conus regius is one of the about 10 conid species widely distributed in the western Atlantic (Rosenberg 2009) and is found in a considerable variety of coastal habitats on rocks (Diaz & Puyana 1994), rocky reefs (Warmke & Abbott 1962) or similar reef structures (Abbott 1974), calcareous environments (Tunnell et al. 2010) and sediments as well as under rocks (Bandel 1976, Redfern 2013), gravel and calcareous algae bottoms (Rios 1994) associated with coral reefs (Bandel 1976), coral fragments (D'Asaro 1970a), dead corals and cavities under semispherical coral colonies, sea-grass bottoms or even buried in the sand (Bandel 1976). This species typically lives in these habitats on the coast of northeastern Brazil (Haddad et al. 2009), even on substrates exposed to the atmosphere at low tide. The coast of the state of Bahia, including the Abrolhos Archipelago is recognized as an area of important diversity of *Conus* due to the considerable environmental complexity of the coastline and islands, which results in a large variety of marine habitats (Coltro 2004). However, *C. regius* and congeners are currently rather rare in the habitats of Itapuã beach and adjacent areas of the coast due to environmental degradation and the intensive collection by fishermen for the sale of shells and as a food source.

Egg masses of *Conus regius* have been rarely studied in the western Atlantic (see D'Asaro 1970a: 434–435, fig. 9F–H, Bandel 1976: 184–185, fig. 9). In previous studies, egg capsules of the species was found only on coral fragments in Florida (D'Asaro 1970a) as well as under *Acropora* fragments and hollow hemispherical corals in Colombia and Curaçao (Bandel 1976). Unlike these studies, the specimen observed herein was in an unprotected site with a mixed substratum of gravel, sand and rocks (Figure 2B). Geraldo Oliveira has studied mollusks on the coast of Bahia (especially Itapuã beach) for approximately 40 years and this is the first time that the researcher has found a female specimen in oviposition and egg masses in the region.

For *Conus regius*, D'Asaro (1970a: fig. 9F–H) and Bandel (1976: fig. 9) also reported the arrangement in closely spaced rows of egg capsules and figured they with essentially the identical shape to those studied herein (Figures 2C and 3). Bandel (1976) also found an arrangement in the number of capsules (four to ten in rows) similar to that of the present study. However, the capsules examined herein had a more conspicuous and frequent pattern of transversal wrinkles (Figures 2C and 3). Additionally, the egg capsules in this study (mh – about 12 to 14 mm) have intermediate dimensions to those analyzed by Bandel (1976: mh – 10 to 11 mm) and described by D'Asaro (1970: mh – 15 to 16.5 mm) for a specimen of *C. regius* from Florida.

Among the species of the western Atlantic, *Conus regius* has an capsule morphology somewhat similar to those of *C. ermineus* Bom, 1778 (see Bandel 1976: fig. 12A–B; Penchaszadeh 1985: fig. 4) and *C. mus* Hwass, 1792 (see Lebour 1945: fig. 31a), especially in the outline, conspicuous pattern of transversal wrinkles and elongated, narrow escape aperture. On the other hand, the egg capsules of *C. regius* differ in shape from those previously observed for *Conasprella puncticulata* (Hwass in Bruguière, 1792) (see Bandel 1976: fig. 10), *C. stearnsii* (Conrad, 1869) (see D'Asaro 1986: fig. 4D–F), *Conus anabathrum* Crosse, 1865 (see D'Asaro 1986: fig. 4A–C), *C. largilliertii* Kiener, 1847 (see Bandel 1976: fig. 11) and *C. spurius* Gmelin, 1791 (see Penchaszaded 1985: fig. 1A–D), especially in the rather regular outline and narrower escape aperture and peduncle.

The coastal ecosystems of Brazil exhibit an important biodiversity of Conidae (Coltro 2004, Rios 2009) that has been insufficiently studied, especially with regard to reproductive aspects. The conid fauna and the entire coastal invertebrate community have been drastically affected by multiple anthropogenic impacts (Migotto & Marques 2006, Peters et al. 2013). The destruction and pollution of habitats constitute the most serious global threat to marine biodiversity (Gomes et al. 2000, Amaral & Jablonski 2005, Migotto & Marques 2006, Peters et al. 2013). In particular, populations of Conidae have undergone considerable decline in recent times, with a number of species currently on the IUCN Red List of Threatened Species (IUCN 2017). In 2011, the IUCN assembled a committee to assess the threat levels of more than 600 species of *Conus*, with the results in the paper prepared by Peters et al. (2013) (Dr. Alan J. Kohn, personal communication, august 2017). The IUCN committee voted not to deem *Conus regius* as "threatened", but rather with status "Least Concern" (IUCN 2017). Conids have undergone population reduction especially in the study site due to intensive collection by fishermen for the purposes of selling the shells and as a food source. To reverse the decline of such populations, local governments need to develop conservation strategies and act more effectively to impede the capture of threatened mollusks, especially conids that are recognized as having economic importance in terms of a food source and the extraction of compounds of medical and pharmacological interest.

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Morphological characterization of fruit, seeds and seedlings of white-seal (*Chrysophyllum rufum* Mart. -Sapotaceae)

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Abstract: *Chrysophyllum* is the second largest genus of Sapotaceae, with 81 species distributed in the neotropics. Little data are found in the literature regarding the morphology of seedlings and the early development of this genus. This study aims to morphologically characterize the fruit, seeds and seedlings of *Chrysophyllum rufum* Mart. Fruits were collected from individuals present in two fragments of the Atlantic Forest, Pernambuco. A sample of 100 seeds and 100 fruits was randomly selected to obtain the morphological data. The seeds were sown in plastic trays in a greenhouse. The fruits are bacoid, obovoid and globose with one or two functional seeds per fruit. The seeds are obovate, with the shape of the hilum ranging from elliptical transverse to oblong transverse. The embryo is cotyledonar, with a spatulated form. The cotyledons are foliaceous and whitish-translucent. The endosperm is abundant and whitish. Germination is epigeal, phanerocotylar and unipolar. The seedling has different characteristics than those of the adult individual, such as the shape and leaf consistency, type of leaf margin, type of venation variation, number of pairs of secondary veins, trichome coloring and abundance of latex.

Keywords: *morphological description, germination, propagules, Atlantic Rainforest.*

Caracterização morfológica do fruto, sementes e morfofunção de plântula do lacre-branco (*Chrysophyllum rufum* Matius - Sapotaceae).

***Resumo*:** *Chrysophyllum* é o segundo maior gênero de Sapotaceae, com 81 espécies distribuídas nos neotrópicos. Dados encontrados na literatura sobre a morfologia dos propágulos e desenvolvimento inicial do gênero ainda são escassos. O presente estudo tem como objetivo caracterizar morfologicamente o fruto, a semente e a plântula de *Chrysophyllum rufum* Mart.. Os frutos foram coletados de indivíduos presentes em fragmentos de floresta Atlântica, Pernambuco. Uma amostra de 100 frutos e 100 sementes foi selecionada aleatoriamente para obtenção dos dados morfológicos. As sementes foram semeadas em bandejas plásticas, em casa de vegetação. Os frutos são do tipo bacóide, obovóides a globosos, com uma ou duas sementes funcionais por fruto. As sementes são obovadas, hilo com forma variando de transversal elíptica a transversal oblonga. O embrião é cotiledonar, com forma espatulada. Os cotilédones são foliáceos, esbranquiçado-translúcido. O endosperma é abundante e esbranquiçado. A germinação é epígea, fanerocotiledonar e unipolar. A plântula apresenta caracteres diferentes do indivíduo adulto, tais como a forma e consistência foliar, tipo de margem foliar, variação do tipo de venação, número de pares de veias secundárias, coloração do tricoma e abundância do látex.

Palavras-chave: *descrição morfológica, germinação, própagulos, floresta Atlântica.*

Introduction

Chrysophyllum is the second largest genus, in number of species, of the Sapotaceae family, with 81 species distributed through the neotropics (Pennington 1990). The genus is represented in Brazil by 31 species, including nine subspecies, distributed along the national territory in different phytogeographic domains (Pennington 1990, Carneiro et al. 2015). The taxonomy of the genus appears to be quite undefined and complex, with species characterized by difficult morphological delimitation. Recent studies show that *Chrysophyllum* does not constitute a monophyletic group either in relation to the generic concept nor in the sections proposed in the last revision of the family (Pennington 1990, Swenson & Andeberg 2005, Swenson et al. 2008). In accordance with this, interest in studying the morphology of the fruit, seed and seedling of *Chrysophyllum rufum* Mart. has arisen. This species, commonly known as white-seal in the state of Pernambuco, Brazil, has shrubs or trees from 8 to 20 m, with small flowers (2-3 mm), green sepals covered by dense golden-rusty trichomes, and delicate, cream-green petals. According to the National Environmental Council (Conama 2011), *C. rufum* is an indicator species for different successional stages of the vegetation in the Atlantic Forest and is primarily found in the middle and advanced stages of regeneration. Only in the Pernambuco area does *C. rufum* occur in the initial stages of regeneration (Conama 2011). In small patches of the Atlantic Forest in Pernambuco, the remaining individuals of *C. rufum* are frequently found as part of regrowth, a phenomenon deserving of more attention by scientific research: human pressure in different Brazilian ecosystems, combined with a lack of data about the plant's conservation status, are currently serious obstacles to maintaining the species. In Brazil, *C. rufum* occurs in the northeastern (Alagoas, Bahia, Ceará, Paraíba, Pernambuco, and Sergipe) and southeastern (Espírito Santo, Minas Gerais, and Rio de Janeiro) regions, at the phytogeographic Caatinga dominium, Cerrado and the Atlantic Forest (Pennington 1990, Carneiro et al. 2015). Despite being a well collected species, little data are available in the literature regarding the morphology of seedlings and the early development of the plants. Thus, this study proposes a morphological characterization of the fruit, seed and seedling of *C. rufum* (white-seal), with consideration that studies following this approach may, besides highlighting characteristics of taxonomic value, serve as support for ecological research related to fauna and flora interactions and the recognition of species seedlings in studies of banked seed and seed rain, among others.

Material and Methods

1. Local collection

The fruits of *C. rufum* were collected from three individuals present in two fragments of the Atlantic Forest. The first fragment is popularly known as "mata do frio" and is located in the municipality of Paulista, which encompasses Parque Natural Municipal do Frio (Natural Park), the first Conservation Unit of this category in the region. The second fragment is the Ecological Park São José, located at the municipality of Igarassu/Pernambuco (7º40'21.25"– 7º55'50.92"S e 34º54'14.25"–35º05'21.08"W); it is one of the largest reserves of the Atlantic Forest in the Brazilian Northeast and property of the Usina São José (USJ).

2. Collection and processing of plant material

Mature fruits were collected directly from trees or on the ground and stored in paper bags. After collection, they were transported to the Laboratory of Floristic Coastal Ecosystems (Laboratório de Florística de Ecossistemas Costeiros - LAFLEC) at the Universidade Federal Rural de Pernambuco (UFRPE), where they were selected properly (discarding immature fruits and/or those with injury/damage by animals), described and photographed. In the laboratory, 100 fruits and 100 seeds were analyzed. The fruits were analyzed in relation to color, brightness, hairiness, dehiscence, shape, form, size (length x width), consistency and texture of pericarp and number of seeds per fruit. The seeds were observed for size (length x width), shape, color, brightness and hairiness of tegument, shape and heel position, and embryo characteristics. The terminology used to describe fruits and seeds follows the guidelines of Barroso et al. (1999). After processing the fruits and obtaining the seeds, seeds were sown in plastic trays (28 × 15 × 8 cm) in a greenhouse. As substrate for germination, we used the soil collected from the forest fragments where the individuals of *C. rufum* were found. From the pool of germinated individuals, the most vigorous individuals were selected to carry out the morphological description of seedlings, these being described using the elements suggested by Roderjan (1983): hypocotyl, cotyledons, epicotyl, eophyll (protophyll) and root. The terminology employed was based on Radford et al. (1974), Duke & Polhill (1981), Oliveira (1993) and Camargo et al. (2008). It is important to mention that the seeds presented a type of dormancy, shown by slow and discontinuous germination and a reduced number of germinated seeds.

Results and Discussion

1. Morphological description of fruits and seeds

The studied species presents bacoid fruits, light green when immature, which turn brownish-red at later stages and purple when ripe (Figure 1). The fruit originates from a superior ovary, typically six-locular and in rare cases seven-locular, with one ovule per locule. However, not all ovules complete their development. The shape of the fruit varies from obovoid to globose with a round apex and acute-obtuse base, and presents a depression in the base due to the seed shape (Figure 1). The size of the fruits vary from 9.35 to 14.25 mm long and from 8.34 to 12.83 mm wide, with one or two well developed seeds. It is important to note that few fruits present two well developed seeds with one apparently viable embryo (Figure 1). However, five to six small and non-functional seeds (Figure 1) can be found inside the fruit. Six to seven persistent and pubescent sepals are observed at the fruit base, with pedicels 5.34 to 6.79 mm long and with pubescent sepals presenting malpighiaceous ferruginous trichomes. The epicarp is smooth (though the voucher specimen has a slightly wrinkled appearance), glossy, thin, and glabrous or with thin sparse trichomes. At the apex of the fruit, one observes the persistent stigma forming a small apical (hairy apiculture). The mesocarp is sulcused and soft but slightly fleshy and whitish-purple, without a discernible odor. The endocarp is very thin, membranous, whitish, and not adhered to the seed. The propagule's characteristics may contain elements that define and separate the different taxa. Almeida Jr. et al. (2010) highlight the importance of obtaining characteristics of fruits, seeds and seedlings to support studies on Sapotaceae, helping with the species identification. In addition, studies that demonstrate the morphology of fruits and seeds of native plant species can be of great use in the production of seedlings for forest nurseries, subsidizing ecological research and forest regeneration after anthropogenic disturbances. The seed has dimensions of 9.8 to 11.95 mm in length and 7.06 to 9.9 mm in width, occupying almost the entire internal cavity of the fruit. Only one seed develops completely inside the fruit (Figure 1). The seed shape is obovate, with a smooth brow, glossy, stiff, and glabrous, with color ranging from cream to light brown (sometimes bi-color). The hilum is evident, wide, depressed, and base-ventral, with its shape ranging from elliptical transverse to oblong transverse. Around the hilum, depressions corresponding to "aborted" seeds that had not finished their development could be observed. Contour lines are observed leading down from the seed apex to the base (around the hilum). The embryo is cotyledonar with a spatulated form, perfectly distinguishing between the hypocotyl-root axis and the cotyledons, which are whitish-translucent, with two oblong, planar and membranous embryonic leaves. The endosperm surrounding the embryo is abundant and whitish in color. The cotyledons are thin and foliaceous (Figure 1). Pennington (1990, 1991) emphasized the importance of seed characteristics to species separation, especially the shape (if laterally compressed or not, the size and shape of hilum, and the presence or absence of endosperm as well as its abundance in the seed). In addition, features such as these, in conjunction with other morphological data, have provided support for the author to subdivide *Chrysophyllum* into five sections. It is also worth noting that the author describes the existence of only one seed per fruit, but the present study verifies that eventually, it is possible to find more than one well developed and viable seed.

2. Morphological description of seedling and morfofunction

Germination began 20 – 34 after sowing, with radicle protusion and subsequent formation of the hypocotylar strap (Figure 2). The seedling has epigeal germination, phanerocotylar and unipolar, with axis positioned between the cotyledons. The hypocotyl is slightly elongated (1.5 to 2.5 cm) and starts light green before becoming brown and lignified/glabrous.

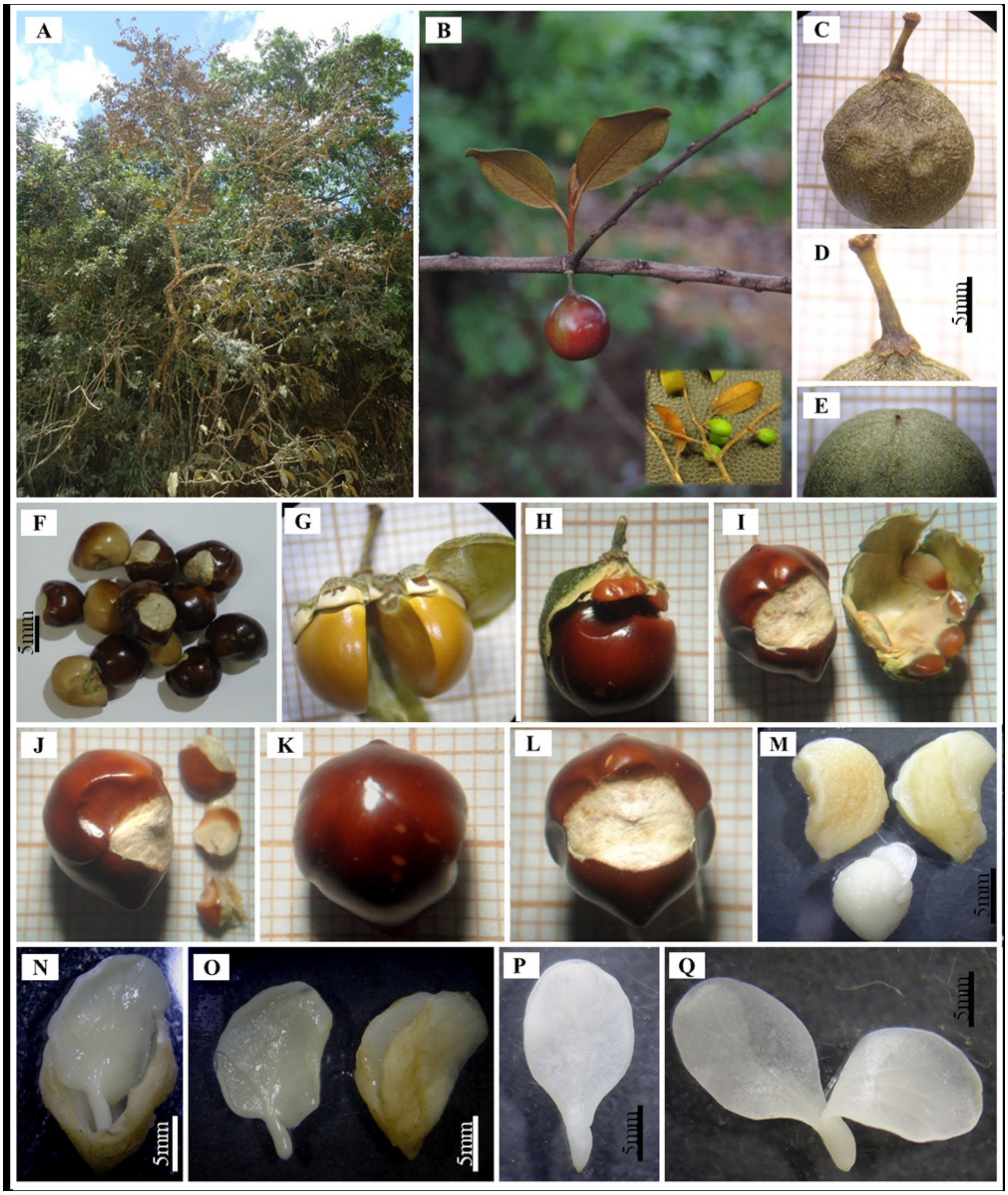

Figure 1. Images showing individuals of the species of *C. rufum*: A – habitat; B – fruit in maturation phase, with details showing fruits still immature; C, D and E: detail of dry fruit, base and apex, respectively; F – color and seed shape; G and H: number of seeds developed and viable per fruit; I and J: viable seed, more details showing undeveloped seeds; K and L: seed apex and base, displaying the hilum; M: endosperm covering the axis hypocotyl-root; N, O and P: endosperm and embryo detail; Q – detail showing the two foliaceous cotyledons.

Foliaceous cotyledons are 1.53 to 1.66 cm and photosynthetic and have a petiole that is greenish, opposite, glabrous and 0.26- to 0.31-cm in length. The epycotyl is 0.4 to 0.55 cm long and greenish, becoming light brown with indument pubescent-depressed and with white trichomes becoming ferruginous during its development (Figure 2). Internodes range from 4.64 to 8.91 mm and are greenish in color, with indument and texture similar to that of the epycotyl. The first leaves (16.61-27.10 x 9.14-9.84 mm) are simple, oblanceolate, cartaceous, alternating, and slightly spiraled, while the petiole (2.27 to 3.90 mm) is light green, not canaliculated, and pubescent-depressed, with ferruginous trichomes. Leaf blades are green, smooth on both sides, glossy and glabrescent, with rare ferruginous trichomes near the base and in the mid rib. In the bud and in the beginning of leaf development, trichomes are whitish, becoming ferruginous to golden; leaf bases are cuneate to slightly cuneate, with a cuspidade-acute apex, entire margin (rarely with ciliated margin, trichomes caduceous), and brochidodromous venation. In the beginning of the development of the first leaves (eophyll and metaphyll), only the primary and the secondary venation (arched near the margin) are evident. The following leaves present five to ten pairs of secondary veins, rare intersecondary and reticulate terciary veins, and a small amount of viscous white latex. A greenish apical bud, always formed by leaf sketches of different sizes, parallels a slightly curved, pubescent indument, with white to ferrugineous malpighiaceaous trichomes

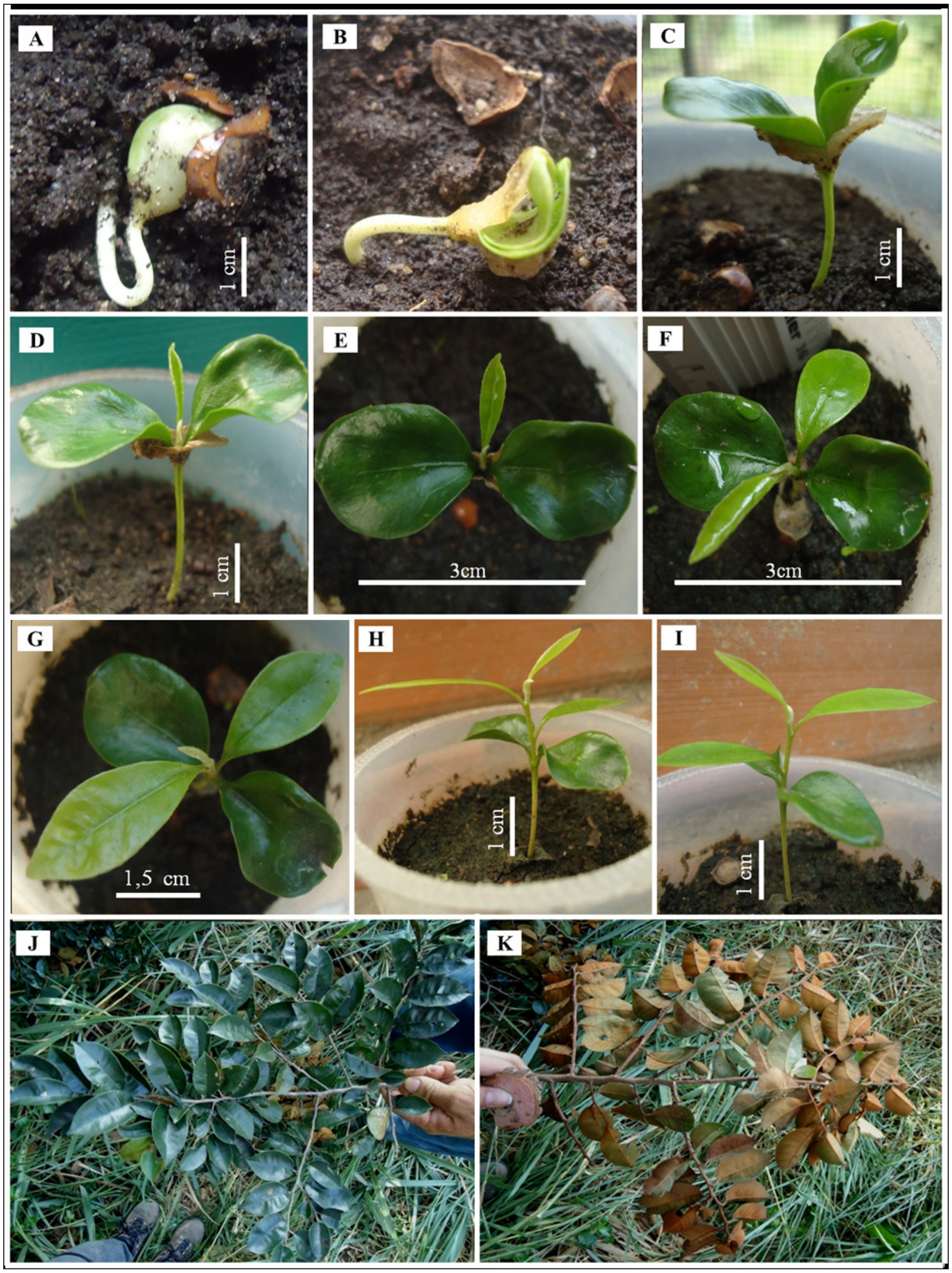

Figure 2. Germination and initial development of *C. rufum*: A and B: hypocotylar strap and early lifting of cotyledons; C – total lift of hypocotyl and expansion of foliaceous photosynthetic cotyledons; D and E: formation of first eophyll; F: formation of second eophyll; G, H and I: beginning of formation of the third eophyll until its complete expansion; J and K: branch of adult individual of *C. rufum,* showing the morphological differences (abaxial and adaxial side, respectively) from the seedling stage.

and largely asymmetric axes. The importance of seedling morphology studies is that many characteristics of young individuals are lost during their development, making the identification of those that germinate in forest areas difficult (especially for those developing research with soil seed bank and seedlings analysis) (Table 1, Figure 3). In the case of the species analyzed here, *Chrysophyllum rufum*, it was possible to observe some characteristics present in the seedling that can distinguish immature from adult individuals. The discussion about the importance of assessing the initial phase of plant organisms is not recent (Rizzini 1965, Ng, 1978, Vogel 1980, Candolle 1985, Miquel 1987). Studies related to the morphology of seedlings are useful in the restoration of anthropic areas or after disturbances and openings of forest clearings, as allowing the correct delimitation of taxa, even young individuals, contributes to support forestry strategies and, consequently, to accelerate the management of conservation actions (Almeida Jr. et al., 2010, Barreto & Ferreira 2010, Lima et al., 2010, Amorim et al., 2006, Guerra et al., 2006). In the case of *C. rufum*, the leaf variation, the amount and color of indumenta and embryo characteristics, and fruit and seed morphology all contribute to the identification of the species in its natural environment and aid its delimitation.

Table 1. Primary differences observed in leaf morphology between seedling and adult individuals of *Chrysophyllum rufum* (Sapotaceae).

Characters	Seedling	Adult individual
Leaf shape	Oblanceolate	Oblong-elliptic (lanceolate)
Leaf consistency	Cartaceous	Coriaceous
Leaf apex	Cuneate to closely cuneate	Acute-acuminate
Leaf base	Cuneate to closely cuneate	Base obtuse-cuneate
Leaf margin	Entire or ciliated (trichomes caduceus)	Strongly revolute
Type of venation	Barely visible initially, then brochidodromous	Eucamptodromous-brochidodromous
Nº of pairs of secondary veins	5 to10	8 to 11
Indument of abaxial face	Glabrescent/ glabrous	Densely tomentose
Color of trichomes	Golden-whitish	Ferruginous to dark brown
Latex	Small amounts	More abundant

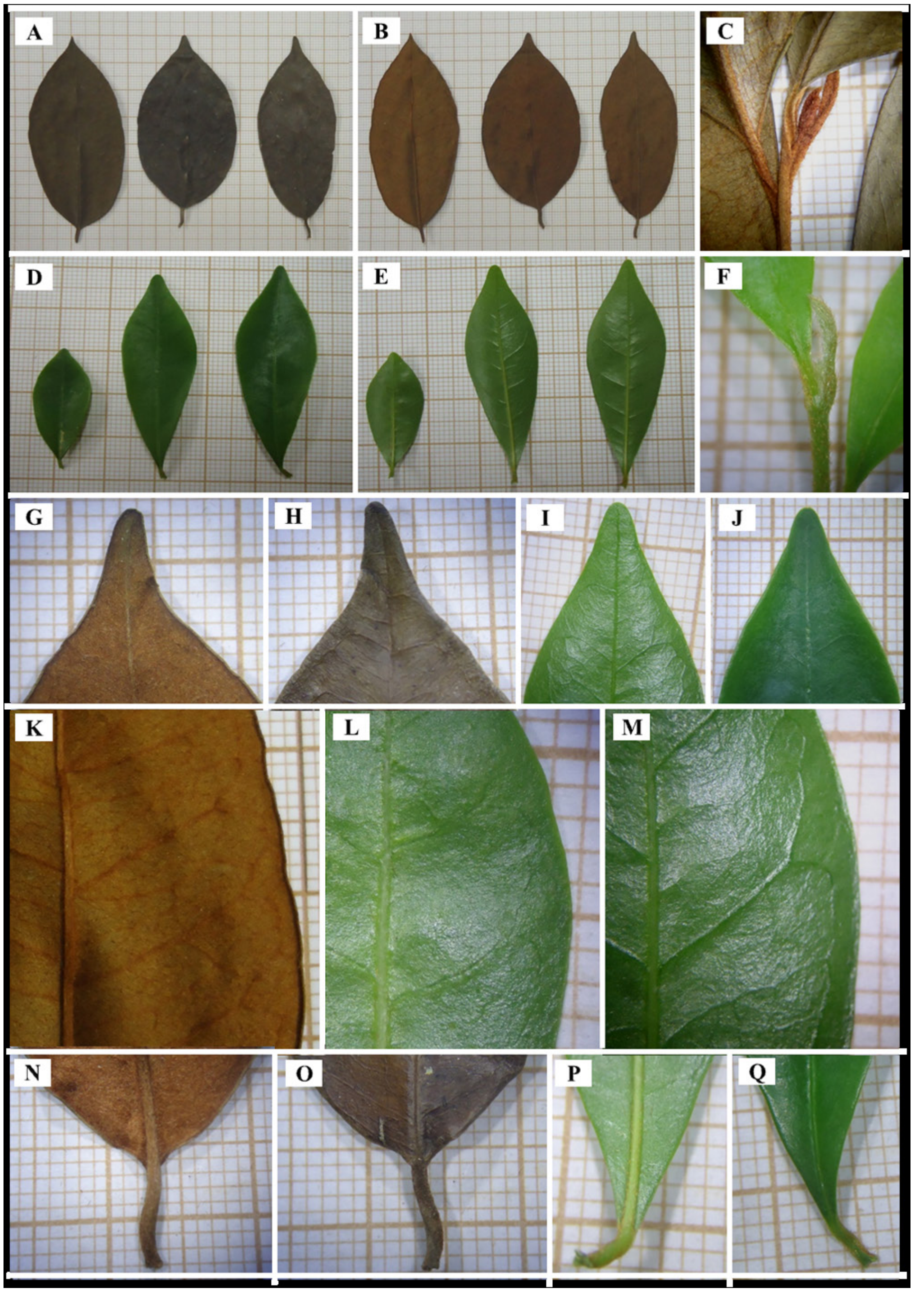

Figure 3. Comparison of leaf morphology between adult individual and seedling: A, B and C: leaf shape (abaxial and adaxial side, respectively) and bud of adult individual; D, E and F: leaf form (abaxial and adaxial side, respectively) and bud of seedling; G, H, I and J: leaf apex of adult individual and seedling; K: leaf venation of adult individual; L and M: leaf venation during seedling development; N, O, P, Q: leaf base of adult individual and seedling.

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Author Contributions

Liliane Ferreira Lima: collection and species identification, analysis and monitoring of the experiment in the greenhouse and laboratory, orientation methodology, and theoretical contributions; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Renata Gabriela Vila Nova de Lima: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Angélica Cândida Ferreira: support during field collection, practical execution of the experiment in the greenhouse and laboratory, and assistance with personnel and scientific development; substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation.

Eduardo Bezerra de Almeida Junior: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Carmen Sílvia Zickel: support for orientation methodology and scientific theoretical contributions; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Conflicts of interest

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

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Inventory

Preliminary dragonfly (Odonata) species list from the Pampa biome in Rio Grande do Sul, Brazil, with ecological notes for 19 new records for the State

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Abstract: An inventory of Odonata was carried out in the southern half of the state of Rio Grande do Sul, Brazil, in the Pampa biome. Originally, this biogeographical region was covered mostly by open fields and grassland, with sections of higher vegetation surrounding water bodies and rocky hills. Today the landscape is fragmented due to agricultural activities, mainly cattle farming, rice crops and forest plantations. Our survey was conducted in three municipalities from this region, between March 2015 and April 2016. Aiming at a general overview of the species composition, our sampling sites were selected on a wide basis, including lakes, bogs, temporary water bodies, small streams and river sections. Eighty two species of Odonata were collected comprising 40 genera and seven families. The dominant families were Libellulidae (56,1%), Coenagrionidae (24,5%) and Aeshnidae (7,3%). We found a diverse odonate assemblage, adding 19 new species records for the state of Rio Grande do Sul.

Keywords*:* *Anthropogenic threats, ecology, grassland, Neotropics, southern fields.*

Lista preliminar de libélulas (Odonata) do Bioma Pampa no Rio Grande do Sul, Brasil, com notas ecológicas para 19 novos registros para o estado

***Resumo*:** Um inventário de Odonata foi desenvolvido na metade Sul do estado do Rio Grande do Sul, Brasil, no Bioma Pampa. Originalmente, esta região biogeográfica era coberta principalmente por campos abertos e pastagens, com seções de vegetação mais alta nas imediações de corpos d'água e elevações rochosas. Atualmente a paisagem se encontra fragmentada devido a atividade agrícola, entre as principais, a criação de gado, cultivo de arroz e silvicultura. A pesquisa foi desenvolvida em três municípios da região, entre Março de 2015 e Abril de 2016. Buscando uma visão geral da composição de espécies, os locais de amostragem selecionados incluíram lagos, banhados, corpos d'água temporários e seções de rio. Oitenta e duas espécies foram coletadas compreendendo 40 gêneros e 7 famílias. As famílias dominantes foram Libellulidae (55,1%), Coenagrionidae (24,5%) e Aeshnidae (7,3%). Nós encontramos uma grande diversidade nas comunidades de Odonata, adicionando 19 novos registros de espécies para o estado do Rio Grande do Sul.

Palavras-chave: *Ameaças antropogênicas, ecologia, pastagens, região neotropical, campos sulinos.*

Introduction

One of the biggest problems towards conservation measures is the lack of knowledge on species distribution, scientifically known as the Wallacean shortfall (Cardoso et al. 2009). Jetz et al. (2012) stated that even when looking at the best known species, knowledge on their occurrence is substantially lower than the amount of available information of other important environmental variables. Insects is the least known group concerning their distribution, mostly due to their high species richness, lack of taxonomic expertise and lack of sampling (Diniz-Filho et al. 2010). In the Neotropical region, and specifically in Brazil, there are still whole biomes which are almost unknown regarding insects (Oliveira et al. 2017). An example is the Pampa biome: extending from the southern half of the state of Rio Grande do Sul (29° S), southwards through the whole Uruguayan territory and within Argentina to the temperate Patagonian steppes, and ending at 39ºS (Roig & Flores 2001). It covers only around 2% of the Brazilian territory but more than 63% of the Rio Grande do Sul State area (IBGE 2016). Although scarce, data from this region has proven high diversity and high levels of endemism, at least regarding the flora (MMA 2002, Behling et al. 2004). The Pampa is under extreme pressure from several human activities such as agriculture, cattle farming, and forestry by the conversion of grasslands into extensive plantations of *Eucalyptus*, *Pinus* and *Acacia* (Bencke 2009, Overbeck et al. 2009, Roesch et al. 2009). Official government data from 2008, shows that only 36% of the original vegetation remains in a fragmented mosaic (MMA 2009). In Brazil, the officially protected areas of the Pampa grasslands cover only about 0.5% of the total biome, thus, more actions are needed to recover and preserve this threatened environment (Overbeck et al. 2009). For conservation purposes, information on species diversity becomes more relevant every day, as well as knowledge on range of distribution (Oliveira et al. 2017). Knowing where species occur and their abundance is fundamental for setting up conservation priorities and red listing. Making information available to scientists and the public is also central to overcome the prevailing lack of distribution knowledge (Jetz et al. 2012). Thus, inventories can provide and improve ongoing and future management efforts (Lewis 2006). Concise information is since long needed for biologists and decision makers to prioritize specific areas for the preservation of biodiversity (Kerr et al. 2000). The Odonata fauna in Brazil is moderately known in terms of species occurrence and distribution: only 29% of the country's territory is adequately surveyed, according to De Marco & Vianna (2005), but this number is probably outdated. For the southeast and central regions several species lists are available (e.g., Costa et al. 2000, Costa & Oldrini 2005, Anjos-Santos & Costa 2006, Calvão et al. 2014, Bedê et al. 2015), while in the southern part of Brazil these studies are more scarce, the most recently published are Kittel & Engels (2014) and Renner et al. (2015, 2016a, 2016b), which were all conducted in the Atlantic Forest biome. Thus, we were motivated to increase the knowledge of species occurrence to the Pampa biome, the southernmost part of Brazil, where no survey of Odonata has previously been made.

Material and Methods

1. *Study area*

We sampled 63 localities distributed in four municipalities: Alegrete (AL, N = 10), Manoel Viana (MV, N = 9), Santana da Boa Vista (SB, N = 8) and São Francisco de Assis (SF, N = 36); within two different regions (1 and 2) of the Pampa biome (Figure 1). In total, the three municipality areas encompass more than 13,000,000 km^2 (IBGE 2016). The climate is Temperate (Cfb Köppen), with mean annual temperatures between 13°C and 17°C and altitudes from 50 to 200 m a.s.l. Annual precipitation mean is between 1,200 and 1,600 mm (INPE 2014). Our sites were distributed among several types of standing and running water including all types of habitats that could be considered to be used as reproduction sites for Odonata. We included small temporary pools or flooding areas, perennial waters such as bogs, swamps, ponds, lakes and rivers of various sizes. These sites included Rivers/streams (*N* = 27), Lakes (*N* = 18) and Temporary waters (*N* = 18), see below.

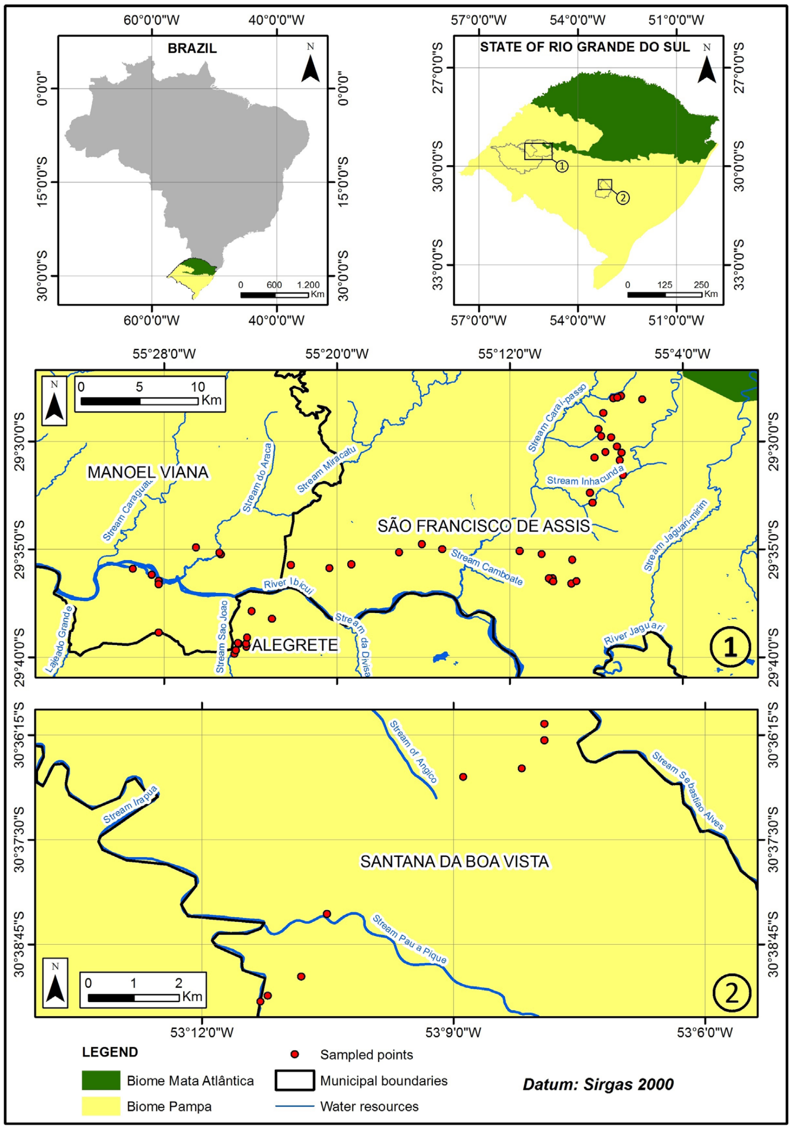

FIGURE 1: Map of Brazil and the Pampa biome domain in Rio Grande do Sul, rectangular insets from the two sampling regions (1 and 2), red dots marking the sampling sites.

2. *Data collection*

We sampled adult dragonflies from March 2015 to May 2016, the majority of the sampling sites were visited four times during this period, excluding winter due to the lack of activity of adult Odonata in the low temperatures (below 0° C) during that season. Other sites were visited only once, e.g. temporary water and flooded areas. The sampling method followed Renner et al. (2015): hand-held insect nets by a team of two people, in sunny days, during the peak time of Odonata activity (between 09:00 h to 16:00 h). Each site was sampled during 30 minutes, by walking the edge and marginal zones, the distances walked were dependent on the size and type of waterbodies, varying from ca. 50 to 250 m perimeters. We focused on adults only, since the majority of the larvae are still unknown (e.g., Garrison et al. 2006). Adults are often dispersing long distances (Corbet 1999), and we expect that some of our specimens might therefore derive from other regions. Our aim, however, was not to discern vagrants from reproducing species, but to get an account of species present in the area.

All specimens collected were preserved in 96% ethanol, and later determined to species level according to Garrison et al. (2006, 2010), Heckman (2006, 2010) and Lencioni (2006); species data were compared to the original species descriptions if needed and difficult species were kindly identified with help from Dr. R. W. Garrison, Sacramento, CA, U.S.A. For systematic classification, we followed Dijkstra et al. (2013, 2014). After identification, the specimens were deposited in the Museu de Ciências Naturais da Univates, Lajeado, Rio Grande do Sul, Brazil (MCNU). The collection authorization process was issued by ICMBio, under the number 50624-1. Beyond the municipalities abbreviations (mentioned above), we used the official abbreviations for the Brazilian states, as follows: AM (Amazonas) BA (Bahia), CE (Ceará), ES (Espírito Santo), GO (Goiás), MG (Minas Gerais), MS (Mato Grosso do Sul), MT (Mato Grosso), PA (Pará), PI (Piauí), PR (Paraná), RJ (Rio de Janeiro), RS (Rio Grande do Sul), SC (Santa Catarina), SE (Sergipe), SP (São Paulo) and TO (Tocantins).

To quantify our sampling effort, we choose to present a rarefaction curve (Mao tau) and the Jakkinfe 1 estimator. It is a general statistical technique for reducing the bias of an estimator by removing subsets of the data and recalculating the estimator with the reduced sample. Specifically, Jackknife1 depends only on the uniques (species found in only one sample) because the richness estimated is changed only when a sample that contains one of these species is deleted from a subset of samples (Gotelli & Colwell, 2011). We also quantified our sampling effort by using a Jackknife estimation of total species richness according to Smith & van Belle (1984).

Results

1. *Sampling sites*

We sampled many different environments ranging from temporary water bodies (small sites) to big river sections. Some of these places were in good environmental conditions, only marginally affected by human disturbances, and notable by the presence of species considered rare by us. These were small erosion sites with temporary water flow, small streamlets/swamps where cold and clear water was flowing from the underground (Figure 2a), temporary flooding zones at a major river (Figure 2b) and also swampy areas with varied and well vegetated edge zones (Figure 2c). Most of the present threats to such unique environments are related to the expansion of forestry practices, which are growing notably and quickly in the regions where the studies took place.

2. *Species List*

Eighty-two species belonging to 40 genera and seven families were collected in the three municipalities (Table 1). Seven specimens, newly emerged males and/or females, were impossible to determine to species level; so these were classified as “sp.”. Among these we found two specimens of an undescribed species belonging to the family Coenagrionidae. This species is currently under description by Dr. J. Muzón (La Plata, Argentina, personal communication), and no further information is therefore given here. Libellulidae was the dominant family (56,1%, N = 46) followed by Coenagrionidae (24,5%, 20) and, Aeshnidae (7,3%, 6), as shown in Table 2. The richest genus was *Erythrodiplax*, represented by eight species, this genus was also the most abundant in all seasons, occurring in virtually all sampling sites along with *Pantala flavescens* Fabricius, 1798, a known migratory and widely distributed disperser (Troast et al. 2016). Some species occurred only once (uniques) in this study and were considered regionally rare, for example: *Minagrion waltheri* Selys, 1876, *Brechmorhoga nubecula* Rambur, 1842, *Macrothemis lutea* Calvert, 1909 and *Dasythemis venosa* Burmeister, 1839. The rarefaction curve, which was based on the sampling events data, gave us a view of the sampling effectiveness of this study (Figure 3), tending to reach its asymptote. According to Smith & van Belle (1984) calculations using Jackknife as estimator, we expect 101 species to occur in the region, meaning we reached 81% of the total estimated diversity.

3. New records

Excluding the single undescribed species, we found 19 new records for the Rio Grande do Sul State; some of these species we considered rare and some seem to be well distributed in South America. Here we present some habitat preferences and ecological aspects of these species, based on the literature found and on our field observations.

FIGURE 2: Some of the sampling sites which were remarkable by good environmental conditions: (a) Swampy area, with slowly flowing clear water in SF; (b) Flooding area near to Ibicuí River, MV; (c) Swamp close to a forested edge zone in SB.

TABLE 1: Preliminary species list from the Pampa biome in Rio Grande do Sul, Brazil. Municipalities of occurrence and voucher/collection numbers. New records for the state are marked with *.

Suborder	Family	Species	Municipality	Collection ID
Zygoptera	Calopterygidae	*Hetaerina rosea* Selys, 1853	AL, MV, SB, SF	ZAUMCN1119
		**Mnesarete lencionii* Garrison, 2006	SF	ZAUMCN1120
		Mnesarete pudica (Hagen *in* Selys, 1853)	SF	ZAUMCN1121
	Coenagrionidae	*Acanthagrion cuyabae* Calvert, 1909	AL	ZAUMCN1122
		Acanthagrion gracile Rambur, 1842	AL, MV, SB, SF	ZAUMCN1123
		Acanthagrion lancea Selys 1876	AL, MV, SF	ZAUMCN1124
		Argentagrion ambiguum Ris, 1904	AL, MV, SF	ZAUMCN1125
		Argia albistigma (Hagen *in* Selys, 1865)	MV, SB, SF	ZAUMCN1126
		**Argia lilacina* Selys, 1865	AL, MV, SF	ZAUMCN1127
		Argia modesta Selys, 1865	SF	ZAUMCN1128
		Argia sp.	SB	ZAUMCN1129
		Homeoura chelifera Selys, 1876	AL, MV, SB, SF	ZAUMCN1130
		Ischnura capreolus Hagen, 1861	AL, MV, SB, SF	ZAUMCN1131
		Ischnura fluviatilis Selys, 1876	AL, MV, SB, SF	ZAUMCN1132
		**Minagrion waltheri* Selys, 1876	SF	ZAUMCN1133
		Neoneura leonardoi Machado, 2005	AL	ZAUMCN1134
		Oxyagrion hempeli Calvert, 1909	AL, MV, SB, SF	ZAUMCN1135
		**Oxyagrion rubidum* Rambur, 1842	SF	ZAUMCN1136
		Oxyagrion terminale Selys, 1876	AL, MV, SB, SF	ZAUMCN1137
		Telebasis corallina Selys, 1876	SB, SF	ZAUMCN1138
		Telebasis theodori Navás, 1934	MV, SB, SF	ZAUMCN1139
		Telebasis willinki Fraser, 1948	AL, SF	ZAUMCN1140
		*Gen. nov. sp. nov. (under description)	AL	ZAUMCN1141
	Heteragrionidae	*Heteragrion triangulare* (Hagen *in* Selys, 1862)	SF	ZAUMCN1142
	Lestidae	*Lestes bipupillatus* Calvert, 1909	SB, SF	ZAUMCN1143
Anisoptera	Aeshnidae	*Castoraeschna* sp.	SF	ZAUMCN1144
		Limnetron debile Karsch, 1891	SB	ZAUMCN1145
		Remartinia luteipennis Burmeister 1839	SF	ZAUMCN1146
		Rhionaeschna bonariensis Rambur, 1842	SB, SF	ZAUMCN1147
		Rhionaeschna planaltica Calvert, 1952	SF	ZAUMCN1148
		Staurophlebia reticulata Burmeister, 1839	SF	ZAUMCN1149
	Gomphidae	*Aphylla theodorina* Navás, 1933	SB, SF	ZAUMCN1150
		Aphylla molossus Selys, 1869	MV	ZAUMCN1151
		**Archaeogomphus densus* Belle, 1982	SB	ZAUMCN1152
		Progomphus basistictus Ris, 1911	MV, SF	ZAUMCN1154
		Progomphus sp.	MV	ZAUMCN1153
	Libellulidae	*Brachymesia furcata* Hagen, 1861	SB, SF	ZAUMCN1155
		**Brechmorhoga nubecula* Rambur, 1842	SF	ZAUMCN1200
		**Dasythemis venosa* Burmeister, 1839	SF	ZAUMCN1156
		Dasythemis mincki mincki Karsh, 1890	SB, SF	ZAUMCN1157
		Diastatops intensa Montgomery, 1940	MV, SB, SF	ZAUMCN1158
		**Diastatops obscura* Fabricius, 1775	AL	ZAUMCN1159
		**Dythemis nigra* Martin, 1897	SF	ZAUMCN1160
		Elasmothemis sp.	SF	ZAUMCN1161
		Erythemis peruviana Rambur, 1842	SF	ZAUMCN1162
		Erythemis plebeja Burmeister, 1839	SF	ZAUMCN1163
		**Erythemis vesiculosa* Fabricius, 1775	AL, MV	ZAUMCN1164
		Erythemis sp.	SF	ZAUMCN1165
		Erythrodiplax atroterminata Ris, 1911	AL, MV, SB, SF	ZAUMCN1166
		Erythrodiplax hyalina Förster, 1907	AL, MV, SB, SF	ZAUMCN1167
		**Erythrodiplax lygaea* Ris, 1911	SF	ZAUMCN1168
		Erythrodiplax media Borror, 1942	AL, MV, SB, SF	ZAUMCN1169
		Erythrodiplax melanorubra Borror, 1942	AL, MV, SB, SF	ZAUMCN1170
		Erythrodiplax nigricans Rambur, 1842	AL, MV, SB, SF	ZAUMCN1171
		Erythrodiplax paraguayensis Förster, 1905	AL, MV, SB, SF	ZAUMCN1172
		Erythrodiplax sp.	AL, MV, SB, SF	ZAUMCN1173

TABLE 1: Continued...

Suborder	Family	Species	Municipality	Collection ID
		**Gynothemis venipunctata* Calvert, 1909	SF	ZAUMCN1174
		**Idiataphe longipes* Hagen, 1861	SB, SF	ZAUMCN1175
		**Macrothemis heteronycha* Calvert, 1909	MV, SB, SF	ZAUMCN1176
		Macrothemis imitans Karsch, 1890	SB, SF	ZAUMCN1177
		**Macrothemis lutea* Calvert, 1909	MV	ZAUMCN1178
		Macrothemis marmorata Hagen, 1868	AL, MV, SB, SF	ZAUMCN1179
		Miathyria marcella (Selys *in* Sagra, 1857)	AL, MV, SB	ZAUMCN1180
		Micrathyria hesperis Ris, 1911	AL, MV, SF	ZAUMCN1181
		Micrathyria longifasciata Calvert, 1909	SF	ZAUMCN1182
		Micrathyria ocellata Martin, 1897	MV, SB, SF	ZAUMCN1183
		**Micrathyria spuria* Selys, 1900	SB	ZAUMCN1184
		Micrathyria tibialis Kirby, 1897	MV, SF	ZAUMCN1185
		Micrathyria sp.	SF	ZAUMCN1186
		Nephepeltia flavifrons Karsch, 1889	AL, MV, SF	ZAUMCN1187
		**Orthemis aequilibris* Calvert, 1909	AL, MV, SF	ZAUMCN1188
		Orthemis ambinigra Calvert, 1909	SF	ZAUMCN1189
		**Orthemis attenuata* Erichson, 1848	AL, MV	ZAUMCN1190
		Orthemis discolor Burmeister, 1839	AL, MV, SB, SF	ZAUMCN1191
		Pantala flavescens Fabricius, 1798	AL, MV, SB, SF	ZAUMCN1192
		Perithemis icteroptera (Selys *in* Sagra, 1857)	AL, MV	ZAUMCN1193
		Perithemis mooma Kirby, 1889	AL, MV, SB, SF	ZAUMCN1194
		Tauriphila argo Hagen, 1869	MV	ZAUMCN1195
		**Tholymis citrina* Hagen, 1867	MV	ZAUMCN1196
		Tramea abdominalis Rambur, 1842	MV	ZAUMCN1197
		Tramea binotata Rambur, 1842	AL, MV, SB, SF	ZAUMCN1198
		Tramea cophysa Hagen, 1867	AL, MV, SF	ZAUMCN1199

TABLE 2: Number of Odonata species collected per family in Rio Grande do Sul within the Pampa biome.

FAMILY	No.	%
Zygoptera		
Calopterygidae	3	3,6
Coenagrionidae	20	24,5
Heteragrionidae	1	1,2
Lestidae	1	1,2
Anisoptera		
Aeshnidae	6	7,3
Gomphidae	5	6,1
Libellulidae	46	56,1
TOTAL	82	100%

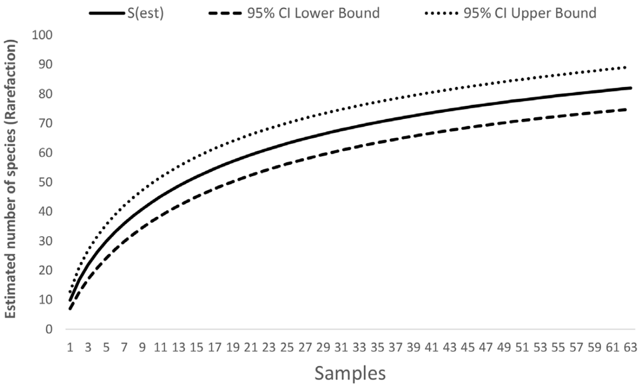

FIGURE 3: Rarefaction curve showing the efficiency of our sampling, stabilization expected to be reached if we continue our efforts. S(est): estimated species number; 95% CI: confidence interval, upper and lower.

3.1 - Mnesarete lencionii Garrison, 2006 (Calopterygidae)

Rare, at least for the latitude where our survey took place; this species is thought to be distributed all over Southeastern Brazil (Garrison 2006). The species resembles the congeneric *M. pruinosa* (Hagen *in* Selys, 1853), differing from it by the presence of a dark pseudopterostigma. Only one male was captured, it was found in a small fast flowing rivulet used for drainage of rice fields. At the same place we also found other calopterygids such as *Hetaerina rosea* Selys, 1853, which at the time of sampling, was abundant in the area. This species of *Mnesarete* was recorded from Argentina and Paraguay (Garrison 2006), and from Brazil from MG (Vilela et al. 2016) and SP (Garrison 2006).

3.2 - Argia lilacina Selys, 1865 (Coenagrionidae)

An inconspicuous coenagrionid due to its diminutive size (less than 30 mm), whose females shows pale brownish to green coloration and males are a bit more colored, varying from grayish blue to black. This species was quite common and we collected more than 80 specimens. It was mostly found in small streams flowing in open/field areas. According to Takiya et al. (2016), this species was previously recorded for Bolivia, Paraguay, and Argentina, and in Brazil for CE, TO, MT, GO, MG, MS, ES, SP and RJ.

3.3 - Minagrion waltheri Selys, 1876 (Coenagrionidae)

This *Minagrion* species was considered a rare species in our dataset, it occurred in small numbers along the margins of lentic waters, such as swamps and marshes (Garrison et al. 2010). It occurs in areas dominated by cattails (*Typha*), preferring slow moving clear water streams (Santos 1956, 1965). Our specimens occurred at only one sampling site, which had the same environment as that provided by Santos (1956, 1965). The clean water seemed to pertain to an underground flow giving it zero or almost zero turbidity. The previously known records for this species are from MG (Bedê et al. 2015) and SP (Selys 1876; Santos 1965). We thus expand its distribution more than 1,200 km southwards. The species may well occur in between these two regions, but there are no records, probably due to lack of sampling.

3.4 - *Oxyagrion rubidum* (Rambur, 1842) (Coenagrionidae)

This species was locally common, alongside with *O. terminale* Selys, 1876, and difficult to identify in the field due to the resemblance of the two species. The environment where it was found was characterized by dense marginal vegetation on rivers, streams and ponds. This species is known from Uruguay (von Ellenrieder et al. 2009), Paraguay (Heckman 2010), Argentina and Chile (Muzón et al. 2014). From Brazil there is only one record in SP (Costa et al. 2000).

3.5 - *Archaeogomphus densus* Belle, 1982 (Gomphidae)

This is a small gomphid, pale colored which makes it inconspicuous in the forest and fields. It is known by its agile and rapid flight that makes it difficult to collect (Belle 1982). This genus is easily identified by its unique characters: two latero-dorsal hooks on the male S10 (Garrison et al. 2006). We consider this species as poorly known since the only records found are the original description from Argentina and MG by Belle (1982). Our unique specimen (male) was caught in a rocky fast flowing stream with sandy margins and covered by low vegetation.

3.6 - *Brechmorhoga nubecula* Rambur, 1842 (Libellulidae)

This species was considered rare to our sampling efforts, since it was found on only one occasion in a fast flowing stream, with a shaded marginal zone. It could be easily confused with some species belonging to the *Macrothemis* genus. According to Kompier (2015) it can be overlooked due to its secretive habitats, which was proven true by our sampling experience. According to Heckman (2006) this species occurs in almost all southern American countries, and in Brazil there are records from CE (Takyia et al. 2016), RJ (Assis et al. 2004, Kompier 2015), MG (Souza et al. 2013, Bedê et al. 2015) and SP (Costa et al. 2000).

3.7 - *Dasythemis venosa* Burmeister, 1839 (Libellulidae)

This forest species was found only in well preserved environments, such as small forest fragments, in shaded areas or natural clearings (at fallen trees), perching on dry twigs above the water. Records of this species are from Argentina and Paraguay (Heckman 2006) and from Brazil there are records from SP (Costa et al. 2000) and MG (Souza et al. 2013).

3.8 - *Diastatops obscura* (Fabricius, 1775) (Libellulidae)

A conspicuous species that has dark colored wings and butterfly-like flying style. Our specimens were found in rich marginal vegetation of streams and lakes. The species can be easily confused with its congener *Diastatops intensa* Montgomery, 1940, which also occurred at the same localities. This species is known from many countries in South America, including Paraguay and Argentina (Heckman 2006); from Brazil there are records from, MG (Bedê et al. 2015, Vilela et al. 2016), MS (Dalzochio et al. 2011), MT (Calvão et al. 2014), RJ (Kompier 2015) and SP (Costa et al. 2000). The species probably occurs in all Brazilian states, but was not previously detected in RS due to lack of sampling.

3.9 - *Dythemis nigra* Martin, 1897 (Libellulidae)

At our sampling sites this species seemed to be rare, found on only one location, a small slow-flowing clear water stream. Our specimen was caught perching on twigs in a shaded marginal zone close to the water. The distribution records for this species, according to Takiya et al. (2016), are from Mexico, Panama, Trinindad and Tobago, Colombia, Venezuela, Guyana, Suriname, French Guiana, Ecuador, Peru, Paraguay, Argentina and Brazil: AM, BA, CE, ES, GO, MG, MS, MT, PA, PE, RJ, SC and SP.

3.10 - *Erythemis vesiculosa* Fabricius, 1775 (Libellulidae)

A very conspicuous species, characterized by its flying habits when it is hovering at low height above the water surface. Numerous specimens were observed, mostly at the lakes, and male-to-male territorial disputes and fight behavior was observed on several occasions. This species also has a wide distribution since there are records from many countries in South America. In Brazil the records are from several states: MG (Bedê et al. 2015), MS (Dalzochio et al. 2011), MT (Calvão et al. 2014), RJ (Assis et al. 2004, Kompier 2015) and SP (Costa et al. 2000).

3.11 - *Erythrodiplax lygaea* Ris, 1911 (Libellulidae)

This is a tiny libellulid species whose males have bright yellowish colors that make the identification easy in the field. In our survey it occurred only on two locations, which were slow flowing water areas fed by small streams, corroborating the information provided by Costa et al. (2001) describing the larvae of the species. It is known to occur in Brazil (Pirassununga, SP), Paraguay and Argentina (Jurzitza 1981, Costa et al. 2001, Garrison et al. 2006).

3.12 - *Gynothemis venipunctata* Calvert, 1909 (Libellulidae)

A species which is easily identifiable in the field by the yellowish or amber spots on the male wing bases. Our six specimens were caught flying in a similar motion to that of *Macrothemis*, at about 1.5 m height, above open field areas, corroborating the observations made by Garrison (1983). Also, some were seen at a distance, flying in swarm-like formations consisting of some 5 to 10 specimens close to tree tops of about 10 m height. The species is known from Venezuela (De Marmels 1983) and in Brazil from MS (Costa et al. 1998), RJ (Kompier 2015) and SP (Costa et al. 2000).

3.13 - *Idiataphe longipes* Hagen, 1861 (Libellulidae)

We considered this species as common only in the peak of the summer season (Dec - Feb), since it was found in large numbers, usually perching on dry twigs along the water's edge. It seems to prefer lakes with diverse marginal or aquatic vegetation. There are records from several countries of South America: Colombia, Peru, Venezuela, Paraguay and Guyana. From Brazil the records are from the following states: SP (Costa et al. 2000), RJ (Kompier 2015), ES (Heckman 2006) and MG (Bedê et al. 2015).

3.14 - *Macrothemis heteronycha* Calvert in Ris, 1909 (Libellulidae)

Most of our 14 specimens were caught at temporary waters or small creeks with sandy and rocky bottom. Its flight behavior resembles in some ways that of *Gynothemis venipunctata*; a slow and fragile style, very different form the great majority of dragonflies. Also, opposite to most of its congeners, *M. heteronycha* was found mostly in open areas instead of forest with closed canopy. According to the literature, it occurs in Paraguay, Argentina (Ris 1913, Garrison & von Ellenrieder 2006) and Brazil: SP, RJ, MG, MS, ES and SC (Costa et al. 2000, Dalzochio et al. 2011).

3.15 - *Macrothemis lutea* Calvert, 1909 (Libellulidae)

A very interesting and unexpected finding. Our specimens were caught in tandem along a flooding area near to a river sand bank (Figure 2b). At first sight they were thought to be gomphids, due to the long abdomen (big overall size for a *Macrothemis* species) and general appearance. It is a rare species described from Brazil, known to occur in the state of SE which is the species type locality (Calvert 1909) and recently registered in CE (Nobre & Carvalho 2014). Here the distribution of the species is expanded southwards about 4,000 km.

3.16 - *Micrathyria spuria* Selys, 1900 (Libellulidae)

A common species, easily identified by the abdominal markings and the dorsally whitish cerci. We found this species mostly in lakes and swamps with abundant marginal vegetation. It showed the typical *Micrathyria* behavior of perching on tips of emergent vegetation or dry twigs. The females we caught were found away from the water bodies, perching and foraging around trees and bushes. The species is known to occur in Venezuela (De Marmels 1983), Paraguay and Argentina (von Ellenrieder 2009) and Brazil: MG, MS, PR, RJ and SP (Costa et al. 2000, 2002).

3.17 - *Orthemis aequilibris* Calvert, 1909 (Libellulidae)

Most of our specimens were found in the same localities as *M. heteronycha*; temporary waters or small perennial rocky creeks. Some of them were found away from the water. This species has records from Panama, Colombia, Peru, Venezuela, French Guiana, Guyana, Surinam, Bolivia, Paraguay, and from Brazil it has been recorded in BA, ES, MG and RJ (Costa et al. 2000) and AM, CE, MS, PA and PI (Takiya et al. 2016).

3.18 - *Orthemis attenuata* Erichson, 1848 (Libellulidae)

The males resemble a bit the darker species of the genus *Erythemis*, while the females have a typical and unique color scheme: dark brown with yellowish stripes. Most of our specimens were caught in a temporary river flood pool, which was muddy at the time of our sampling efforts, in the spring season. Strong male-to-female harassment was observed, as well as male-to-male territorial disputes. There are records from several countries in South America (von Ellenrieder 2012); in Brazil it is known to occur in PA (Pinto & Carvalho 2009), RJ (Kompier 2015), BA, ES and MT (von Ellenrieder 2012).

3.19 - *Tholymis citrina* Hagen, 1867 (Libellulidae)

This species is known by its crepuscular habits and erratic flight, usually found over marshes hunting mosquitoes (Paulson 2001). Our specimens were caught during the first minutes of our sampling efforts early in the morning (09:00 am), flying at irregular intervals in shaded areas which made them difficult to see and to capture with insect nets. This species was only found in the flooded areas, near to the Ibicuí River. In Brazil the records are known from the following states: MS (Costa et al. 1998), MT (Juen et al. 2014), RJ (Costa et al. 2002) and SP (Costa et al. 2000).

Discussion

Conservation strategies depend basically from information about diversity distribution, biogeography, population and community ecology. Therefore, inventories play a key role for the development of such measures. In this survey, we increased the knowledge on the Odonata fauna of Brazil's Pampa biome. We found 82 species, reflecting the diversity of our sampling sites; including several types of aquatic systems, most of them located in man-influenced areas, mostly by agriculture. Of these species, no less than 19 were new to the state and one new species not described in this paper. Naturally the number of species in the area will increase by including more sample sites and resampling ours, but never-the-less we already can see a highly diverse fauna in the region. We registered a large number (46) of Libellulidae species, which could be the result of many widespread generalists occurring in the mosaic of open fields and agriculture areas with little riparian fragmented forest, a landscape which is known to favor the fast and agile flying dragonflies, supporting the findings of Machado (2001). As suggested by Corbet (1999), there are different responses from Anisoptera and Zygoptera to environmental conditions, since the latter are known as low range dispersers (Vieira & Cordero-Rivera 2015). In general, there are clear relations between environmental factors (biotic and abiotic) and species composition, these factors acting as determinants of presence and absence of some species due to ecological and physical restrictions (e.g., Paulson 2006, Juen et al. 2007). This is naturally an oversimplified division as Zygoptera is a taxonomical and not an ecological unit and contains both good (Flenner & Sahlén 2008) as well as weak (Lorenzo-Carballa et al. 2015) dispersers, but as an average assumption it is valid also when analysing large scale species patterns (Heiser & Schmitt 2013). We found many rare species in the study (singletons n = 20), all having a restricted occurrence. We consider the following possible explanations: first, we found a high number of species of Coenagrionidae (20), some of which can mirror good ecological conditions even in a highly fragmented region, acting then as bioindicators (Clausnitzer 2003, Suhling et al. 2006, Samways & Sharrat 2010, Renner et al. 2016a). Following this idea, in the open grassland with longer dispersal distances in the open, more exposed surroundings between suitable habitats (Juen et al. 2007), fewer of the smaller bodied species have the possibility to occur. This is due to some genera having ecological restrictions related to aquatic vegetation (i.e. plant diversity) and water quality (e.g., *Argia*, *Homeoura*, *Oxyagrion*) as stated by Garrison et al. (2010). Second, in well preserved areas or large forested areas, higher number of Zygopterans such as Heteragrionidae, Calopterygidae and again coenagrionids are expected to be found, many of which have specific environmental restrictions and ecological needs, mostly regarding to diversity of plants and vegetal structures in the riparian areas (Juen et al. 2014, Carvalho et al. 2013). This fact can explain the occurrence of *Minagrion waltheri* (Coenagrionidae) in only one locality in SA, which is a well-preserved area that keeps its natural features, as well crystal clear water and high diversity of aquatic/riparian vegetation (Figure 2a). These specialized species can function as powerful tools when distinguishing priority areas for preservation, as many of such restricted species have been proposed to be good indicators of environmental quality (Clausnitzer 2003, Sahlén 2006, Koch et al. 2014).

When compared to other studies from the Neotropics (De Marco et al. 2014, Monteiro et al. 2013) our collection efficiency captured only 81% of the expected diversity, tending to reach its asymptote, implying that the actual number of species in the region could be as high as 101 (cf., Figure 3). We have a relatively low number of sampling sites and, hence, cannot see the full picture of the odonate diversity in the Pampa biome, given its dimensions. However, we tried to include every kind of aquatic environment which could shelter any species of Odonata, from small puddles of temporary water to big rivers, lakes and perennial bogs; more than one of each. To our surprise, we found several interesting habitats in which we recorded most of the species that we considered rare. These habitats could be starting point for future conservation actions to be taken for diversity maintenance in the area. Special focus should therefore be given to the environments whose original features are still preserved, such as small rivulets flowing in ravines, swampy and well vegetated areas supplied by underground waters and forest remnants with waterbodies (Figures 2a, b, c).

Future studies should, if possible, include a bigger selection of environments from more municipalities to obtain a more complete sample of the communities. In addition, more frequent sampling during the seasons might also contribute to the inventory, given that some species are related to specific conditions to be active, e.g. crepuscular flight, which occur among many Aeshnidae that are active only in the twilight. There are also species that are active during rainy conditions (Garrison 1989, Wasscher 1990, Corbet 1999), some of which might occur in the Pampa region.

The knowledge achieved in other biomes of Brazil is much deeper than that from the Pampa. But this biome is considered a highly biodiverse system (Overbeck et al. 2009), and in this context, species surveys can supply valuable initial information for the actions needed to preserve and restore these environments. With this survey we tried to add further information and improve the knowledge of the group Odonata from the Pampa biome of southern Brazil, showing that even under such fragmented and altered conditions diversity is still high; this fact acknowledges that future measurements of conservation and restoration are needed.

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Author's contribution

Samuel Renner: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Eduardo Périco: Contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Gerson Júnior Ely: Contribution to data collection.

Göran Sahlén: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Article

Successional stage effect on the availability of tree cavities for cavity-nesting birds in an Atlantic Forest park from the state of São Paulo, Brazil

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Abstract: The availability of suitable cavities and substrate for hole construction can limit the populations of birds that rely on tree hollows to reproduce. Several studies have focused on the effects of types of habitats, and types of human disturbances on cavities abundance, but the effect of successional stages in the Atlantic Forest has been poorly addressed. In this study we aimed to compare the availability and biometric characteristics of tree cavities between primary and mid-successional stage sites in an Atlantic Forest area from southeastern Brazil. Based on nest characteristics described in the literature, we inferred if hollows present in secondary sites could harbor the large secondary-nester Atlantic Forest birds, with special attention to toucans and hawks. From September 2016 to April 2017, 96 cavities were sampled, 67 in the primary, and 29 in the secondary plots. Our data revealed that the communities of cavity-nester birds in mid-successional stage areas may be more affected by the reduced cavity availability than by cavities quality, as the number of cavities in these sites was less than half than that found in mature forests. We also provide evidences that the largest cavity nesters, such as the biggest hawks, might have limited nesting possibilities in mid-successional stage areas especially due to small cavity entrances, which may have important reflects in conservation strategies.

Keywords: *Bird conservation, nesting ecology, cavity-nesters*

Efeito do estágio sucessional na disponibilidade de cavidades para aves que nidificam em ocos de árvores em um parque da Mata Atlântica do Estado de São Paulo, Brasil

Resumo: A disponibilidade de cavidades adequadas e o substrato para a escavação de ocos podem limitar as populações de aves que dependem das cavidades de árvores para se reproduzir. Vários estudos se concentraram nos efeitos dos tipos de habitats e em tipos de distúrbios antrópicos sobre a disponibilidade de cavidades, mas o efeito da sucessão ecológica na Mata Atlântica é muito pouco investigado. Neste estudo, buscamos comparar a disponibilidade e algumas características biométricas das cavidades de árvores entre áreas em estágio primário e secundário-médio na Mata Atlântica do sudeste do Brasil. Baseado nas características dos ninhos das espécies que ocorrem na área de estudo, também deduzimos se as cavidades que estão presentes em áreas secundárias poderiam abrigar as maiores aves que nidificam em cavidades, mas que são incapazes de escavá-las, com atenção especial aos tucanos e falcões. De setembro de 2016 a abril de 2017, 96 cavidades foram amostradas, 67 em áreas primárias e 29 em parcelas em locais de floresta secundária. Nossos dados revelaram que a comunidade de aves dependentes de ocos para nidificação em áreas em estágio médio de sucessão podem ser mais afetadas pela baixa disponibilidade de cavidades do que pela qualidade das cavidades, já que o número de cavidades nesses locais foi menos da metade do que o encontrado em florestas maduras. Também evidenciou-se que os nidificadores de cavidades de maior porte, como os grandes falcões, podem ter possibilidades de nidificação limitadas nas áreas de estágio médio de sucessão, especialmente devido ao tamanho reduzido das entradas de cavidades, o que pode ter importantes reflexos em estratégias de conservação.

Palavras-chave: *conservação de avifauna, ecologia reprodutiva, nidificadores de cavidades*

Introduction

Bird species that use tree cavities for nesting can be classified as i) excavators, or primary cavity nesters, and ii) non-excavators, or secondary cavity nesters. Excavators build their own cavities usually in dead trees or branches and are represented mainly by the woodpeckers, whereas the non-excavators depend on pre-existing cavities such as natural holes present in live or decaying trees, or those created by the excavators (e.g. toucans, woodcreepers, and parrots) (Martin & Eadie 1999, Jackson & Jackson 2004). As predation is often the main cause of nest failures (Li & Martin 1991, Wesolowski 2002, Miller 2002, Cockle & Bodrati 2009, Wiebe & Swift 2001), suitable cavities for birds reproduction are expected to present: a small entrance capable to avoid the passage of predators that are bigger than the nesting bird; depth enough to avoid predators to reach nest contents, and hard walls to impede nest access by predators through wood destruction (Wesolowski 2002).Therefore, the availability of adequate natural cavities and of substrate for holes construction can limit the populations of birds that rely on tree hollows to reproduce (Lõhmus & Remm 2004, Cockle et al. 2008, 2010).

Many types of anthropogenic activities are known to impact the density and quality of tree cavities, and the resulting decline of cavity-nesting species has been reported for bird communities all over the world (for a review, see Cornelius et al. 2008). For instance, the selective logging of the largest trees in areas of mature forest has often been accompanied by the depletion of the largest birds that depend on big cavities to reproduce (Bai et al. 2003, Politi et al. 2010, Cockle et al. 2011a). A similar effect occurs in secondary forests, where the density of large cavities tends to decrease due to the smaller diameter of the trees (Willis & Oniki 2001, Cornelius et al. 2008, Cockle et al. 2011a). Further, timber management practices based on the exploitation of decaying trees have caused disastrous declines in cavity-nesting birds in Europe and North America (Dobkin et al. 1995, Aitken et al. 2002, Franco et al. 2005). These are evidences that forest type, successional stage, and degree of human disturbance are key elements for cavity nests availability and for the persistence of cavity-nesting birds (Cornelius et al. 2008).

The Atlantic Forest is one of the most threatened biomes and one of the five main hotspots on earth (Myers et al. 2000). Besides, it is the second richest area in bird species in the world (Remsen et al. 2015, Piacentini et al. 2015). The biome's original area covered more than one million square kilometers (SOS Mata Atlântica and INPE 2016), but only about 11.7% is left, and 42% of the remaining area consists of fragments smaller than 250 ha that suffer varying degrees of disturbance and threats (Ribeiro et al. 2009). In this scenario, cavity nesting species may be particularly vulnerable, and understanding the factors that influence the persistence of these species is urgently needed (Cockle et al. 2010, 2015). Cockle et al. (2010) demonstrated that in the subtropical Atlantic Forest of Argentina there were nine times fewer cavities in logged than in areas of unmanaged primary forest, and Cockle et al. (2015) showed that in the same region, when trees with desirable characteristics were maintained, several species of cavity nesting birds could succeed in reproduction even in rural areas. These studies have focused mainly on types of habitats, and on types of human disturbances, but the effect of successional stages in the same habitat has been poorly addressed in the Atlantic Forest. This is an important knowledge gap because secondary forests and/or small fragments currently correspond to 32-40% of what is left from the Atlantic Forest (Ribeiro et al. 2009).

In this study we aimed to compare the availability and characteristics of tree cavities between primary and mid-successional stage sites in an Atlantic Forest area from southeastern Brazil, and based on nest characteristics described in the literature, we inferred if hollows present in secondary sites could harbor the large secondary-nester Atlantic Forest birds, with special attention to toucans and hawks. Specifically, we addressed the following questions: i) Does cavity availability differ between mid-successional stage and primary Atlantic Forest sites? ii) Does the amount of dead trees differ between these areas? iii) Do cavity measurements differ between these successional stages? iv) Could cavities in the mid-successional stage sites harbor the entire guild of cavity-nesting Atlantic Forest birds?

Materials and Methods

1. Study area

This study was conducted in Carlos Botelho State Park - PECB (24° 06' 55" - 24° 14' 41" S and 47° 47' 18" - 48° 07' 17" W), state of São Paulo, Brazil. The park holds 37.644 ha, mainly of primary forests, and together with a number of contiguous conservation units it composes one of the largest remaining patches of Atlantic Forest, which totals more than 1.1 million ha. Altitudes are from 20 to 1,000 m a.s.l.; average temperatures from 18-20°C, and mean annual precipitation is 1,676 mm (777-2,264 mm) (Beisiegel & Mantovani 2006). This is one of the few Atlantic Forest parks that still preserves the original faunal assemblage, including jaguars, cougars, ocelots, and tapirs (Brocardo et al. 2012), and Antunes et al. (2013) have recorded 331 bird species in PECB, of which 52 (15.7%) are cavity nesters. Although the PECB is mostly covered by mature forests, 33.3% of the area is represented by recovery-zones in different successional stages. Our survey was conducted in the upper part of the park, in the municipality of São Miguel Arcanjo (714-837 m in altitude), where the natural vegetation is classified as submontane Atlantic Forest. Our sampling sites were accessed by using the called ''service road'', a 28 km path that connects the main administrative base of the park to center of the conservation unit, in which only the use by researchers and park staff is permitted (see Oliveira Jr. et al. 2011). Primary forest areas were reached by choosing random points in the service road, and secondary habitats were investigated in a clearly distinguishable recovery area called ''aceiro''. The later is located in one of the limits of the park and has approximately 136 ha, being bisected by an approximately 3 km secondary trail. This area was deforested mainly for charcoal production and it has been in regeneration since 1982, when PECB was created. Currently it can be generally classified in mid-successional stage (see methods bellow).

2. Successional stage classifications and data analyses

Cavities were sampled in primary and mid-successional stage areas by establishing five 0.5 ha plots in each type of habitat. To determine plot locations, we first assorted numbers from the first 7000 m of the service road, and from the 3000 m of the aceiro sub-trail to generate reference sites. In these sites, we assorted the side of the road or trail and entered the forest until reaching areas that appeared to have the desirable characteristics. Then, in each of these sites we created one 0.5 ha plot, subdivided in 25 m wide stripes, demarked with strings.

To confirm the successional stage, we established three 10x10 m sub-plots, one in each corner, and one in the middle of each plot, arranged diagonally. In these sub-plots we obtained diameters at breast high and height of woody trees with DBHs above 5 cm (Carvalho et al. 2015). Mid-successional stage was considered when DBHs of trees averaged across the three subplots were between 10 and 20 cm, and heights between 4 - 12 m (Resolução Conjunta SMA Ibama/SP 1994, hereafter Ibama 1994). Primary, or advanced-successional stage was considered when average DBHs and heights were above 20 cm and 10 m, respectively, and when woody vines, adult individuals of the palm *Euterpe edulis* Mart., and of the samambaiaçu *Cyathea* spp. were present (Ibama 1994, Lima et al. 2011).

In each plot, we quantified the number of natural cavities, the number of woodpecker's holes, and the numbers of dead trees or branches. The minimum diameter considered for the counting of dead trees or branches was 5 cm. We have defined a cavity as a depression in a woody tree (living or dead), which had a plateau to support an oologic chamber and had a closed cover. Cavities that were up to 7 m high were accessed using ladder for biometric analyses (Rendell & Robertson 1989, Kerpz & Smith 1990, Aitken & Martin 2004, Martin et al. 2004). Measurements included: tree diameter at cavity height, smaller entrance diameter, internal diameter, and depth (Marini et al. 2002, Remm et al. 2006, Tamungang et al. 2016). When a cavity presented more than one entrance, we considered the measurements of the larger one as this is the one that would permit easier access by predators. As in many cavities entrance is not round shaped, but irregular, we chose measuring the smaller entrance diameter as this is what may restrict animal entrance. Height above ground was also estimated for all of the cavities.

The mean numbers of cavities (natural plus woodpecker's holes), the mean numbers of woodpecker's cavities, and of decaying trees or branches were compared between habitats using univariate *t*-tests with $\log_{10}$ data correction. The same procedure was used to compare cavities height above ground, but pooling together the data of all of the plots from each habitat. The proportion of natural versus woodpecker holes between habitats was compared through *G*-test. To compare cavity measurements between habitats, we first preformed a Principal Component Analysis (PCA) to reduce the number of variables and to seek for potential graphical clusterings that could be correlated to each type of habitat. Data was transformed using *z-score* (Gotelli & Ellison 2011) and we used variance-covariance matrix. Then, the new scores provided for the main axis were compared between habitats by *t*-test. All of the analyses were performed using the software Past3 (Hammer et al. 2001), with 95% significance level.

Based on the birds survey of Carlos Botelho State Park (Antunes et al. 2013), we listed all of the forest secondary cavity-nester species. Then, biometric data of their nests were consulted in literature in order to investigate if cavities with minimum dimension requirements were available in the analyzed plots. Only species that presented at least two nests described, measures of cavity smaller entrance diameter, and depth, with values range (maximum and minimum) were selected. A graphical comparison was made between the values of the smaller entrance opening and cavities depth, which proved to be the most important characteristics selected by birds in several studies (Wesolowski 2002, Cockle et al. 2008, Cockle & Bodrati 2009).

Results

From September 2016 to April 2017, we found a total of 96 cavities, being 67 in the primary, and 29 in the secondary plots. The number of cavities per plot varied from five to 21 in the primary (13.4 ± 6.34), and from one to 13 (5.8 ± 5.07) in the secondary habitats, being the average number of cavities significantly higher in the primary forest ($t = 2.05$, $P = 0.036$). On the other hand, the number of decaying trees or branches was significantly higher in the secondary habitats ($t = -2.92$, $P = 0.0096$), being 90 in the primary (11 to 30; 18.0 ± 7.17), and 156 in the secondary habitats (21 to 42; 31.2 ± 7.66) (Fig. 1). Of the 67 cavities found in the primary plots, 61 (91%) were in live trees or branches, and six (9%) were in decaying structures, of which five (7.5%) were typical woodpecker's excavations. Of the 29 cavities found in the secondary plots, 22 (76%) were in live trees or braches, and seven (24%) were in decaying structures, of which six (20.6%) were woodpecker's excavations. Neither the proportion of natural versus woodpecker holes ($G = 3.21$, $P = 0.07$), nor the average numbers of woodpeckers cavities ($t = 0.11$, $P = 0.45$) have different between habitats (Figure 1), and 100% of the holes excavated by woodpeckers occurred in decaying substrate.

Cavities average heights above ground were 8.17 ± 7.96 (0.34 - 25; $n = 67$) in the primary, and 3.52 ± 2.48 (0.56 - 10; $n = 26$) in the secondary plots, being significantly different ($t = 2.08$, $P = 0.02$). Of the 96 cavities, 51 were accessible but one was not measured because of the presence of bees in primary forest, and three were not considered because they were full of water, all of them in secondary forest, indicating that they were inadequate for birds reproduction. Then, measurements were obtained for 47 cavities, being 32 in the primary plots and 15 in the secondary habitats (Table 1). In the PCA, 95.4% of the variation was explained by the first two axes, with the first axis concentrating 78.72% of the variation. There was no graphical evidence for clustering (Figure 2), and the *t*-test performed to compare the new scores of the main axis (component 1) was not significant ($t = 0.0$, $P = 0.50$), indicating that cavities presented similar characteristics in the two types of habitats.

Of the 62 cavity-nester bird species recorded for PECB, only nine had nest measurements available in the literature that attended our selection criteria for depth (Figure 3), and seven for entrance diameter (Figure 4) comparisons. These graphical comparisons permitted to observe that cavities from both habitats provided minimal depth requirements for nesting of all of the analyzed species, but in the mid-successional stage areas the cavities we found might exclude the large Collared Forest Falcon, *Micrastur semitorquatus* (approximately 50 cm) based on entrance diameters.

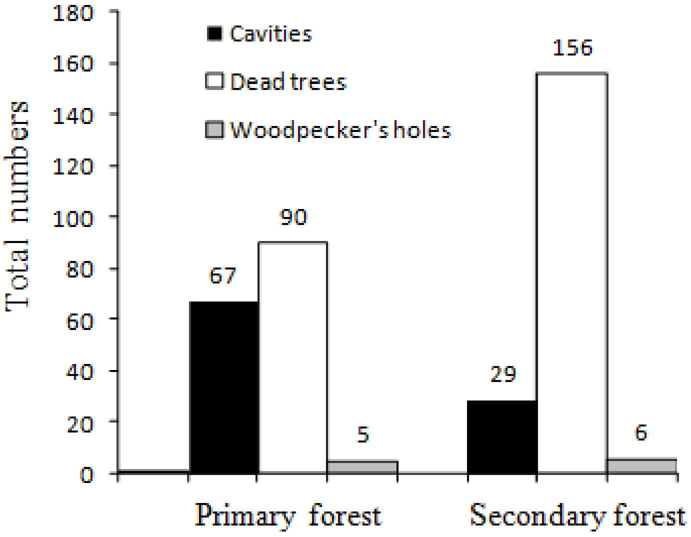

Figure 1. Numbers of cavities (natural cavities plus woodpecker's roles), numbers of dead trees or branches, and numbers of woodpecker's holes found pooled across five 0.5 ha plots in primary and mid-successional stage Atlantic Forest areas.

Table 1 - Average and standard deviation values for cavities measures in different successional stages.

Successional stage	Smaller entrance diameter	Internal diameter	Height above ground	DBH at nest height	Cavity depth
Primary	6.63 ± 3.9	12.93 ± 6.91	182.29 ± 118.51	36.02 ± 18.92	36.14 ± 40.98
Mid-successional	4.79 ± 2.32	10.94 ± 5.20	194.15 ± 101.45	23.76 ± 7.29	13.29 ± 34.21

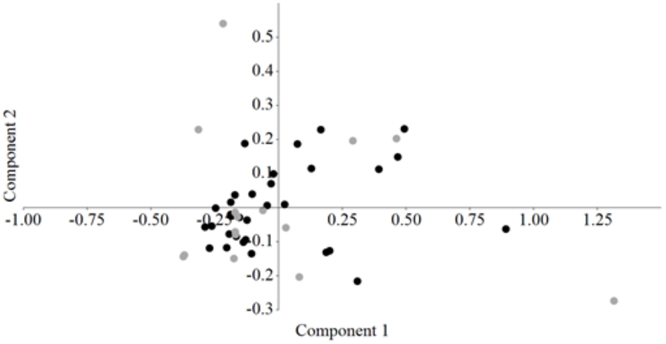

Figure 2. Principal Component Analysis (PCA) evidencing the distribution of cavities based on their measurements (tree diameter at cavity height, smaller entrance diameter, internal diameter, and depth). The new scores distributed between the two main axes did not reveal any clustering tendency between primary (black dots), and mid-successional stage (gray dots) Atlantic Forest areas.

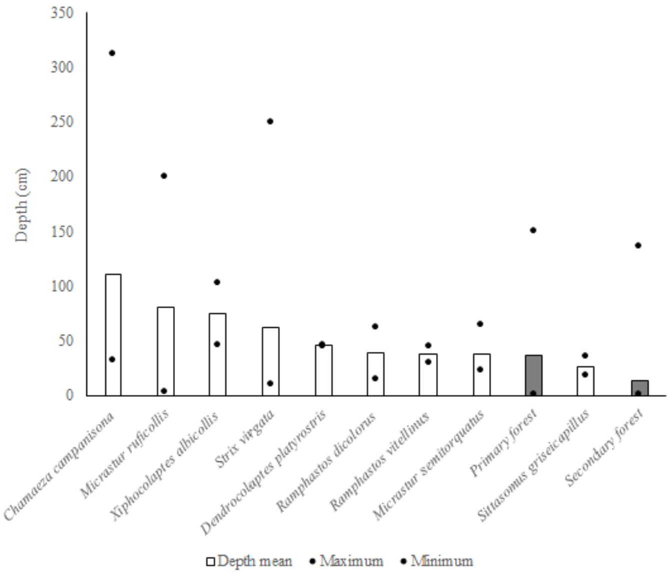

Figure 3. Comparisons of cavity nests depth found for bird species occurring in PECB, and depths found for cavities available in primary and mid-successional stage Atlantic Forest areas (Lange 1967, Lill 1968, Thorstrom et al. 1990, 2000, Thorstrom 2001, Gerhardt 2004, Cockle & Bodrati 2009, Jesus & Mikish 2009, Bodrati et al. 2012, Jesus et al. 2012, Cockle & Bodrati 2013, Holt et al. 2016).

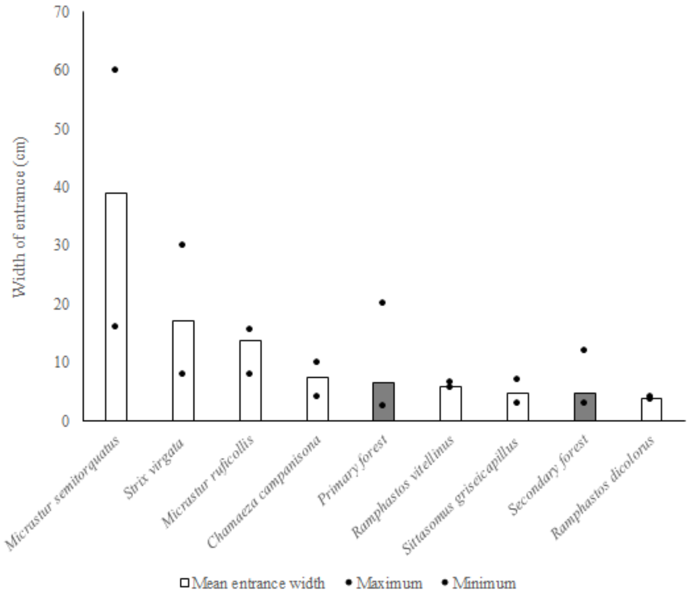

Figure 4. Comparisons of the smaller entrance diameters of cavities used by bird species occurring in PECB, and diameters found for cavities available in primary and mid-successional stage Atlantic Forest areas (Lange 1967, Lill 1968, Thorstrom et al. 1990, 2000, Thorstrom 2001, Gerhardt 2004, Cockle & Bodrati 2009, Jesus & Mikish 2009, Bodrati et al. 2012, 2014, Jesus et al. 2012, Cockle & Bodrati 2013, Holt et al. 2016).

Discussion

Our main prediction of reduced number of cavities in mid-successional stage areas was corroborated and our data suggested that only advanced successional stage, or primary areas, may present the density of cavities expected for natural Atlantic Forest habitats. Notably, the Brazilian legislation (Ibama 1994) do not make any distinction between advanced successional stage and primary Atlantic Forests, then both must be representative of the original conditions. To our knowledge, this is the first work to provide evidences for such successional effect in a Neotropical humid forest, and it is consistent with studies carried out in Europe and Thailand where the number of cavities was higher in older conifer forests and mixed deciduous forest, respectively (Van Balen et al. 1982, Pattanavibool & Edge 1996). Hypotheses that attempt to explain the greater number of cavities in mature forests involve the greater biodiversity, greater structural complexity, and higher forest productivity (Cornelius et al. 2008).

The greater number of dead trees and branches in secondary sites was also consistent with other studies, not only from the Atlantic Forests (Aleixo 1999, Carvalho et al. 2007), but also from temperate forests (Kirby et al. 1998). According to Ibama (1994) and Carvalho et al. (2015), in mid-succesional regeneration stage the fast growing pioneer trees die as they get replaced by primary species during the ecological succession process, which may explain the greater amount of dead trees in this type of secondary formation (Kirby et al. 1998, Carvalho et al. 2015). Further, the decomposition of dead substrate must be faster in primary areas due to increased humidity and diversity of decomposers, which reduces the number of decaying structures (Cornelius et al. 2008). As woodpecker's nests were always constructed in decaying substrate, the lack of difference in the proportion of natural versus woodpecker's nests between successional stages was unexpected, and may be explained by the low representativeness of woodpecker's cavities in general. In North America, for instance, woodpeckers were responsible for constructing 77% of the available cavities (for a review, see Cockle et al. 2011b), which is much more than the 28.1% (pooled between habitats) observed here. Then, the differences in cavities availability between habitats observed here involved mostly the numbers of natural holes in live trees, and they may be more common in the largest and older trees found in primary forests.

Although cavity characteristics have not diverged significantly between habitats, our graphical comparisons revealed that hollows in primary habitats exhibited the minimal depth and entrance diameter requirements for birds nesting, including the largest cavity-nester species present in the PECB, such as hawks, toucans and owls. In the mid-succesional stage sites, on the other hand, entrance diameter seemed to be a restrictive characteristic for the largest cavity nester birds.

It is important to note that, although most of the bird species considered in our comparisons could theoretically nest in cavities from both successional stages, here we have not considered other important parameters that are often involved in nesting habitat and nesting site choice by birds, for instance, canopy closure, and vegetation density (Cockle et al. 2015). Then, based on cavities characteristics alone our analyses certainly tend to overestimate the usefulness of the cavities.

In general, our data revealed that in the recovery area we studied, the communities of cavity-nester birds may be more affected by the reduced cavity availability than by cavities quality, as the number of cavities in mid-succesional stage sites was less than half than that found in mature forests. We also provide evidences that the largest cavity nesters, such as the biggest hawks, might have limited nesting possibilities in mid-seccessional stage areas. Management strategies currently used to overcome these problems involve the distribution of nest boxes and the enlargement of the entrances of part of the available cavities (Kuniy et al. 2006, Smallwood et al. 2009, Olah et al. 2014). In face of the evidences provided here, we suggest that these strategies might be considered for the preservation of the guild of Atlantic Forest cavity-nester birds in areas classified in mid- or lower successional stages.

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Author contributions

Michele Viana Katayama: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Paulo Victor Queijo Zima: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intelectual content.

Daniel Fernandes Perrella: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to critical revision, adding intelectual content.

Mercival Roberto Francisco: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

Conflicts of interest

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

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Helminth parasites of Phyllodactylidae and Gekkonidae lizards in a Caatinga ecological station, northeastern Brazil

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Abstract: We investigated the parasites of five lizard species belonging to Phyllodactylidae (*Phyllopezus pollicaris* and *Gymnodactylus geckoides*) and Gekkonidae (*Hemidactylus agrius*, *Lygodactylus klugei* and *Hemidactylus brasilianus*) families in a semiarid region of Brazil. Six nematode species were identified: *Parapharyngodon alvarengai* and *Spauligodon oxkutzcabiensis* (Pharyngodonidae), *Physaloptera lutzi* (Physalopteridae), *Skrjabinelazia intermedia* (Seuratidae), *Trichospirura* sp. (Rhabdochonidae) and *Piratuba* sp. (Onchocercidae), and a cestode species, *Oochoristica* sp. (Linstowiidae). The most prevalent species were *Spauligodon oxkutzcabiensis*, which infected *P. pollicaris* (75%), and *Parapharyngodon alvarengai*, which infected *G. geckoides* (29%). South American lizards were identified as being new hosts for the *Trichospirura* genus (a usual parasite of mammals), and there were 16 new occurrences of parasite species in the five lizard species studied herein.

Keywords: *Squamata, Nematoda, Cestoda, geckos, semiarid*

Helmintos parasitas de lagartos Phyllodactylidae e Gekkonidae em estação ecológica na Caatinga, nordeste do Brasil

Resumo: Nós investigamos os parasitas de cinco espécies de lagartos pertencentes às famílias Phyllodactylidae (*Phyllopezus pollicaris* e *Gymnodactylus geckoides*) e Gekkonidae (*Hemidactylus agrius*, *Lygodactylus klugei* e *Hemidactylus brasilianus*) em região semiárida do Brasil. Seis espécies de nematoides foram encontrados: *Parapharyngodon alvarengai* e *Spauligodon oxkutzcabiensis* (Pharyngodonidae), *Physaloptera lutzi* (Physalopteridae), *Skrjabinelazia intermedia* (Seuratidae), *Trichospirura* sp. (Rhabdochonidae), *Piratuba* sp. (Onchocercidae) e uma espécie de cestódeo, *Oochoristica* sp. (Linstowiidae). As espécies de maiores prevalências foram *S. oxkutzcabiensis*, a qual infectou *P. pollicaris* (75%) e *P. alvarengai* a qual infectou *G. geckoides* (29.%). Nós documentamos novo registro de hospedeiro para lagartos na América do Sul pertencente ao gênero *Trichospirura*, o qual é comum em mamíferos, e 16 novas ocorrências de espécies parasitas nas cinco espécies de lagartos aqui estudadas.

Palavras chave: *Squamata, Nematoda, Cestoda, geckos, semiárido*

Introduction

Helminths are parasites that infect the internal and external organs of most invertebrate and vertebrate groups (Fergunson 1942; Round 1968; Salgado-Maldonado et al. 2005; Hamann et al. 2006a, b; Ávila et al. 2012). Among this wide diversification of hosts, there is a great diversity of parasites in South American lizards (Ávila & Silva 2010), and several studies have recorded variation in parasite richness and diversity (Bursey & Goldberg 2004; Bursey et al. 2005; Anjos et al. 2013; Araujo-Filho et al. 2014; Brito et al. 2014a, b; Galdino et al. 2014; Sousa et al. 2014).

Knowledge of the lizard-associated helminth fauna has increased through research concerning (i) records of new hosts (Bursey & Goldberg 2004; Bursey et al. 2005; Ávila & Silva 2010; McAllister et al. 2011; Ávila et al. 2012), (ii) descriptions of new parasite species (Bursey et al. 2003; Pereira et al. 2012), and (iii) influence of biotic and abiotic variables on helminth diversity and abundance (Sharpilo et al. 2001; Brito et al. 2014a, b; Galdino et al. 2014).

Phyllodactylidae and Gekkonidae are two lizard families of the Gekkota clade that are phylogenetically closely related taxa (Sites et al. 2011). Both families occur in Brazil with, respectively, 13 and six nominal species (Costa & Bérnils 2015), and only *Gymnodactylus geckoides* Spix, 1825, *Phyllopezus periosus* Rodrigues, 1986, *Phyllopezus pollicaris* (Spix, 1825) (Phyllodactylidae), *Hemidactylus mabouia* Moreau de Jonnes, 1818, and *Hemidactylus agrius* Vanzolini, 1978 (Gekkonidae) occur within the Caatinga biome. *Hemidactylus brasilianus* (Amaral, 1935) and *Lygodactylus klugei* (Smith, Martin & Swain, 1977) (Gekkonidae) often inhabit the Caatinga biome (Vitt 1995; Rocha et al. 2011; Andrade et al. 2013). These species are sit-and-wait foragers, have nocturnal habits (except for the diurnal *L. klugei*) and an insectivore diet (Vitt 1995; Colli et al. 2003; Rocha & Rodrigues 2005; Mesquita et al. 2006; Sousa 2010; Recorder et al. 2012; Albuquerque et al. 2013; Passos & Rocha 2013; Passos et al. 2015). The studies reporting helminth parasitism in South America for both families have been conducted by Anjos et al. (2005), Ávila & Silva (2010), Ávila et al. (2010), Ávila & Silva (2013), Brito et al. (2014a), Sousa et al. (2014), Cazorla & Morales Moreno (2015) and Bezerra et al. (2016).

Parasites are good indicators of healthy ecosystems, which is essential to studies of conservation and maintenance of host populations (Marcogliese 2004, 2005), which may reflect the anthropic influence in the environment in which they reside (Hamman et al. 2006b). Thus, characterization of the parasite population of a certain area of the Caatinga biome is essential, especially for future studies on species conservation.

The current study characterizes the helminth richness of parasitic species and the parameters of parasitic infection (prevalence, mean intensity of infection and range) in lizards of the Phyllodactylidae and Gekkonidae families collected at the Aiuaba Ecological Station, northeastern Brazil.

Material and Methods

Lizards were collected at the Aiuaba Ecological Station (ESEC Aiuaba), municipality of Aiuaba, state of Ceará, northeastern Brazil (6°36'27"S and 40°08'00.9"W, 466m asl, datum SIRGAS 2000) (Figure 1). The sampled area is within the Caatinga biome, which is characterized by xerophytic plants, shrubs, thorny trees and open areas (Andrade-Lima 1981). The climate in this biome is semiarid, hot tropical, with an average annual rainfall of 562.4 mm, average temperature ranging from 24 ºC to 26 ºC and a rainy season from February to April (IPECE 2015).

We conducted manual collections through active searching over four sampling expeditions, two in 2014 (September and November) and two in 2015 (February and April) with authorization provided by the "Sistema de Autorização e Informação em Biodiversidade" (SISBIO order number 43753-1). The lizards were euthanized with a lidocaine lethal dose. Their snout-vent length (SVL) was measured with a digital caliper (± 0.01mm). Thereafter they were labelled, fixed with 10% formaldehyde and preserved in 70%

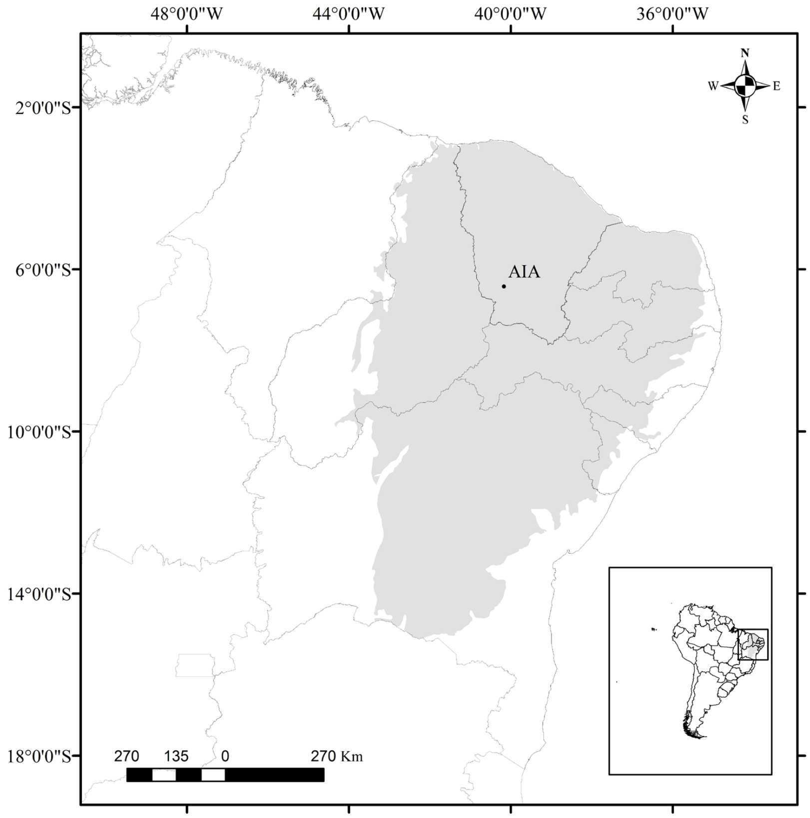

Figure 1. Geographic map featuring the Aiuaba Ecological Station (AIA), state of Ceará, northeastern Brazil. In gray, the Caatinga biome as conceived by IBGE (2004).

ethyl alcohol. Lizard specimens were deposited in the Herpetological Collection of the Regional University of Cariri, municipality of Crato, state of Ceará, Brazil. The hosts were dissected under a stereomicroscope and their body cavity, lung, stomach, small intestine and large intestine was analyzed in search of helminths. The lizard's gonads were analyzed to determine their gender and sexual maturity. We considered as mature males those with developed testicles and a convoluted epididymis, and as mature females those that had vitellogenic follicles in their oviducts and/or eggs in their ovaries.

The nematodes were cleared in a Hoyer solution and the cestodes were colored in Carmim and cleared in a Hoyer solution as well for identification (Everhart 1957). The parasites were mounted on temporary slides for identification, and their morphology was compared with specimens present in the parasitological collection of the Universidade Regional do Cariri – URCAP, and bibliographic reference on the description of the parasite species found that did not have specimens in the collection. Subsequently, they were deposited in the Parasitological Collection of the Regional University of Cariri (Appendix 1). The prevalence and the mean intensity of infection for each endoparasite species were calculated for each lizard species according to Bush et al. (1997).

Results

We sampled a total of 355 lizards specimens, distributed in the five focused species of this study. Phyllodactylidae: *Phyllopezus pollicaris*, 132 specimens, 57 males (SVL = 64.2 ± 8.4 mm) and 75 females (SVL = 62.5 ± 11.9 mm); *Gymnodactylus geckoides*, 71 specimens, 30 males (SVL = 37.7 ± 3.7 mm) and 41 females (SVL = 37.5 ± 4.1 mm). Gekkonidae: *Hemidactylus agrius*, 63 specimens, 28 males (SVL = 47.1 ± 3.8 mm) and 35 females (SVL = 47.8 ± 3.7 mm); *Lygodactylus klugei*, 65 specimens, 25 males (SVL = 27.5 ± 2.5 mm) and 40 females (SVL = 28.1 ± 2.4 mm); and *Hemidactylus brasilianus*, 24 specimens, 11 males (SVL = 45 ± 5.9 mm) and 13 females (SVL = 43.6 ± 5.7 mm). All lizards were in full sexual maturity.

We found six nematode species [*Parapharyngodon alvarengai* Freitas, 1957, *Spauligodon oxkutzcabiensis* (Chitwood, 1938), *Physaloptera lutzi* Cristofaro, Guimarães & Rodrigues, 1976, *Skrjabinelazia intermedia* Freitas, 1940, *Trichospirura* sp. Smith & Chitwood, 1967 and *Piratuba* sp. Lent & Freitas, 1941] and one cestode of the genus *Oochoristica* Lühe, 1898 that could not be identified at the species level (Table 1).

Helminth parasites found in this study have different life cycles. While *P. lutzi*, *Oochoristica* sp., *Piratuba* sp., *Trichospirura* sp. and *S. intermedia*

Table 1. Helminth parasites collected in lizards belonging to Phyllodactylidae and Gekkonidae families from the Aiuaba Ecological Station, municipality of Aiuaba, state of Ceará, northeastern Brazil. Number of Infected hosts = (NI), Prevalence = P (%), Mean Intensity of Infection = MII and Range (R). (*) New records of hosts.

Parasite	Location in host	Host (NI)	P (%)	MII (R)
NEMATODA				
Pharyngodonidae				
Parapharyngodon alvarengai	large intestine	*Phyllopezus pollicaris* (6)	4.54%	1.33 (1-2)
	large intestine	*Hemidactylus brasilianus**(2)	8.33%	1 (1)
	small intestine; large intestine	*Hemidactylus agrius* (8)	12.69%	1.87 (1-4)
	small intestine; large intestine	*Gymnodactylus geckoides* (21)	29.57%	1.66 (1-5)
Spauligodon oxkcutzcabiensis	small intestine; large intestine	*Phyllopezus pollicaris* (99)	75%	26.02 (270)
	large intestine	*Hemidactylus brasilianus** (2)	8.33%	5.5 (3-8)
	large intestine	*Hemidactylus agrius** (11)	17.46%	1.9 (1-4)
	large intestine	*Gymnodactylus geckoides* (1)	1.40%	18 (18)
	large intestine	*Lygodactylus klugei** (4)	6.15%	3 (1-9)
Physalopteridae				
Physaloptera lutzi	stomach; large intestine	*Phyllopezus pollicaris** (4)	3.03%	1.25 (1-2)
	stomach	*Hemidactylus brasilianus** (1)	4.16%	1 (1)
	stomach	*Hemidactylus agrius* (1)	1.58%	6 (6)
	stomach; large intestine	*Gymnodactylus geckoides* (9)	12.67%	2 (1-4)
	stomach	*Lygodactylus klugei* (1)	1.53%	1 (1)
Seuratidae				
Skrjabinelazia intermedia	small intestine; large intestine	*Phyllopezus pollicaris** (1)	0.75%	2 (2)
	large intestine	*Hemidactylus brasilianus** (4)	16.66%	1.25 (1-2)
	large intestine	*Hemidactylus agrius** (17)	26.98%	1.64 (1-4)
Rhabdochonidae				
Trichospirura sp.	gallbladder	*Phyllopezus pollicaris** (13)	9.84%	3.69 (1-11)
	gallbladder	*Hemidactylus brasilianus** (2)	8.33%	3.5 (2-5)
	gallbladder	*Hemidactylus agrius** (12)	19.04%	2.58 (1-7)
	gallbladder	*Gymnodactylus geckoides** (4)	5.63%	1.5 (1-3)
Onchocercidae				
Piratuba sp.	body cavity	*Gymnodactylus geckoides** (1)	1.40%	1 (1)
CESTODA				
Linstowiidae				
Oochoristica sp.	small intestine	*Phyllopezus pollicaris* (2)	1.51%	1.5 (1-2)
	small intestine	*Hemidactylus brasilianus** (1)	4.16%	2 (2)
	small intestine	*Hemidactylus agrius** (1)	1.58%	1 (1)

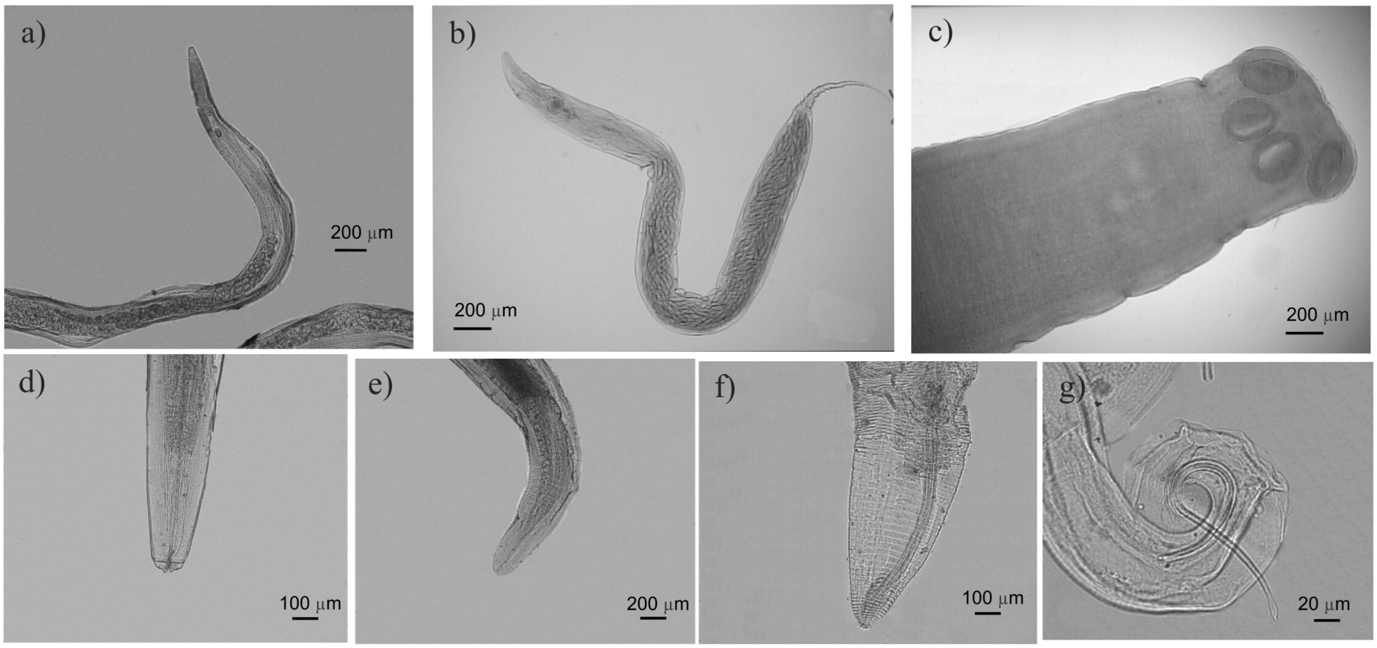

Figure 2. Helminths of the gastrointestinal tract of geckos from the Aiuaba Ecological Station (AIA), state of Ceará, northeastern Brazil: (a) *Skrjabinelazia intermedia* (posterior view); (b) *Spauligodon oxkutzcabiensis* (entire specimen); (c) *Oochoristica* sp. (anterior view); (d) *Physaloptera lutzi* (anterior view); (e) *Piratuba* sp. (anterior view); (f) *Parapharyngodon alvarengai* (anterior view); (g) *Trichospirura* sp. (posterior view).

have heteroxenic life cycles, which requires more than one host to complete its life cycle, *P. alvarengai* and *S. oxkutzcabiensis* have monoxenic life cycles, not requiring more than one host (Illgen–Wilcke et al. 1992, Anderson, 2000, Lhermitte et al. 2007) (Figure 2).

Discussion

Among the parasite species identified, there were 16 new records of hosts distributed among the five lizard species sampled in this study. We found three parasite species in *P. pollicaris*, four in *H. agrius*, two in *G. geckoides*, six in *H. brasilianus* and one in *L. klugei*.

Spauligodon oxkutzcabiensis was first recorded with *Thecadactylus rapicauda* (Houttuyn, 1782) (Phyllodactylidae). In South America, *S. oxkutzcabiensis* was recorded in association with the following lizards (Ávila et al. 2010; Goldberg et al. 2010; Goldberg & Bursey 2010; Sousa et al. 2014): *G. geckoides*, *Hemidactylus mabouia* (Moreau de Jonnès, 1818), *Microlophus occipitalis* (Peters, 1871), *Phyllodactylus reissi* (Peters, 1862), *P. inaequalis* Cope, 1876, *P. johnwrighti* Dixon & Huey, 1970, *P. microphyllus* (Cope, 1876), *Phyllopezus lutzae* (Loveridge, 1941) [= *Bogertia lutzae*] and *Tropidurus guarani* Alvarez, Cei & Scolaro, 1991 [= *T. spinulosus*]. *Spauligodon oxkutzcabiensis* was present in all five lizard species sampled in this research. It is, therefore, considered here as the lizard's generalist endoparasite for Phyllodactylidae and Gekkonidae, adding *H. agrius*, *H. brasilianus* and *L. klugei* to the list of new records of hosts.

Parapharyngodon alvarengai was recorded as being hosted by *Ameivula ocellifera* (Spix, 1825) [= *Cnemidophorus ocellifer*], *Ameiva ameiva* (Linnaeus, 1758), *Amphisbaena ridleyi* Boulenger, 1890, *Brasiliscincus heathi* (Schmidt & Inger, 1951), *G. geckoides*, *Phyllopezus pollicaris*, *P. periosus*, *Trachylepis atlantica* (Schmidt, 1945), *Tropidurus hispidus* (Spix, 1825), *T. semitaeniatus* (Spix, 1825) and *Hemidactylus agrius* Vanzolini, 1978 (Anjos et al. 2011; Ávila et al. 2012; Brito et al. 2014b). We observed *P. alvarengai* infecting *P. pollicaris*, *G. geckoides*, *H. agrius* and *H. brasilianus*.

Physaloptera lutzi infected all lizards sampled in the current study. Although it had been originally described as a parasite of *A. ameiva*, we recorded both *P. pollicaris* and *H. brasilianus* as new hosts for this species. Other records of hosts for *P. lutzi* were also reported in the literature (Anjos et al. 2011; Ávila et al. 2010, 2012; Brito et al. 2014b): *Ameivula abaetensis* (Dias, Rocha & Vrcibradic, 2002) [= *Cnemidophorus abaetensis*], *Ameivula littoralis* (Rocha, Araújo, Vrcibradic & Costa, 2000) [= *Cnemidophorus littoralis*], *A. ocellifera*, *Enyalius bilineatus* Duméril & Bibron, 1837, *Eurolophosaurus nanuzae* (Rodrigues, 1981), *Liolaemus alticolor* Barbour, 1909, *L. ornatus* Koslowsky, 1898, *L. quilmes* Etheridge, 1993, *Tropidurus guarani*, *T. semitaeniatus*, *T. hispidus*, *T. itambere* Rodrigues, 1987, *T. torquatus* (Wied, 1820), *H. agrius*, *L. klugei*, *G. geckoides*, *Micrablepharus maximiliani* (Reinhardt & Lütken, 1862) and *Salvator merianae* (Duméril & Bibron, 1839) [= *Tupinambis merianae*]. Given that *P. lutzi* has a heteroxenic life cycle, its intermediate host is probably a food item that is consumed by all sampled lizards in our study area.

Regarding the *Trichospirura* genus, our data revealed *P. pollicaris*, *G. Geckoides*, *H. agrius* and *H. brasilianus* lizards as new host records for South America. Only primates from the following genera had been previously recorded as hosts for *Trichospirura*: *Callicebus* Thomas, 1903 (Orihel & Seibold 1971; Pacheco et al. 2003), *Callithrix* Erxleben, 1777 (Smith & Chitwood 1967; Resende et al. 1994), *Saimiri* Voigt, 1831 (Orihel & Seibold 1971), *Aotus* Illiger, 1811 (Orihel & Seibold 1971), *Callimico* Miranda Ribeiro, 1922 (Orihel & Seibold, 1971) and *Saguinus* Hoffmannsegg, 1807 (Cosgrove et al. 1968).

The *Skrjabinelazia* genus was found infecting geckos (Lhermitte et al. 2007; Anjos et al. 2011). Nevertheless, records for *S. intermedia* were restricted so far to a teid *Ameivula nativo* (Rocha, Bergallo & Peccinini–Seale, 1997) [= *Cnemidophorus nativo*] (Menezes et al. 2004), twice in Tropiduridae [*Tropidurus torquatus* and *T. guarani*, Vicente et al. 1993] and once for Dactyloidae *Dactyloa punctata* (Daudin, 1802) [= *Anolis punctatus*] (Ávila & Silva 2010). Our data provided three host records for *S. intermedia*: *P. pollicaris*, *H. agrius*, and *H. brasilianus*.

Little is known about the biology of the genus *Oochoristica*. Most studies on the genus were limited to describe new species and record new hosts (Ávila & Silva 2010; Brito et al. 2014b; Sousa et al. 2014). Our study reports three records of hosts (*P. pollicaris*, *H. agrius* and *H. brasilianus*) with two new infection records for the genus *Oochoristica*.

Piratuba is a genus that belongs to the Onchocercidae family. It has a widespread distribution throughout South America, in which other studies reported that lizards from the genera *Tropidurus*, *Kentropyx* Spix, 1825, *Plica* (Linnaeus, 1758), *Polychrus* Merrem, 1820, *Dactyloa* (Daudin, 1804) [= *Anolis*] and *Ameiva* Meyer, 1795 were infected by parasites of the referred genus (Ávila & Silva 2010). We recorded a specimen *Piratuba* sp. infecting *Gymnodactylus geckoides*, which corresponds to a new record for *Piratuba*.

Currently, there are 23 genera of helminths recognized as parasites of the lizard families Phyllodactylidae and Gekkonidae (Anjos et al. 2005; Ávila & Silva 2010; Ávila et al. 2010; Avila & Silva 2013; Brito et al. 2014a; Sousa et al. 2014; Cazorla & Morales Moreno 2015; Bezerra et al. 2016; this study); for the Caatinga biome, about 10 species of helminths have already been reported with a prevalence above 50% (Anjos et al. 2005; Avila et al. 2012; Brito et al. 2014b; Sousa et al. 2014; this study), and there were four helminth species with prevalences below 60% for deserts and coastal areas in Peru (Goldberg & Bursey 2010); two species had prevalences of 33.3% in the Brazilian Amazon forest (Ávila & Silva 2013); two species had prevalences up to 22.8% in the Atlantic Forest (Ávila et al. 2010; Almeida-Gomes et al. 2012); and three helminth species had prevalences below 15% for the tropical thorny mountain areas of Venezuela (Cazorla & Morales Moreno 2015). Notwithstanding that some genera and species of helminths were previously reported for different biomes as parasites of geckos, as *S. oxkutzcabiensis* and all species of the genera *Oochoristica* and *Parapharyngodon*, almost all prevalences registered for them were below that obtained for the Caatinga biome [e.g., *Oochoristica* sp. and species of the genus *Parapharyngodo*n presented up to 55% prevalence, while *S. oxkutzcabiensis* did not exceed 8% in coastal environments of the tropical thorny mountain areas in Peru and Venezuela (Goldberg & Bursey 2010; Cazorla & Morales Moreno 2015)]. These results suggest that the different environmental conditions of distinct biomes may favor one parasitic species over another, such as *S. oxkutzcabiensis*. The presence of *Trichospirura* in lizards of the genus *Sceloporus* in Mexico and *H. brookii haitianus* Meerwarth, 1901 in Hispaniola (Goldberg et al. 2003; Powell et al. 1990), suggests that the new host records documented here may not be species-specific of the studied biome. Therefore, additional studies are necessary in the different biomes to determine the true helminth diversity of these two families of lizards.

The current study increases to 16 the number of new host records in association with the families Phyllodactylidae and Gekkonidae in the Caatinga biome. However, the development of new research on other areas of the biome is essential to enhance our scientific knowledge of the diversity of helminths associated with lizards.

Supplementary material

The following online material is available for this article:

Appendix 1: Voucher specimens of helminth parasites and respective host lizards collected in the Aiuaba Ecological Station, northeastern Brazil.

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Author Contributions

Adonias Aphoena Martins Teixeira: contribution to data collection; contribution to identification of parasites and lizards; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Antonio Marcos Alves Pereira: contribution to data collection; contribution to identification of parasites and lizards; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Diêgo Alves Teles: contribution to data collection; contribution to identification of parasites and lizards; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

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Vandeberg Ferreira Lima: contribution to data collection; contribution to identification of parasites and lizards; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Waltécio de Oliveira Almeida: contribution to data collection; contribution to identification of parasites and lizards; contribution to data analysis and interpretation; contribution to manuscript preparation and critical revision.

Conflicts of interest

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

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inventory

Inventory of benthic marine and estuarine algae and Cyanobacteria for Tabasco, México

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Abstract: Studies of benthic marine and estuarine algae as well as Cyanobacteria are in their early stages in the littoral of the state of Tabasco. Sixty seven taxa of seaweeds are currently known from Tabasco. The inventory of these organisms was made based on samples obtained in six localities, three of them in marine and three in estuarine environments. We found 147 species and subspecific taxa with Rhodophyta having the greatest number with 84, followed by Chlorophyta 44, and finally Phaeophyceae 19. In addition, the 26 species of Cyanobacteria collected are recorded for the first time for the coast of Tabasco since there are no previous reports. The presence of 115 species of benthic marine and estuarine algae is reported for the first time for Tabasco. Most of the new records are Rhodophyta (53.9%), followed by Chlorophyta (29.5%) and Phaeophyceae (16.6%). Of the 115 new records of algae for Tabasco, *Gayliella fimbriata* and *Grateloupia subpectinata* are new records also for the Mexican Atlantic. The highest number of species was recorded for Sanchez Magallanes breakwater, while the lowest number of taxa was recorded for Mecoacan Lagoon. According to the Feldmann and Cheney indexes the algal flora of the coast of Tabasco is tropical. The greatest diversity was found during the rainy season.

Keywords*: Tabasco, Mexico, new records, marine, estuarine, algae, Cyanobacteria.*

Inventário de algas bentônicas marinhas e estuarinas e cianobactérias para Tabasco, México

Resumo: Estudos sobre algas marinhas, estuarinas e cianobactérias para o litoral do estado de Tabasco encontram-se apenas nos estágios iniciais. Neste estudo, um inventário desses organismos foi feito com base em amostras obtidas em seis localidades, três delas marinhas e três em ambientes estuarinos. Os resultados indicaram a presença de 147 táxons, com Rhodophyta apresentando maior diversidade (84), seguido de Chlorophyta (44) e, finalmente, Phaeophyceae (19). Além disso, 26 espécies de Cyanobacteria são registradas pela primeira vez para a costa de Tabasco. A presença de 115 espécies de algas marinhas e estuarinas bentônicas é relatada peloa primeira vez para Tabasco. A maioria dos novos registros são de Rhodophyta (53.9%), seguido por Chlorophyta (25.9%), e Phaeophyceae (16.6%). Dos 115 novos registros de algas para Tabasco, *Gayliella fimbriata* e *Grateloupia subpectinata* são novos registros para a costa atlântica do México. O maior número de espécies foi localizado no paredão de Sanchez Magallanes, enquanto o menos número de táxons foi localizado na Lagoa Mecoacán. De acordo com os índices de Feldmann e Cheney, a flora de algas da costa de Tabasco é tropical, e a maior diversidade foi encontrada durante a estação chuvosa.

Palavras-chave*: Tabasco, México, registros novos, marinhos, estuarinos, algas, cianobactérias.*

Introduction

The Gulf of México is characterized by a relatively shallow but well developed continental shelf with an extensive system of generally deep, hard banks of varying origin and composition (Fredericq et al. 2009). In spite of the extension of the Gulf of Mexico coast, in the Mexican portion it has been scarcely studied from the phycological point of view. The phycological literature (Ortega et al. 2001; Wynne 2017) highlights the limited records for the coast of Tabasco. Orozco-Vega & Dreckmann (1995) reported six taxa for the Laguna Mecoacan, an estuarine environment; and Ramirez (1996) recorded 24 species of red algae (Rhodophyta) for the Sanchez-Magallanes breakwater. On the other hand, Dreckmann & De Lara (2000) described the vegetative and reproductive structures of *Gracilaria caudata* (Rhodophyta) for Laguna Mecoacan. Sentíes & Dreckmann (2013) updated information about the marine and estuarine algae for Tabasco, and recorded 50 taxa of algae for the Tabasco littoral. Recently, Quiroz-González et al. (2017) reported 17 Chlorophyta as new records for this coast. Of the five publications mentioned above, the most complete is that of Senties & Dreckmann (2015), who published an inventory of marine and estuarine algae recorded until 2000 for the coast of Tabasco; also sampled in four localities: Playa El Bellote, Playa El Cangrejo, Laguna Mecoacan and

Sánchez Magallanes breakwater, recording 50 taxa of algae, of which Rhodophyta had the highest number (38), followed by Phaeophyceae (7) and Chlorophyta (5). Quiroz-González et al (2017) carried out several collections in seven localities: Playa Miramar, Pico de Oro, Playa Paraiso, Poblado Chiltepec, Playa de Dos Bocas, Playa Las Brisas and Sanchez Magallanes breakwater; their study was based exclusively on Chlorophyta and the authors reported 17 new records of this group for the coast of Tabasco. In conclusion, it is evident that the length of the coast of Tabasco has not been equally inventoried and there are even portions which have not been studied at all, and the different groups of marine and estuarine algae have not been equally studied either.

Collectively, our knowledge about the cyanobacteria of the Tabasco coast is also limited. Cyanobacteria have been omitted in most studies conducted on the Mexican Atlantic benthic algae. Among the few reports that have been published is that of Mateo-Cid et al. (2013).

The aims of this study are to provide an updated revision of the diversity and distribution of the benthic marine and estuarine algae and Cyanobacteria of three new localities of the coast of Tabasco (San Pedro Centla, Laguna La Machona, La Machona), besides Sánchez Magallanes breakwater, Playa Paraíso breakwater and Laguna Mecoacan. We include records of new algae and cyanobacteria for Tabasco, as well as data about their distribution and temporal variation during the dry, rainy summer and rainy winter seasons (nortes). Using the Feldmann and Cheney indexes, the phycoflora of the coast of Tabasco will be classified as a function of the latitudinal gradient, and thus establish the basis for future taxonomic, ecological and biogeographic studies.

Materials and methods

1. Study site and collections

The shoreline of the state of Tabasco is between 092˚ 28 ‘and 094˚ 10’ W, and 17˚15’ and 18˚ 39’ N, located in the southeast meso-region of Mexico, bounded on the north by the Gulf of Mexico (Figure 1); the south by the coastline of Tabasco; to the east northeast by the state of Campeche; and to the west by the state of Veracruz (Hernández-Santana et al., 2008). The Tabasco coastline presents a morphological predominance of low sandy beaches. The coast of Tabasco has a warm humid weather type Am (f): one with a higher annual average temperature of 22 ° C. Rains occur in the summer months and the precipitation of the driest month is less than 60 mm; the percentage of winter rain is higher than 10.2% of the annual total (García y Vidal-Zepeda, 1990a), with minimum extreme values between 20 and 22 ° C in January, and maximum between 30 and 34 ° C in May (García y Vidal-Zepeda, 1990b). Sampling was carried out in the littoral of Tabasco in August and November 2015 and in April and June 2016. The collections were made in the intertidal zone, 0.5 to 1.5 meters deep for sampling sites were chosen by the type of substrate and of access to the localities. A total of 120 samples was collected at six localities: Sanchez Magallanes breakwater (marine environment), La Machona (marine environment), Playa Paraiso breakwater (marine environment), Laguna La Machona, Laguna Mecoacan and Pedro Centla (these three localities of estuarine environment) (Figure 1). Data on the GPS coordinates, type of substrate, depth and date of collection in which organisms were obtained are shown in Table 1. Algae were preserved in a 5% formalin/sea water solution. Small fragments were cut manually with a double-edged blade, and stained with aniline blue and hematoxilin-eosine for anatomical observations and measurements. All specimens of red and brown macroalgae were carefully checked under the microscope for epiphytes. Using a stereoscopic microscope Zeigen HG571405 and an optical microscope OLYMPUS CX3 epiphytic algae were found growing on macroalgae as *Gracilaria, Grateloupia, Cladophora* and *Chnoospora*, among others. Thalli were thoroughly reviewed from the base to the apical portion locating filamentous epiphytes and articulated or fleshy macrophytes. After that using a double- edged blade, longitudinal scrapings of 2 cm of each thallus were performed in order to locate microscopic algae (Mateo-Cid et al., 2013).

Semi-permanent slides were prepared using corn syrup/water 1:1 with a trace of phenol added to prevent fungal growth. The identification at the

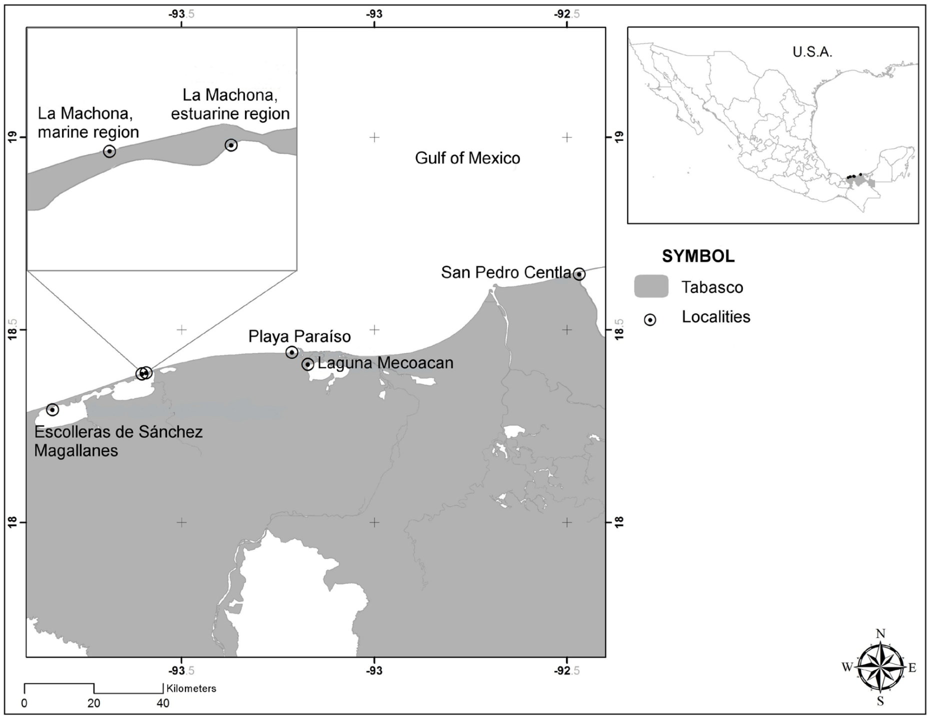

Figure 1. Map of the study area and sampling stations.

Table 1. Location of sampling sites, substrate, environment, depth and collection dates.

Locality	GPS Coordinates	Substrate	Environment	Depth of collection	Collection dates
1. Sanchez Magallanes breakwater	18º17’26” N, 93º50’03” W	Rocks and artificial substrates	Marine	0-1 m	August and November, 2015; April and June 2016
2. La Machona	18º38’51” N, 93º60’61” W	Rocks	Marine	0 m	November 2015, April and June 2016
3. Laguna La Machona	18º17’56” N, 93º51’03” W	Aquatic vascular plants and artificial substrates	Estuarine	0-1 m	November 2015, April and June 2016
4. Playa Paraíso breakwater	18º23’33” N, 93º12’52” W	Rocks	Marine	0 m	August and November, 2015; April and June 2016
5. Laguna Mecoacan	18º24’37” N, 93º10’21” W	Rocks and artificial substrates	Estuarine	0-2 m	November 2015, April and June 2016
6. San Pedro Centla	18º38’41” N, 92º28’07” W	Cement floor and artificial substrates	Estuarine	0-1 m	November 2015, April and June 2016

specific level was based mainly on Taylor (1960), Schneider & Searles (1991), Littler & Littler (2000), Cho et al. (2008), Dawes & Mathieson (2008) and Won et al. (2009). For the determination of Cyanobacteria Gomont (1892), Anagnostidis & Komárek (1988), Komárek & Anagnostidis (1995), and Komárek et al. (2014) were used. The sequence of the floristic list follows the order proposed by Komárek (2010) for Cyanobacteria, Wynne (2017) and Guiry & Guiry (2017) for red, brown and green algae. Most of the material analyzed is deposited and available for study in the phycological section of “Herbario Escuela Nacional de Ciencias Biológicas at the Instituto Politécnico Nacional, Mexico”.

For the study of descriptive phytogeography, Feldmann (1937) proposed the R / P index (number of Rhodophyta species divided by the number of Phaeophyceae species) useful for classifying the flora of a given region as a function of the latitudinal gradient. Thus, a value of the ratio R / P> 4 is characteristically found in tropical regions, while R / P <2 corresponds with a phycoflora of cool temperate regions. On the other hand, Cheney (1977) included the Chlorophyta (C) based on the one previously mentioned: (R + C) / P and verified that values of the ratio (R + C) / P> 6 are obtained in tropical floras, while those in temperate-cold seas are <3.

Results

1. New records of algae and cyanobacteria for Tabasco

A total of 173 taxa was identified, 84 of them are Rhodophyta, 44 Chlorophyta, 19 Phaeophyceae as well as 26 Cyanobacteria. The taxa identified from our collections are listed in Table 2 with sampling sites, seasonality, marine or estuarine environment and type of substrate where the organisms were collected, where the reference comes from, either from bibliography or collected by the authors, observations on new records for Tabasco and the Atlantic coast of Mexico and herbarium number. Many of the new algae records located in this study are epiphytes and they are not reported frequently in the floristic lists of Mexican coast of Gulf of Mexico (Ortega et al. 2001, Mateo-Cid et al. 2013, Senties & Dreckmann 2013, Wynne 2017).

Two red algae, *Gayliella fimbriata* (Setchell & N.L. Gardner) T.O. Cho & S.M. Boo and *Grateloupia subpectinata* Holmes are new records for the Atlantic coast of Mexico. Twenty six Cyanobacteria, 62 Rhodophyta, 18 Phaeophyceae and 34 Chlorophyta are new records for Tabasco. These new records are indicated in Table 2. The following families were the best represented in number of species in the study area: Rhodomelaceae (14), Ceramiaceae (12), Gracilariaceae (7), Cladophoraceae (19), Bryopsidaceae (8) and Ulvaceae (7). These families collectively include 45.57% of the total floristic diversity registered. The highest species richness was recorded in sampling sites with rocky substrate. The locality with the greatest diversity of species and subspecific taxa corresponds to the Sanchez Magallanes breakwater with 123 species, followed by the Playa Paraíso breakwater with 75, Laguna La Machona 35, La Machona 31, San Pedro Centla 26 and finally Laguna Mecoacan with 20 species (Figure 2).

Species and subspecific taxa such as *Jania ungulata* f. *brevior* Yendo, *Centroceras gasparrinii* (Meneghini) Kützing, *Spyridia clavata* Kützing, *Polysiphonia scopolorum* var. *villum* (J. Agardh) Hollenberg, *Peyssonnelia armorica* (P.L. Crouan & H.M. Crouan) Weber-van Bosse, *Hecatonema floridanum* (W.R. Taylor) W.R. Taylor, *Herponema tortugense* (W.R. Taylor) W.R. Taylor, *Rosenvingea orientalis* (J. Agardh) Børgesen, *Blidingia minima* (Nägeli ex Kützing) Kylin, *Cladophora crispula* Vickers and *Pseudobryopsis blomquistii* Diaz-Piferrer are considered unusual because few records are in the floristic lists of the Mexican coast of the Gulf of Mexico (Ortega et al. 2001, Mateo-Cid et al. 2013, Senties & Dreckmann 2013, Wynne 2017).

Thirty of the 67 species previously recorded for Tabasco (Orozco-Vega & Dreckmann 1995, Ramirez 1996, Dreckmann & De Lara-Isassi 2000, Senties & Dreckmann 2013, Quiroz-González et al., 2017) were found in this study. The other 115 species (table 2) are considered new records for Tabasco.

New records for the Atlantic coast of Mexico

Gayliella fimbriata and *Grateloupia subpectinata* of the coast of Tabasco, Mexico were recorded for the first time.

Description

Gayliella fimbriata (Setchell & N.L. Gardner) T.O. Cho & S.M. Boo (Figures 3a-d)

Type locality: Eureka, near La Paz, Lower California Mexico (Setchell & Gardner 1924: 777).

Basionym: *Ceramium fimbriatum* Setchell & N.L. Gardner 1924

Representative specimens examined: Sánchez Magallanes (Mendoza González & García López, 26.viii.2015, ENCB 22091, Vegetative).

Habit and anatomy: Thalli are 0.5–1.0 cm high, consisting of prostrate axes giving rise to erect axes (Figure 2). Erect axes bear forcipate incurved and complanate apical regions, main filaments 85-95 µm diameter at the nodes. Axial cells are spherical to cylindrical. The acropetal corticating filaments are 3–4 cells long, while the basipetal ones are 2–3 cells long. Branches are regularly alternate. Gland cells usually develop from cortical cells of acropetally and rarely basipetally corticating filaments, becoming strongly protruding, and are clavate, 60-65 µm x 30-34 µm.

Reproductive thalli were not found in our collections.

Comments: A particularly interesting case is the finding of *Gayliella fimbriata*, because this species was first described by Setchell and Gardner (1924) for the Bay of La Paz (Gulf of California, Mexico) as *Ceramium fimbriatum* and was subsequently transferred by Cho et al. (2008) to *G. fimbriata*. The presence of characteristic gland cells on the cortical cells and the dimensions of thalli agree with those recorded in specimens from La Paz, BCS, Mexico (Setchell & Gardner, 1924, Dawson, 1962).

Table 2. Marine and estuarine algae and Cyanobacteria of the coast of Tabasco (The abbreviations are explained at the end of the table).

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
CYANOBACTERIA							
Cyanophyceae							
Oscillatoriales							
Oscillatoriaceae							
1. *Blennothrix lyngbyacea* (Kützing ex Gomont) Anagnostidis et Komárek	1,6	W, S, R	M,E	Epi, As	A	NRT	21883
2. *Lyngbya confervoides* C. Agardh ex Gomont	1,4,6	W,S, R	M,E	As	A	NRT	21769 21963 21992
3. *L. majuscula* Harvey ex Gomont	1,3	R	M,E	Epi, Avp	A	NRT	21937 21962
4. *L. salina* Kützing ex Gomont	1	W, R	M	Epi	A	NRT	21968
5. *L. semiplena* J. Agardh ex Gomont	1,6	W, R	M,E	Epi	A	NRT	22010 22014
Homoeotrichaceae							
6. *Ammatoidea aegaea* Anagnostidis & Pantazidou	1	R	M	Epi	A	NRT	22014
Phormidiaceae							
7. *Coleofasciculus chthonoplastes* (Thuret ex Gomont) M. Siegesmund, J.R. Johansen & T. Friedl	5, 6	W	E	R, Epi	A	NRT	22173
Nostocales							
Aphanizomenonaceae							
8. *Nodularia harveyana* Thuret ex Bornet & Flahault	1	R	M	Epi	A	NRT	22014
Rivulariaceae							
9. *Calothrix confervicola* C. Agardh ex Bornet & Flahault	1	R	M	R, Epi	A	NRT	21880
10. *C. parietina* Thuret ex Bornet & Flahault	1,2,4	S, R	M	Epi	A	NRT	21921
Scytonemataceae							
11. *Scytonematopsis crustacea* (Thuret ex Bornet & Flahault) Koválik & Komárek	1,4	R	M	Epi	A	NRT	21910
Pseudanabaenales							
Pseudanabaenaceae							
12. *Leptolyngbya marina* (N.L. Gardner) Anagnostidis	1,2,3,4,6	W, R	M,E	Epi	A	NRT	21875 22111
Chroococcales							
Chroococcaceae							
13. *Chroococcus ercegovicii* Komárek & Anagnostidis	6	W	E	Epi	A	NRT	22173
14. *C. turgidus* (Kützing) Nägeli	1	S, R	M	Epi	A	NRT	22014
15. *Pseudocapsa maritima* Komárek	6	W	E	Epi	A	NRT	22173
Pleurocapsales							
Dermocarpellaceae							
16. *Cyanocystis hemisphaerica* (Setchell & N.L. Gardner) Kaas	1	R	M	Epi	A	NRT	21991
17. *Stanieria sublitoralis* (A. Lindstedt) Anagnostidis & Pantazidou	1,2	S, R	M	Epi	A	NRT	21921
Entophysalidaceae							
18. *Entophysalis conferta* (Kützing) F.E. Drouet & W.A. Daily	1,2,3,4,6	W, S, R	M,E	Epi	A	NRT	21772 21922
Xenococcaceae							
19. *Xenococcus cladophorae* (Tilden) Setchell & N.L. Gardner	3,5	W	E	Epi	A	NRT	22160
20. *X. minimus* Geitler	1	R	M	Epi	A	NRT	22311
21. *X. gilkeyae* Setchell & N.L .Gardner	1	W, R	M	Epi	A	NRT	21906
22. *X. pyriformis* Setchell & N.L. Gardner	5	W	E	Epi	A	NRT	21995

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
Synechococcales							
Chamaesiphonaceae							
23. *Chamaesiphon rostafinskii* Hansgirg	1	R	M	Epi	A	NRT	22111
Merismopediaceae							
24. *Aphanocapsa litoralis* Hansgirg	1	S, R	M	Epi	A	NRT	22152
25. *A. marina* Hansgirg	6	R	E	Epi	A	NRT	22117
26. *Synechocystis minuscula* Woronichin	1,6	W, R	M,E	Epi	A	NRT	21998
RHODOPHYTA							
Bangiophyceae							
Bangiales							21906
Bangiaceae							21996 22133
27. *Bangia atropurpurea* (Mertens ex Roth) C. Agardh	1,3,5	W, R	M,E	As, R	A	NRT	
28. *B. fuscopurpurea* (Dillwyn) Lyngbye	6	W, S	E	As	A	NRT	21766 21848 22071
Compsopogonophyceae							
Erythropeltales							
Erythrotrichiaceae							
29. *Erythrotrichia carnea* (Dillwyn) J. Agardh	1,2,3,4,5	W, R	M,E	Epi	A, B		21770
30. *Sahlingia subintegra* (Rosenvinge) Kornmann	2,4	S, R	M	Epi	A	NRT	21913 22072
Stylonematophyceae							
Stylonematales							
Stylonemataceae							
31. *Chroodactylon ornatum* (C. Agardh) Basson	1,4	R	M	Epi	A	NRT	21970
32. *Stylonema alsidii* (Zanardini) K.M. Drew	1,4	S, R	M	Epi	A	NRT	21875 22062
Florideophyceae							
Acrochaetiales							
Acrochaetiaceae							
33. *Acrochaetium flexuosum* Vickers	1,3	W, R	M,E	Epi	A	NRT	21766 22063
34. *A. microscopicum* (Nägeli ex Kützing) Nägeli	1,2,4,6	W, S, R	M,E	Epi	A	NRT	21772 21849 22072
35. *Acrochaetium sancti-thomae* Børgesen	1	S	M	Epi	A	NRT	22093
36. *A. sagraeanum* (Montagne) Bornet	4	S	M	Epi	A	NRT	TAB-ENCB/A2
37. *A. secundatum* (Lyngbye) Nägeli	4	S	M	Epi	A	NRT	TAB-17/A1
Colaconematales							
Coleconemataceae							
38. *Colaconema dasyae* (Collins) Stegenga, I. Mol, Prud'homme van Reine & Lokhorst	1	R	M	Epi	A	NRT	21948 22064
39. *C. daviesii* (Dillwyn) Stegenga	1	S	M	Epi	A	NRT	22152
40. *C. hallandicum* (Kylin) Afonso-Carillo, Sanson, Sangil & Diaz-Villa	3	R	E	As	A	NRT	TAB-ENCB-C3
41. *C. hypneae* (Børgesen) A.A. Santos & W.N. Moura	1,2,3,4	S, R	M,E	Epi	A	NRT	21769 21898 21913 22082 21934
42. *C. savianum* (Meneghini) R. Nielsen	5	W	E	Epi	A	NRT	21995
Corallinales							
Corallinaceae							
43. *Hydrolithon farinosum* (J.V. Lamouroux) D.L. Penrose & Y.M. Chamberlain	4	S, R	M	Epi	A	NRT	21922
44. *Jania adhaerens* J.V. Lamouroux	1	R	M	R	A	NRT	22459

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
45. *J. capillacea* Harvey	4	R	M	R	A	NRT	22457
46. *J. ungulata* f. *brevior* Yendo	4	R	M	Epi	A	NRT	21206
47. *J. rubens* (Linnaeus) J.V. Lamouroux	4	S, R	M	R	A	NRT	22458
48. *Lithophyllum prototypum* (Foslie) Foslie	4	S	M	R	A	NRT	21621
49. *L. stictiforme* (Areschoug) Hauck	4	S, R	M	R	A	NRT	21620
Ceramiales							
Callithamniaceae							
50. *Aglaothamnion boergesenii* (Aponte & D.L. Ballantine) L'Hardy-Halos & Rueness	1	R	M	R	A	NRT	TAB-15-49/06
51. *Callithamnion corymbosum* (J.E. Smith) Lyngbye	1	S, R	M	Epi, R	A, B		22011 22042
Ceramiaceae							
52. *Antithamnionella boergesenii* (Cormaci & G.Furnari) Athanasiadis	1	R	M	Epi	A	NRT	22088
53. *An. elegans* (Berthold) J.H. Price & D.M. John	1	R	M	Epi	A	NRT	21929 22042
54. *Callithamniella tingitana* (Schousboe ex Bornet) Feldmann-Mazoyer	1	R	M	Epi	A	NRT	22138
55. *Centroceras gasparrinii* (Meneghini) Kützing	1,4	S, R	M	R, Cm	A	NRT	22043 22044 22045
56. *Cn.* *hyalacanthum* Kützing	1,4	R	M	Epi	A	NRT	21874 22047
57. *Cn. micracanthum* Kützing	1,4	W, S, R	M	R	A	NRT	22039
58. *Ceramium brevizonatum* var. *caraibicum* H.E. Petersen & Børgesen	4	R	M	R	A	NRT	22038
59. *C. cruciatum* F.S. Collins & Hervey	1,4	R	M	R	A	NRT	21883 22056
60. *C. leutzelburgii* Schmidt	5	S, R	E	Epi	A	NRT	22040
61. *Gayliella flaccida* (Harvey) T. O. Cho & L. J. McIvor	1,4	S, R	M	Epi	A	NRT	21874 22037
62. *G. fimbriata* (Setchell & N.L. Gardner) T.O. Cho & S.M. Boo	1	R	M	Epi	A	NRA	22091
63. *G. mazoyerae* T.O. Cho, Fredericq & Hommersand	1	R	M	Epi	A	NRT	22025
64. *G. transversalis* (Collins & Hervey) T.O. Cho & Fredericq	1	R	M	Epi	A	NRT	22111
Spyridiaceae							
65. *Spyridia clavata* Kützing	1	S	M	R	A	NRT	21977 21978
66. *S. hypnoides* (Bory de Saint-Vincent) Papenfuss	1	S	M	R	A, B		22090 22091
Wrangeliaceae							
67. *Wrangelia argus* (Montagne) Montagne	1,2,4	W, S, R	M	Epi, R, As	A	NRT	21973 21974 21975
Dasyaceae							
68. *Dasya pedicellata* (C. Agardh) C. Agardh	5	S	E	Cm	A, B		22034
69. *Heterosiphonia crispella* (C. Agardh) M.J. Wynne	4	R	M	Epi	A	NRT	22009
Delesseriaceae							
70. *Caloglossa leprieurii* (Montagne) G. Martens	6	W, S	E	Cm	A	NRT	22075
Rhodomelaceae							
71. *Acanthophora muscoides* (Linnaeus) Bory	1	W	M	R	A	NRT	21767
72. *A. spicifera* (Vahl) Børgesen	1,2,3,4	W, S, R	M,E	R	A, B		21769 21999 22000
73. *Bostrychia radicans (*Montagne) Montagne	6	S	E	As	A	NRT	TAB-15-D/11

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
74. *Bryocladia cuspidata* (J. Agardh) De Toni	1,2,4	W, S, R	M	R	A, B		21990 22013
75. *B. thyrsigera* (J. Agardh) F. Schimtz	1,2,3,4	S, R	M,E	R	A	NRT	21906 22010 22012
76. *Chondria collinsiana* M. Howe	1	R	M	R	A	NRT	22140
77. *Melanothamnus ferulaceus* (Suhr ex J. Agardh) Díaz-Tapia & Maggs	1,2	S, R	M	R, Epi	A, B		21981 21983
78. *M. sphaerocarpus* (Børgesen) Díaz-Tapia & Maggs	4,5	W, R	M,E	As, Epi	A, B		21980 22005
79. *Polysiphonia atlantica* Kapraun & J.N. Norris	4,5	W, R	M,E	Epi	A	NRT	22005 22115
80. *P. havanensis* Montagne	1,4,6	W, S, R	M, E	Epi, As	A, B		22015 22114 22117
81. *P. schneideri* B. Stuercke & D.W. Freshwater	1	W	M	As	A	NRT	22140
82. *P. scopolorum* var. *villum* (J. Agardh) Hollenberg	1	R	M	Epi	A	NRT	22147
83. *P. subtilissima* Montagne	1,4,5	W, S, R	M,E	Cm, As	A, B		22002 22003 22113
84. *Yuzurua poiteauii* var. *gemmifera* (Harvey) M.J. Wynne	3	W	E	As	A	NRT	22120
Gelidiales							
Gelidiaceae							
85. *Gelidium americanum* (W.R. Taylor) Santelices	2,4,5	W, R	M,E	Cm, R	A	NRT	22023 22025 22032
86. *G. corneum* (Hudson) J.V. Lamouroux	1,2	W	M	R	A	NRT	22026 22027
87. *G. pusillum* (Stackhouse) Le Jolis	1,4	W, S, R	M	R	A, B		22007 22008
Pterocladiaceae							
88. *Pterocladiella sanctarum* (Feldmann & Hamel) Santelices	1	W	M	R	A	NRT	22061
Hildenbrandiales							
Hildenbrandiaceae							
89. *Hildenbrandia rubra* (Sommerfelt) Meneghini	1	R	M	R	A	NRT	21272
Peyssonneliales							
Peyssonneliaceae							
90. *Peyssonnelia armorica* (P.L. Crouan & H.M. Crouan) Weber-van Bosse	4	R	M	Epi	A	NRT	21620
91. *P. rubra* (Greville) J. Agardh	1,4	R	M	R	A	NRT	21348
Gigartinales							
Cystocloniaceae							
92. *Hypnea cornuta* (Kützing) J. Agardh	1	R	M	R	A	NRT	22049
93. *H. musciformis* (Wulfen in Jacquin) J. V. Lamouroux	1,2,4	S, R	M	Epi, R, As	A, B		22076 22078 22081
94. *H. spinella* (C. Agardh) Kützing	1,2,3,4	W, S, R	M,E	R, Epi	A, B		21875 22051 22052
95. *H. valentiae* (Turner) Montagne	1,2	S, R	M	R	A	NRT	22050
Gigartinaceae							
96. *Chondracanthus acicularis* (Roth) Fredericq	1	S	M	R	A	NRT	22108
Phyllophoraceae							
97. *Gymnogongrus griffithsiae* (Turner) Martius	1	W	M	R	A	NRT	22070
98. *G. tenuis* J. Agardh	1	W, R	M	R	A	NRT	21876 22103
Gracilariales							
Gracilariaceae							
99. *Gracilaria blodgettii* Harvey	1,3	W, S	M,E	R	A, B		22121 22122 22130
100. *G. bursa-pastoris* (S.G. Gmelin) P.C. Silva	3	R	E	As, Cm	A	NRT	22123
101. *G. caudata* J. Agardh	1,2,3,5	W, R	M,E	R, Cm	A, B		22068 22069

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
102. *G. cervicornis* (Turner) J. Agardh	1	R	M	R	A, B		22110 22112
103. *G. damaecornis* J. Agardh	1,2	R	M	R	A, B		21852 21997
104. *G. flabelliformis* (P. Crouan & H. Crouan) Fredericq & Gurgel	1,3	S, R	M,E	R, Cm	A	NRT	21874 21875 22109
105. *Gracilariopsis longissima* (S.G. Gmelin) M. Steentoft, L.M. Irvine & W.F. Farnham	1,3	S, R	M,E	R, Cm	A	NRT	22085 22089
Halymeniales							
Halymeniaceae							21766 21995 21996
106. *Grateloupia filiformis* Kützing	1,2,3	W, S, R	M,E	R, Cm	A, B		
107. *G. gibbesii* Harvey	1	S	M	R	A	NRT	22094 22107
108. *G. subpectinata* Holmes	1.3	W,S,R	M, E	R, As	A	NRA	220021 22031
109. *Prionitis pterocladina* M.J. Wynne	1	R	M	R	A	NRT	22065 22067
Rhodymeniales							
Lomentariaceae							
110. *Ceratodictyon variabile* (J. Agardh) R.E. Norris	1,4	R	M	R	A	NRT	22086 22088
OCHROPHYTA							
Phaeophyceae							
Dictyotales							
Dictyotaceae							
111. *Dictyopteris delicatula* J.V. Lamouroux	4	R	M	R, Epi	A	NRT	21996
112. *Dictyota crenulata* J. Agardh	4	S, R	M	R	A	NRT	21958 22148
Asterocladales							
Asterocladaceae							
113. *Asterocladon rhodochortonoides* (Børgesen) S. Uwai, C. Nagasato, T. Motomura & K. Kogame	4	S	M	Epi	A	NRT	21947 22146
Scytothamnales							
Asteronemataceae							
114. *Asteronema breviarticulatum* (J. Agardh) Ouriques & Bouzon	4	R	M	R	A	NRT	21959 21960 21971
Bachelotiaceae							
115. *Bachelotia antillarum* (Grunow) Gerloff	1	R	M	R	A	NRT	21933 21954
Ectocarpales							
Acinetosporaceae							
116. *Feldmannia duchassaingiana* (Grunow) Aisha & Shameel	1,4	R	M	R	A	NRT	22481
117. *F. irregularis* (Kützing) Hamel	1	R	M	R	A	NRT	22482
118. *F. mitchelliae* (Harvey) H.S. Kim	1,2,3,4	S, R	M,E	Epi, Cm	A	NRT	21946 21948 21949 22144
119. *Herponema tortugense* (W.R. Taylor) W.R. Taylor	3	S	E	As	A	NRT	TAB-16-B/01
Chordariaceae							
120. *Hecatonema floridanum* (W.R. Taylor) W.R. Taylor	1	R	M	Epi	A	NRT	21853
121. *Kuetzingiella elachistaeformis* (Heydrich) M. Balakrishnan & Kinkar	1,4	S, R	M	Epi	A	NRT	21935
Ectocarpaceae							
122. *Ectocarpus siliculosus* (Dillwyn) Lyngbye	1,2,3,4	S, R	M,E	R, Cm	A	NRT	21938 21945 21950
Scytosiphonaceae							
123. *Chnoospora minima* (Hering) Papenfuss	1,2,4	S, R	M	R	A	NRT	21956 21961 21970

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
124. *Colpomenia sinuosa* (Mertens ex Roth) Derbès & Solier	1,2,4	S, R	M	R	A	NRT	21942 22136
125. *Rosenvingea floridana* (W.R. Taylor) W.R. Taylor	1	S	M	R	A	NRT	22143
126. *R. intricata* (J. Agardh) Børgesen	1	S, R	M,E	R	A, B		22155
127. *R. orientalis* (J. Agardh) Børgesen	1	S	M	R	A	NRT	22135
Sphacelariales							
Sphacelariaceae							
128. *Sphacelaria rigidula* Kützing	1,2,3	W, R	M,E	Epi, Cm, As	A	NRT	21914 21965 21967
129. *S. tribuloides* Meneghini	1,2,3	R	M,E	R, Cm	A	NRT	21952 22150
CHLOROPHYTA							
Ulvophyceae							
Phaeophilales							
Phaeophilaceae							
130. *Phaeophila dendroides* (P.L. Crouan & H.M. Crouan) Batters	1,2,4,5	W, S, R	M,E	Epi	A	NRT	21863
Ulvales							
Kornmanniaceae							
131. *Blidingia marginata* (J. Agardh) P.J.L. Dangeard ex Bliding	1,6	W, S	M,E	Avp	A, C		21920 21944
132. *Bl. minima* (Nägeli ex Kützing) Kylin	1,4,5,6	W, S	M,E	Epi, Avp	A	NRT	21878 21896 21916 21969
Ulvaceae							
133. *Ulva clathrata* (Roth) C. Agardh.	3	W	E	As	A	NRT	21864
134. *U. compressa* Linnaeus	1,3,4,5,6	W, S, R	M,E	R, Epi, Cm	A, C		21775
135. *U. fasciata* Delile	1,4	S, R	M	R	A, C		21842 21894
136. *U. flexuosa* Wulfen	3,4,6	W, S, R	M,E	R, As	A, C		21778 21845 21882
137. *U. flexuosa* subsp. *paradoxa* (C. Agardh) M.J. Wynne	6	W, S	E	Cm	A, C	NRT	21873 21928
138. *U. intestinalis* Linnaeus	1,4,6	W, S, R	M,E	R, Cm	A, C		21915 21932
139. *U. rigida* C. Agardh	1,4	W, S, R	M	R	A, C		21856 21868
Ulvellaceae							
140. *Ulvella lens* P.L. Crouan & H.M. Crouan	1,2	R	M	Epi	A	NRT	21770
141. *Ul. scutata* (Reinke) R. Nielsen, C.J. O'Kelly & B. Wysor	3	S	E	As	A	NRT	TAB-ENCB/A
142. *Ul. viridis* (Reinke) R. Nielsen, C.J. O'Kelly & B. Wysor	1,4,5	W, R	M,E	Epi	A	NRT	21770 22073
Boodleaceae							
143. *Boodlea composita* (Harvey) F. Brand	4	W	M	R	A	NRT	21901
144. *Cladophoropsis membranacea* (Hofman Bang ex C. Agardh) Børgesen	4	S	M	R	A	NRT	21890
145. *C. sundanensis* Reinbold	1	R	M	R	A	NRT	TAB-ENCB/B1
Cladophoraceae							
146. *Chaetomorpha aerea* (Dillwyn) Kützing	1	S	M	R	A	NRT	21914
147. *Ch. antennina* (Bory) Kützing	1,2,4	W, S, R	M	R	A, C		21771 21855
148. *Ch. linum* (O.F. Müller) Kützing	3.5	W, S	M,E	Epi, Avp	A	NRT	21777 21860
149. *Ch. minima* Collins & Hervey	3	S	E	Epi	A	NRT	21924
150. *Ch. nodosa* Kützing	1,4	W, R	M	R	A, C		21839 21910
151. *Cladophora coelothrix* Kützing	1	R	M	R	A	NRT	21883
152. *Cl. crispula* Vickers	3,6	R	E	As, Cm	A	NRT	21937
153. *Cl. crystallina* (Roth) Kützing	1	R	M	R	A	NRT	TAB/15-54/04
154. *Cl. dalmatica* Kützing	4	S	M	R	A	NRT	21779
155. *Cl. flexuosa* (O.F. Müller) Kützing	1	R	M	R	A	NRT	TAB-15-54/02

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Table 2. Continued...

DIVISION/SPECIES	LOCALITIES	SEASONALITY	ENVIRONMENT	SUBSTRATE	REFERENCE	OBS.	ENCB Herbarium number
156. *Cl. fracta* (O.F. Müller ex Vahl) Kützing	1	S	M	R	A	NRT	21858
157. *Cl. glomerata* var. *crassior* (C. Agardh) Hoek	1	R	M	R	A	NRT	TAB-16-A/01
158. *Cl. hutchinsiae* (Dillwyn) Kützing	1,4	S	M	R	A	NRT	22137
159. *Cl. sericea* (Hudson) Kützing	1,4,6	S, R	M,E	R, Cm	A	NRT	21884 21887 21891
160. *Cl. submarina* P. Crouan & H. Crouan	1	R	M	Cm	A	NRT	21897
161. *C. vagabunda* (Linnaeus) van den Hoek	1,2,4,6	W, S, R	M,E	R, Cm	A, C		21770 21772 21904 21918
162. *Pseudorhizoclonium africanum* (Kützing) Boedeker	1,2,3	W, S	M,E	Epi, As	A	NRT	21860 21886 21926
163. *Rhizoclonium riparium* (Roth) Harvey	1,3,4,5,6	W, S, R	M,E	Epi	A, C		21840 21861
164. *Willeella brachyclados* (Montagne) M.J. Wynne	1,5,6	W, R	M,E	R, Cm	A	NRT	21773 22311
Bryopsidales							
Bryopsidaceae							
165. *Bryopsis halliae* W.R. Taylor	4	R	M	R	A	NRT	21843
166. *B, hypnoides* J.V. Lamouroux	1,4	R	M	R	A	NRT	21895 21919
167. *B. pennata* (Kützing) Collins & Hervey	1,4	R	M	R	A	NRT	21846 21877
168. *B. pennata* var. *leprieurii* (Kützing) Collins & Hervey	1,4	R	M	R	A	NRT	21776 21841
169. *B. pennata* var. *secunda* (Harvey) Collins & Hervey	1	R	M	R	A	NRT	21847 21863 22145
170. *B. plumosa* (Hudson) C. Agardh.	1,4	S, R	M	R	A	NRT	21774 21844
171. *Pseudobryopsis blomquistii* Díaz-Piferrer	4	S	M	R	A	NRT	22149
172. *Trichosolen duchassaingii* (J. Agardh) W.R. Taylor	4	R	M	R	A	NRT	21866 21867
Ulotrichales							
Ulotrichaceae							
173. *Urospora penicilliformis* (Roth) Areschoug	3	W	E	Avp	A	NRT	21848

SIMBOLS: Localities: 1. Sánchez Magallanes 2. La Machona 3. Laguna La Machona 4. Playa Paraíso 5. Laguna Mecoacan 6. San Pedro Centla Seasonality: W (Winter rains) S (Dry) R (Summer rains) Environment: M: Marine E: Estuarine References (Data source) A. This study B. Senties & Dreckmann (2013) C. Quiroz-González, et al. (2017) OBS. (Observations) NRA: New record for Atlantic coast of Mexico NRT: New record for littoral of Tabasco Substrate: R: Rocky As: Artificial Substrate Cm: Cement floor Avp: Aquatic vascular plants Epi: Epiphytic on other algae

This species has been reported from Korea, Vietnam, Japan, China, India, Australia, New Zealand, Hawaii, Mariana Islands, Solomon Islands and Galapagos Islands (Guiry & Guiry, 2017). Therefore this is the first record of *Gayliella fimbriata* for Tabasco and moreover, for the Atlantic coast of America.

Grateloupia subpectinata Holmes (Figures 3 d-j)

Type locality: Japan.

Heterotypic synonyms:

Grateloupia luxurians (A. Gepp & E.S. Gepp) R.J. Wilkes, L.M. McIvor & Guiry 2005: 58

Grateloupia filicina var. *luxurians* A. Gepp & E.S. Gepp 1906: 259

Representative specimens examined: Sánchez Magallanes Breakwater (Mateo Cid, Mendoza González & Valencia Torres, 10.iv.2016, ENCB 22996, female thalli and tetrasporangial thalli); Laguna La Machona (Mateo Cid & Mendoza González, 10.iv.2016, ENCB 22006, female).

Habit and anatomy: The thallus arising from a discoid holdfast is formed by simple or pinnate erect fronds tapering at the ends, mainly compressed, mucilaginous but firm, reddish to blackish purple, sometimes greenish. Thalli are 2.0–7.0 cm high, simple linear compressed and 375-450 µm diameter; cortex 3-5 layers, the cells moniliform or rounded to stellate in

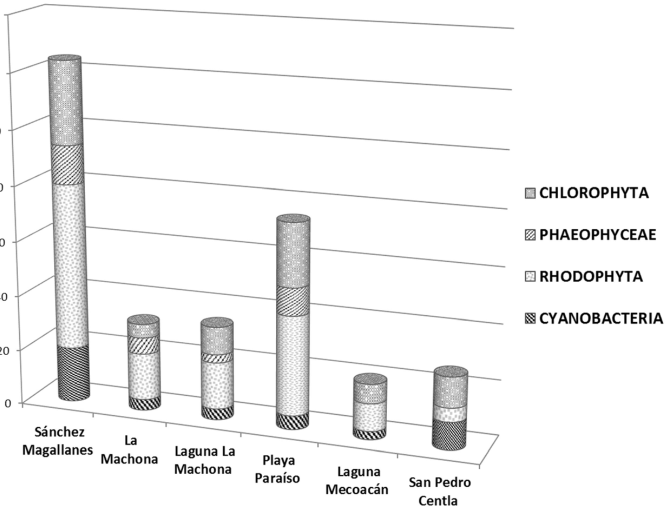

Figure 2. Number of species per division per locality

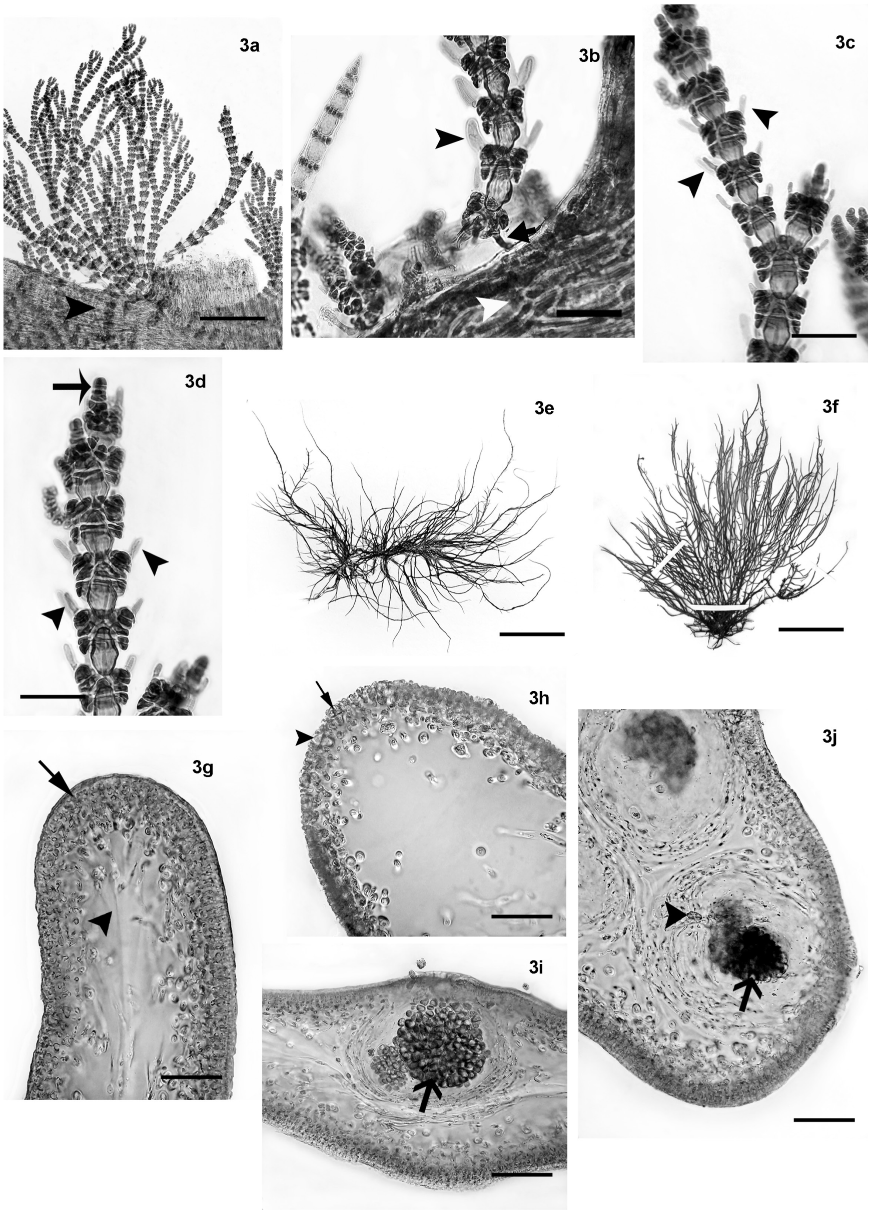

Figure 3. *Gayliella fimbriata:* Sánchez Magallanes (ENCB 22091). 3a. General appearance of the filamentous thallus growing on *Spyridia* sp (arrowhead). Scale 200 µm. 3b: Base of the thallus on the host (white arrowhead). Note rhizoid (arrow), and the prominent gland cells (black arrowhead) on the cortical bands. Scale 135 µm. 3c*:* Detail of verticilar arrangement of gland cells (arrowheads). Scale 25µm. 3d*:* Detail of an apical branch without forcipates apices. Note the apical cell (arrow) and gland cells (arrowheads). Scale 20 µm. 3e. *Grateloupia subpectinata:* Sanchez Magallanes (ENCB 22031), a: tetrasporangial thallus. Scale 1.6 cm. 3f: G. *subpectinata:* Sanchez Magallanes (ENCB 22016), Female thallus. Scale 1.8 cm. 3g: Cross-section through a blade showing cortex (arrow) and developing medulla. (Arrowhead). Scale 150 µm. 3h: Cross-section through a blade showing an immature tetrasporangium (arrowhead) and a mature, cruciately divided tetrasporangium. (Arrow) Scale 95 µm. 3i: Close-up of mature gonimoblast (arrow). Scale 80 µm. 3j: Cross-section through a blade showing the gonimoblasts (arrow) borne on an expanded fusion cell. Note the elongated ampullar filaments (arrowhead). Scale 60 µm.

shape 7-8 µm x 7.5-8.5 µm; medulla filamentous with lax filaments of 3-4 µm in diameter.

Tetrasporophytes and gametophytes isomorphic, the gametophytes dioecious with reproductive structures scattered over the entire thallus except the basal parts.

Tetrasporangia initiated from inner cortical cells, cruciately divided when mature, 14-15 µm wide x 29-30 µm long. Carposporangial ampullae not found; gonimoblasts 60-80 µm immersed inside the medulla and surrounded by branched ampullar filaments and a network of secondary medullary filaments.

Comments: Regarding the dimensions of the thallus, tetrasporangia and gonimoblasts show a smaller size than those recorded in specimens from Japan (Faye et al., 2004). Mexican specimens of *Grateloupia subpectinata* are morphologically similar to *G. filicina* (J.V. Lamouroux) J. Agardh. Nevertheless, both species have a considerable morphological plasticity (Faye et al., 2004, Verlaque et al., 2005). Compressed and branching *Grateloupia* species are notoriously difficult to identify due to their morphological similarity and lack of clear-cut diagnostic characters. The characteristics of our specimens are closer to the concept of *Grateloupia subpectinata*, so it is considered a new record for Tabasco and the Mexican Atlantic. This taxon has been also recorded in the Caribbean Islands (Wynne 2017), Britain, France, Spain and Australia (Guiry & Guiry 2017).

Discussion

1. Floristic composition

Here we report the presence of 115 previously unreported benthic marine and estuarine algae and 26 Cyanobacteria for the continental coasts of Tabasco, two of them, new records for the Atlantic coast of Mexico. Including the recently published reports of species from Tabasco (Senties & Dreckmann 2013, Quiroz-González et al., 2017), the total number of species increases to 194. This represents an increase of 150% (67) updated from the number published in the recent articles of Senties & Dreckmann (2013) and Quiroz-González et al. (2017), who based their analyses on reports in the literature and recent collections obtained by these authors. When analyzing the different taxonomic groups (Table 2), our survey resulted in a considerable increase in the number of Rhodophyta taxa (53.9%), followed by Chlorophyta (29.5%), and Phaeophyceae (16.6%).

Among the new records we can highlight: *Acrochaetium sagreanum, A. microscopicum*, *Antithamnionella boergesenii, An. elegans, Heterosiphonia crispella, Gayliella mazoyerae, G. fimbriata, Peyssonnelia armorica*, *Asterocladon rhodochortonoides*, *Hecatonema floridanum*, *Herponema tortugense, Phaeophila dendroides, Ulvella lens, Ul. scutata,* and *Ul. viridis*. Epiphytes are a very important group in the algal flora, especially in Tabasco, whose coast has many floodplains, extensive sandy beaches, coastal lagoons, breakwaters, and very exposed rocky coasts, lacking of algae.

In this study, a total of 26 taxa of Cyanobacteria were found at six localities of Tabasco and constituted 17.67% of the new records. A list of identified species is given in Table 2. There was a dominance of members of the orders Oscillatoriales and Pleurocapsales (27%), and Nostocales (15%), and all 26 taxa were reported for the first time in studies of the Tabasco coast. Species with wide distribution in the study area are *Lyngbya confervoides*, *Calothrix parietina and Scytonematopsis crustacea*, all of which have been reported in Campeche and Quintana Roo. With regard to the species of Chroococcales and Pleurocapsales, such as *Pseudocapsa maritima*, *Chroococcus ercegovicii*, *Aphanocapsa littoralis*, all were recorded from one locality, while *Entophysalis conferta* was present in five of the six study sites, being the most frequent and abundant of all the cyanobacteria recorded in our study.

In the last 50 years, the presence of cyanobacteria in brackish-water systems has been increasingly reported worldwide. Then, a wide diversity of cyanobacteria species can be found in brackish water systems (Lopes & Vasconcelos 2011). In this study, ten species of Cyanobacteria were observed in San Pedro Centla, locality with estuarine environment, while in the Sanchez Magallanes breakwater 16 species were recorded. Finding such a number of species confirms the ability of cyanobacteria to colonize the substrate available, ability for epiphytism, and to form mats in brackish (estuarine) and marine waters.

Most of these organisms requires culture studies and, when possible also the application of molecular-genetic techniques (Bernecker & Wehrtmann 2009). A detailed taxonomic analysis of these taxa will result in higher species numbers for Tabasco.

2. Type of environment and substrate

According to our study, of the 173 species, 109 develop exclusively in marine environment, 20 in estuarine environment, while 43 were located in both estuarine and marine environments. Most of the Rhodophyta develop in marine rocky areas, whereas members of Chlorophyta are established and developed in both marine and estuarine environments. In the case of brown algae, most of them (14) occur in the marine environment. These results agree with those described by McGlathery et al. (2013), who indicated that brown and red algae are almost exclusively marine species and the Chlorophyta can occasionally be abundant in the lower salt marsh zone in estuaries.

In our study, the localities with rocky substrate with marine water influence present high species richness compared with estuarine localities. A total of 89 species was registered in this type of substrate. The rocky substrate is favorable for the development of a great number of marine tropical seaweeds of Ceramiales, Corallinales, Cladophorales and Bryopsidales. On the other hand, the sampling sites with artificial substrate as plastic, net ropes, fishing lines, textiles and concrete debris present lower richness (25); it may be because the artificial substrate limits growth of marine algae and cyanobacteria because they aren't hard and/or stable (Santelices 1977, Garduño-Solórzano et al. 2005, Mateo-Cid et al. 2013). Moreover, our results agree with the statement by Hartog (1967), who indicates that the flora of the brackish water is poor in species in comparison with those of the sea and the fresh water. Most of the marine organisms are stenohaline and are unable to live permanently in water with a higher or lower salt-content than that of the sea.

3. Epiphytic species

Epiphytes are small size, often microscopic, and their adaptive strategy as epiphytes depends on the permanence of their host and is affected by competition between them for the substrate and light, among other factors. The identification of these species is complicated because their characteristics are difficult to observe. On the other hand, previous works were developed with different objectives, so they have not been similarly inventoried.

Seventy-five epiphytic species (Table 2) were found, 25 of which correspond to Cyanobacteria, 35 to Rhodophyta, six to Phaeophyceae and nine to Chlorophyta. The highest number of epiphytes was observed in the dry season with 45, with a greater number of cyanobacterial species, as well as the families Acrochaetiaceae, Rhodomelaceae and Ceramiaceae, which cover other algae up to almost 90% of their surface. This finding agrees with what is observed in some brown algae and marine phanerogams, in which the level of epiphytism is greater when the surface and biomass of these organisms are larger. This condition is also attributed to the decrease of defense substances against the epiphytes as the host becomes senescent (Ortuño-Aguirre and Riosmena-Rodríguez, 2007).

Most of the epiphytes were frequently found growing on perennial red algae such as *Acanthophora, Bryocladia, Gracilaria,* and *Grateloupia.* It has been suggested that host longevity should be long enough to allow these organisms to complete their life cycle and that this might be a likely reason for the absence of epiphytes on annual and ephemeral algae (Santelices, 1977).

4. Seasonality

Temperature and light determine the latitudinal distribution of seaweeds, and therefore their geographical distribution for they also influence the composition, variation and periodicity of populations at the intertidal and subtidal levels (Santelices, 1977). In our study area summer days are 4 to 5 hours longer that in the winter. Hence, the availability of light for seaweed and estuarine organisms is higher, which influences the species richness of these organisms during the summer (rainy season), with 62 Rhodophyta (49.2%), 15 Phaeophyceae (11.9%), 28 Chlorophyta (22.2%) and 21 Cyanobacteria (16.68%). The lowest specific richness occurred in winter rains ("nortes" in which the rain is greater than 10.2% and temperature values between 20 and 22 ° C) with 29 Rhodophyta (46.77%), 1 Phaeophyceae (1.62%), 19 Chlorophyta (30.65%), and 13 Cyanobacteria (20.96%). Finally, during the dry season 41 Rhodophyta (49.4%), 11 Phaeophyceae (13.3%), 24 Chlorophyta (28.9%), and 7 Cyanobacteria (8.4%) occurred (Figure 4) for our results are similar to those reported in previous studies in the Mexican Atlantic where mentioned that the species richness of algae is higher in the rainy season (Ortega et al., 2001, Callejas-Jimenez et al, 2005, Mateo-Cid et al., 2013).

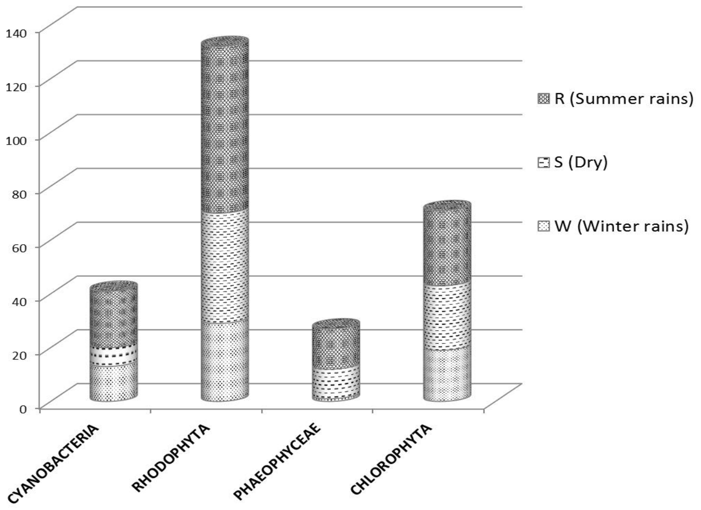

Figure 4. Number of species per division per season

Table 3. Feldmann and Cheney indexes for some marine and estuarine environments of the Gulf of Mexico and Mexican Caribbean coast.

Locality	Feldmann Index	Cheney Index
	(R/P)	(R+C)/P
Isla Mujeres, Quintana Roo	4.50	7.15
Isla Cozumel, Quintana Roo	4.40	7.04
Puerto Morelos, Quintana Roo	3.40	5.60
Coast of Yucatan	6.00	9.00
Coast of Campeche	5.37	9.30
Coast of Tabasco (This study)	4.42	6.73

In the rocky zone of the Sanchez Magallanes breakwater and Playa Paraiso breakwater numerous species of the families Corallinaceae, Rhodomelaceae, Ectocarpaceae, Dictyotaceae and Ulvaceae occurred. Rhodophyta dominates in number, with 60 in Sánchez Magallanes and 36 in Playa Paraíso. In the dry and rainy seasons *Ulva fasciata*, *U. rigida, Cladophora vagabunda* and *Bryopsis pennata* are common; while *Asteronema breviarticulatum* and *Ectocarpus siliculosus* are the most common in the rainy season. It is evident that in the estuarine areas the smallest number of species is presented, the Laguna Mecoacan having the lowest diversity with only 10 Rhodophyta, 7 Chlorophyta and 3 Cyanobacteria.

5. Biogeography

The results of the present study were analyzed applying the Feldmann and the Cheney indexes and compared with findings by Mateo-Cid & Mendoza-González (2007) for Cozumel island; Mendoza-González et al. (2007) of Mujeres Island, Dreckmann et al. (1996) for Puerto Morelos, Huerta-Múzquiz et al. (1987), Ortegón-Aznar et al. (2001, 2009) and Sánchez-Molina et al. (2007) for the coast of Yucatán and Mateo-Cid et al. (2013) for the coast of Campeche. Table 3 shows the data obtained applying the Feldmann and Cheney indexes to the five regions mentioned; the phycoflora of the study area is similar to that of the coast of the state of Quintana Roo, with a value close to that obtained for the Cozumel and Mujeres Islands, both distinctly Caribbean localities.

The indexes used indicate that the marine and estuarine algae of the coast of Tabasco have a predominantly tropical distribution, with tropical elements such as *Melanothamnus ferulaceus*, *Hypnea musciformis, Jania adhaerens, Gracilaria flabelliformis, Gracilariopsis longissima, Dictyopteris delicatula, Dictyota crenulata, Chnoospora minima, Chaetomorpha antennina, Ulva rigida* and *Cladophora flexuosa*, among others. The phycoflora of the coast of Tabasco conforms to the pattern found in all coastlines of the states of the Gulf of Mexico (Ortega et al. 2001, Mateo-Cid et al., 2013).

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Author contributions

A. Catalina Mendoza-González collected and identified specimens, analyzed data and participated in writing the text.

Luz Elena Mateo-Cid collected and identified specimens, participated in writing the text, analyzed data and edited the photographs.

Deisy Yazmín García-López collected and identified the samples and edited map.

All authors contributed to critical revision, adding intellectual content.

Conflicts of interest

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

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