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Article

Synopsis of the knowledge on the Brazilian aplacophorans (Mollusca: Caudofoveata & Solenogastres)

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Abstract: Aplacophorans are well known as exclusively marine benthic molluscs with a vermiform body covered by aragonitic sclerites (also called spicules), whose species are widely distributed from the sublittoral down to the abyss. Currently, only nine species are known from Brazil (one Solenogastres and eight Caudofoveata), but these very few records are no longer a reflection of an existing low diversity. Sampling in deep waters has been conducted recently in oil-rich areas of the Brazilian coast, and the museum collections have now many aplacophoran lots. There is a need to learn about and/or install some microscopical facilities in Brazilian institutions, to form expertise for the investigations on these generally small animals. With studies on taxonomy, phylogeny, biogeography and ecology, important questions will be surely answered about the diversity, distribution, and the relationship among the deep-sea fauna from Brazil and from other places.

Keywords: *Aplacophora, molluscs, biodiversity, Southwestern Atlantic.*

Sinopse do conhecimento sobre os aplacóforos brasileiros (Mollusca: Caudofoveata & Solenogastres)

Resumo: Os aplacóforos são bem conhecidos como moluscos bentônicos exclusivamente marinhos, que possuem um corpo vermiforme coberto por escleritos de aragonita (também chamados de espículas), e cujas espécies são amplamente distribuídas desde o sublittoral a até profundidades abissais. Atualmente, apenas nove espécies são conhecidas para o Brasil (uma de Solenogastres e oito de Caudofoveata), mas esses poucos registros não são um reflexo de uma baixa diversidade existente no nosso litoral. Recentemente, amostragens em águas profundas têm sido feitas em áreas da costa brasileira ricas em petróleo, e as coleções de museus possuem agora muitos lotes de aplacóforos. Há uma necessidade de aprender sobre e/ou instalar algumas condições de infraestrutura nas instituições brasileiras, com o intuito de formar competência para as investigações sobre estes animais geralmente pequenos. Com estudos sobre taxonomia, filogenia, biogeografia e ecologia, questões importantes serão certamente respondidas sobre a diversidade, distribuição e relação entre as faunas de grande profundidades do Brasil e de outros locais do mundo.

Palavras-chave: *Aplacophora, molluscos, biodiversidade, Atlântico sul-ocidental.*

Introduction

Mollusca is a very diverse group of animals, with about 80,000 described living species (Bouchet et al. 2016). They are well known for the beautiful shells that many of its representatives bear (although not all have shells), and also for their ecological and economic importance. In this group there are very popular forms such as land and marine snails and slugs, oysters, mussels, squids and octopuses. They are animals found in almost all types of environments, like the sea bottom or the seawater column, in freshwater and in land ecosystems. This huge biodiversity is grouped into a single phylum, whose monophyly is practically unanimous, based on both morphological (e.g., Salvini-Plawen 1980, Haszprunar 2000) and molecular evidences (e.g., Passamaneck et al. 2004, Kocot et al. 2011, Smith et al. 2011). Most malacologists agree that there are eight living lineages: Solenogastres, Caudofoveata, Polyplacophora, Bivalvia, Monoplacophora, Scaphopoda, Gastropoda and Cephalopoda (Kocot 2013, Giribet 2014, Schrödl & Stöger 2014).

Aplacophora is very distinct from the other molluscs, with characteristics that for a long time have been considered similar to the ones that may have existed in the common molluscan ancestor. Their vermiform body is covered by aragonitic sclerites (also sometimes called spicules) instead of a shell, and the pallial cavity is positioned at the posterior end of the body. Because of these presumably plesiomorphic characters, Aplacophora has been traditionally placed as a sister group of other molluscs (e.g., Salvini-Plawen & Steiner 1996). An alternative hypothesis groups them with Polyplacophora in a clade called Aculifera, which has been recently supported by molecular and phylogenomic data (e. g., Kocot et al. 2011, Smith et al. 2011, Vinther et al. 2012); Aculifera is, in turn, the sister group to Conchifera, which is composed by the remaining molluscs.

Two groups are recognized as aplacophorans, Caudofoveata Boettger, 1956 (or Chaetodermomorpha Pelseneer, 1906) and Solenogastres Gegenbaur, 1878 (or Neomeniomorpha Pelseneer, 1906), distinguished by external and internal characteristics. Both have living species in all oceans, with most of them being distributed between 200 and 3,000 meters depth, although one (*Chevroderma hadalis* Ivanov 1996) was recorded up to about 8,400 meters (Ivanov 1996). They are, in general, small animals, less than five millimeters long, with some few species reaching up to 40 cm (Ivanov & Scheltema 2007). Like other molluscs, a radula is present and the anus is positioned in the mantle cavity, as well as the genital openings and the gills. There is a tetraneural nervous system but a distinct head is lacking, as well as kidneys, or nephridia.

Solenogastres comprises about 280 species (Todt 2013, García-Álvarez et al. 2014), characteristically bearing a ciliated sliding ventral furrow, used for locomotion on both soft bottoms and hard substrates. Within this furrow there is a ciliated epithelium considered homologous to the foot of other molluscs (Scheltema 1978). They form a very diverse aplacophoran group, whose species diversity is currently classified in four orders and 23 families (García-Álvarez & Salvini-Plawen 2007, García-Álvarez et al. 2014). Most of them feed on cnidarians. They are hermaphrodites and the digestive gland is simple and undivided (Scheltema et al. 1994, García-Álvarez et al. 2014). Respiratory organs are either absent or developed as respiratory folds in the mantle cavity.

Caudofoveata is less diverse, composed of about 130 species (Todt 2013, Salvini-Plawen & García-Álvarez 2014), which are classified into only three families: Chaetodermatidae Theel, 1875, Limifossoridae Salvini-Plawen, 1970 and Prochaetodermatidae Salvini-Plawen, 1972 [although two other families were proposed by Ivanov (1981), but not generally accepted (MolluscaBase 2018)]. They are all infaunal and feed on detritus and occasionally on foraminiferans. Their mouth is surrounded as a whole or in part by an oral shield (or oral disc, or pedal shield) that is absent in Solenogastres. The oral shield does not bear sclerites on it but have one or more rows of distinct sclerites surrounding it. It is used in locomotion, and also has a sensory function (Salvini-Plawen & García-Alvarez 2014). In the posterior end of the body, the pallial cavity contains a pair of bipectinate ctenidia, considered homologous with those of other molluscs. They are also distinguished from Solenogastres by the absence of the ventral foot groove, by their dioecious condition and internally by the presence of a well-developed divided digestive tract and a simpler reproductive tract (Scheltema et al. 1994).

In comparison to other molluscs, Aplacophora is poorly known, because, in large part, they occur in deeper waters and are thus less accessible, as discussed by Todt (2013). Moreover, these animals have, in general, small dimensions and studying them requires the observation of important microscopic features, such as the structure of the sclerites, radula and internal organs. For Caudofoveata, the radula and sclerite characters are somewhat sufficient to identify its species, whereas for Solenogastres these and histological characters of the foregut glands are enough for family level, with additional characters of the gonopericardial system being necessary for species assignment. Overall, in addition to Aplacophora being less accessible, their taxonomy requires time-consuming observations and is extremely hard for intrained taxonomists. Thus, among all molluscan groups, the diversity of Aplacophora is one of the least known, related to the small number of specialists involved in their taxonomy. At this moment, there are only few groups of researchers distributed in the United States of America, Spain, Norway, Russia, Japan, Colombia, Germany, Austria, and now in Brazil. It is noteworthy that three of them have recently passed away – Christopher Schander (Norway), Luidfried Salvini-Plawen (Austria) and Amélie Scheltema (USA) – leaving the Aplacophora orphaned of its most important authorities.

In the Atlantic Ocean, Aplacophorans is best known from the north hemisphere, from where there are more abundant records, especially in Europe and USA, including the Gulf of Mexico (e.g., Salvini-Plawen 1967, 1968, 1972, 1996, Scheltema 1985, Scheltema & Ivanov 2000, Schander et al. 2006, Ivanov et al. 2009, Ivanov & Scheltema 2008, 2009, Señaris et al. 2014). There are also species known from the Caribbean, Colômbia and Suriname (e.g., Salvini-Plawen 1972, 1992, Ivanov & Scheltema 2008, Gracia et al. 2013), but further south in the Atlantic American coast records of aplacophoran species are much more scattered. For Brazil, Uruguay and Argentina they are very poorly known, despite the large and diverse coast these countries bear. Three species of Caudofovetata and one of Solenogastres are known from Uruguay (Scheltema 1985, Salvini-Plawen 1992, Scheltema & Ivanov 2000, Scarabino 2003); and for Argentina, six and ten species, respectively (Scheltema & Ivanov 2000, Forcelli 2000, García-Álvarez & Salvini-Plawen 2007). For the Brazilian littoral, the occurrence of aplacophorans is presumably very restricted, based on existing published records. These records are gathered here, additionally pointing out some problems that have hampered the studies of these molluscs in our country.

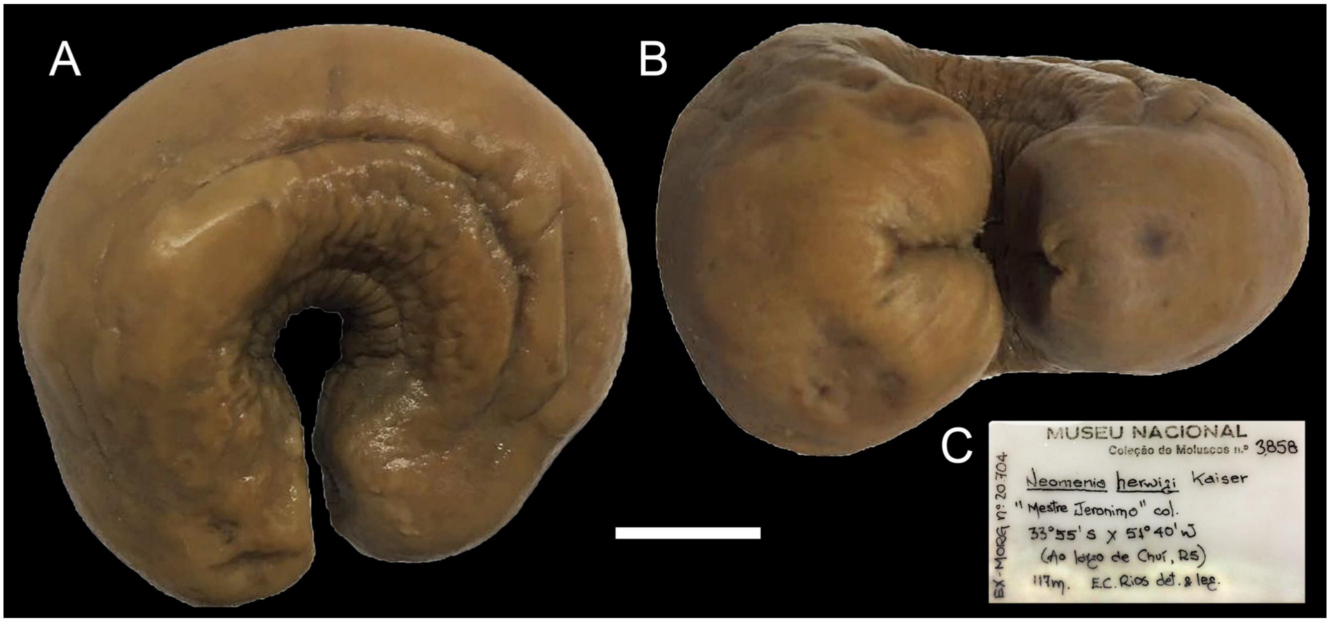
Material and Methods

All literature related to Aplacophora was reviewed, aiming to find information about the species already recorded from Brazil. Apart from this, the molluscan scientific collections of the Museum of Zoology of the State University of Campinas "Prof. Adão José Cardoso" (ZUEC), of the National Museum of Rio de Janeiro (MNRJ), and of the Museum of Zoology of the University of São Paulo (MZUSP) were consulted. Then, pictures of the aplacophoran species already recorded were taken.

Results

The first record of an aplacophoran from Brazil was made by Rios (1980), based on two specimens of *Neomenia herwigi* Kaiser, 1976 (Neomeniidae) collected in the Rio Grande do Sul State (Figure 1). This species was also listed in the subsequent catalogs of Rios (1985, 1994, 2009), and up to now is the single Solenogastres known from our littoral. It is well known as a giant aplacophoran species, for its individuals can reach up to 20 centimeters in length (Rios 1980). It was originally described from Argentinian waters (Kaiser 1976). Apart from this species, there is also only one reference of other Brazilian Solenogastres, "*Proneomenia* (?) sp." (Proneomeniidae), by Simone (2004), collected by the Revizee Project – Score South.

For Caudofoveata, there are more records, but most of them are not very precise, as there are no data about the sampling locality. Rios (1994, 2009), for example, listed two species of Limifossoridae and one of Chaetodermatidae, but all of these were not described in detail, nor are there bibliographic references or biological material on which they were based. In addition to these records, other two can be mentioned: Cruz et al. (1998), who studied the radula of an undescribed species of *Falcidens* Salvini-Plawen, 1968 collected from off the coast of Rio de Janeiro; and Martins (2008), who made great taxonomic discoveries – four species new to science – based on specimens collected in the southeastern and southern coast, but these are not formally published yet. Aside from these incomplete records, there are only eight Caudofoveata species known from Brazilian waters: *Chevroderma turnerae* Scheltema, 1985 and *Spathoderma bulbosum* Ivanov & Scheltema, 2008 (Prochaetodermatidae), originally described by examination of specimens also collected in deep waters from the northeastern coast (Scheltema 1985, Ivanov & Scheltema 2008); and *F. targatus* Salvini-Plawen, 1992, *F. acutargatus* Salvini-Plawen, 1992, *F. australocaudatus* Passos, Corrêa & Todt, 2017 (Chaetodermatidae), *Claviderma amplum* Ivanov & Scheltema, 2008, *C. crassum* Ivanov & Scheltema, 2008, and *C. virium* Corrêa, Miranda & Passos, 2018 (Prochaetodermatidae), recently discovered from the Campos Basin, off the Espírito Santo and Rio de Janeiro States (Corrêa et al. 2014, 2018, Passos et al. 2017). *Chevroderma turnerae* is widely distributed, known as a lower continental rise and abyssal species occurring in the Atlantic and Indian Oceans (Scheltema 1985, Scheltema & Ivanov 2000, Ivanov & Scheltema, 2008). *S. bulbosum* is until now restricted to the continental slope and rise in the eastern Atlantic (Ivanov & Scheltema 2008). The record of *F. acutargatus* from the Campos Basin (Corrêa et al. 2014) represents the extension of occurrence of this species further to the south in the Americas; it was known only from North Carolina to the Caribbean (it remains, however, to be found in other countries from the northern part of South America and in the Brazilian north-eastern coast). *F. targatus* was originally described from the continental shelf of Uruguay (Salvini-Plawen 1992), and before this it was recorded by Scheltema (1990) as an undescribed species from Brazil. Corrêa et al. (2014) suggested that the distribution of this species must be more restricted, maybe for the influence of the delta rivers occurring in the north-eastern and northern Brazil. *F. australocaudatus* was recorded as a very abundant species from the shelf and slope of Campos Basin (Passos et al. 2017); it was compared with *F. caudatus* (Heath, 1918), a species known as to occur on the northeastern coast of the USA. Corrêa et al. (2018) described the three species of *Claviderma* Scheltema & Ivanov, 2000 from the same area, in comparison to other species of the same



MUSEU NACIONAL
Coleção de Moluscos n.º 3858
Neomenia herwigi Kaiser
"Mestre Jeronimo" col.
33°55'S x 51°40'W
(Ao largo de Chuí, RS)
117m. E.C. Rios det. & leg.
EX-MORG nº 20.704

Figure 1. *Neomenia herwigi*. Lateral (A) and ventral (B) view of one of the two individuals used by Rios (1980) to record this species from Brazil. The label (C) shows that this specimen was originally deposited in the collection of the Museu Oceanográfico (MORG 20704); currently, it is in the molluscan collection of the National Museum of Rio de Janeiro (MNRJ 3858). Scale bar = 2 cm.

Figure 2. Caudofoveata species from Campos Basin, southeastern Brazil. A and B are *F. targatus* and *F. acutargatus*, respectively, recorded by Corrêa et al. (2014); C is *F. australocaudatus* (Passos et al. 2017); and D-F are *C. amplum*, *C. crassum*, and *C. virium*, respectively (Corrêa et al. 2018). Scale bars = 1 mm.

genus known from the Atlantic Ocean. Through these comparisons, they noted that species with similar body forms and with sclerites of the same shape occur off the western and eastern Atlantic coasts; these similarities could suggest that these species are sister-groups, sharing the same morphological traits as a result of common ancestry. Corrêa et al. (2014, 2018) and Passos et al. (2017) can be considered as the only more complete studies made on Brazilian aplacophorans, adding detailed data about the structure of the sclerites and radula, as well as about the external morphology and the bathymetric distribution of caudofoveates from Brazil. In Figure 2, all these species recorded by Corrêa et al. (2014, 2018) and Passos et al. (2017) are illustrated.

Discussion

Unlike what happens with other molluscan groups, studies on the taxonomy of Brazilian aplacophorans can be considered in their infancy. Two main problems are now pointed out as impediments for the development of more studies on these animals.

Aplacophoran species are more restricted to deep waters, a part of the marine environment less investigated in Brazil. Oceanographic studies often focus on the continental shelf, especially those interested in the research of the bottom fauna. Just a few large projects have been recently developed to study the slope and the continental rise (and even less the abyss), with most of them related to the activities of oil and gas exploitation in some specific areas. This is the case of the HABITATS and AMBES Projects, which collected a large number of samples from Campos Basin. Indeed, there are only very few Brazilian research institutions able to investigate the marine biota from deep waters, with adequate vessels and equipment for collection of bottom samples from its large and rich coast. This situation is quite distinct from countries of the north Atlantic, where there is a long tradition of investigations of the deep sea. So, these marine organisms from Brazil are just beginning to be studied, the molluscs being one of them, including aplacophorans.

As cited before, the structure of the sclerites, radula and internal organs are essential for the description of aplacophoran species. Light microscopy is traditionally used to study them, and more recently scanning and transmission electron microscopical studies have made great discoveries (Schander et al. 2006, Ivanov & Scheltema 2008, Ivanov et al. 2009, Kingsley et al. 2013, Kocot & Todt 2014), as well as confocal and birefringence microscopy (Scheltema & Ivanov 2004, Schander et al. 2006, Ivanov & Scheltema 2007, Faller et al. 2012, Mikkelsen & Todt 2014), and 3D reconstructions of their anatomy (Todt et al. 2008, Señaris et al. 2014, Bergmeier et al. 2016). In the Brazilian research institutions, these facilities are not always easily available and, as a consequence, aplacophoran research is limited. Up to now, studies on aplacophorans have resulted in only one PhD (Martins 2008) and two MSc theses (Corrêa 2016, Miranda 2016). Fortunately, there is now an increase in interests on know the deep-sea organisms in more detail, and thousands of aplacophoran specimens are waiting studies in Brazilian scientific collections. In these collections, information about the fixation methods are not always available, and so works on molecular biology (as DNA barcoding) have not been undertaken until now.

Conclusion

Concluding, the very few records of aplacophoran species are not a true reflection of a low diversity of these animals in Brazilian waters. On the contrary, they are present and diverse in our littoral, and most likely also in deeper waters, but up to now very few of them were available for research. Thanks to large projects focusing studies on some areas (as those oil-rich ones, like Campos Basin), many samples are now arriving at the universities and museums, with new species and records awaiting to be described. Apart from these basic taxonomic studies, others on ecology, phylogeny, biogeography, and many other themes will surely be developed with the study of these molluscs. How many aplacophoran species are there in Brazil and what is the ecological importance of them in the bottom ecosystem? As the continental shelf of the Brazilian northeast is narrower and shallower, is there some breakup of the aplacophoran diversity in that area? And what is the relationship of our aplacophoran fauna with those from the north Atlantic, the Caribbean, Argentina, or Antarctica? These are some of the most basic questions to be answered by the study of these interesting animals from Brazil, a huge country from where part of its biodiversity is still little known.

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Authors contributions

Flávio Dias Passos: Designed this work and prepared the text.

Marcel Sabino Miranda: Collected the data from the bibliography and made a critical review of the final version of the manuscript. Took the pictures of *N. herwigi*.

Paulo Vinicius Ferraz Corrêa: Collected the data from the bibliography and made a critical review of the final version of the manuscript.

Conflicts of interest

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

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Influence of environmental factors on the bathymetric distribution of the flecked box crab *Hepatus pudibundus* (Herbst, 1785) (Crustacea: Aethroidea) in the Southeastern Brazilian coast

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Abstract: This study evaluated the relationships between environmental factors and the spatio-temporal distribution of *H. pudibundus*, with the hypothesis of differential occupation in coastal areas of southeastern Brazil. The samplings took place monthly in January-December 2000 period, along nine transects from 2 to 40 m of depth, in Ubatuba region, northern coast of São Paulo. We collected 1808 individuals of *H. pudibundus*. The highest abundance was recorded in winter in the transects 10-25 m deep. Abundance was positively correlated with organic matter content and texture sediment (phi values). With the retreat of the South Atlantic Central Water (SACW) in autumn and winter, the sediment swirls, suspending the detritivore and filter-feeding macrofauna, increasing the food availability. Sites characterized by finer sediment offer higher food availability, besides facilitating *H. pudibundus* burying behavior. Due to its opportunistic predatory behavior, this species feeds on a variety of organisms, including mollusks, annelids and foraminifera, which are preys more abundant in the studied area and in sediments of finer grain size.

Keywords: *Brachyura, sediment texture, Decapoda, distribution, Coastal Water.*

Influência de fatores ambientais na distribuição batimétrica do caranguejo de baú *Hepatus pudibundus* (Herbst, 1785) (Crustacea: Aethroidea) na costa sudeste do Brasil

Resumo: Este estudo avaliou as relações dos fatores ambientais e a distribuição espaço-temporal de *H. pudibundus*, com a hipótese de ocupação diferencial em uma região do litoral sudeste do Brasil. As amostragens ocorreram mensalmente no período de janeiro a dezembro de 2000, ao longo de nove transectos de 2 a 40 m de profundidade, na região de Ubatuba, litoral norte de São Paulo. Foram coletados 1808 indivíduos de *H. pudibundus*. A maior abundância foi registrada no inverno nos transectos 10 a 25 m de profundidade. A abundância foi positivamente correlacionada com o teor de matéria orgânica e a textura do sedimento (valores de phi). Durante o outono e inverno com a retração da ACAS ocorre o revolvimento do sedimento e a supensão da macrofauna detritívora e filtradora, aumentando a disponibilidade de alimento. Locais caracterizados por sedimentos mais finos oferecem maior disponibilidade de alimentos, além de facilitar o comportamento de *H. pudibundus* se enterrar. Devido ao comportamento predatório oportunista, esta espécie se alimenta de uma grande variedade de organismos, incluindo moluscos, anelídeos e foraminíferos, que são as presas mais abundantes nas áreas estudadas, principalmente em locais com grãos menores.

Palavras-chave: *Brachyura, textura do sedimento, Decapoda, distribuição, Água Costeira.*

Introduction

Marine resources and their benefits to mankind are still poorly known and, therefore, still being studied. Small, sheltered coastal areas, such as bays and inlets, provide conditions for the establishment of several species with different ecological profiles. Some of these species are of economic interest and/or key elements in the trophic webs (Mantelatto et al. 1995a, Bertini & Fransozo 1999, Fransozo et al. 2016, Mantelatto et al. 2016). Considering that these coastal areas are naturally influenced by environmental factors, and given their reasonable sizes, they can be studied in more detail and serve as models for extend the understanding of human and/or natural impacts in larger scales. The northern coast of São Paulo has been affected by an increasing tourism and urbanization pressure (Burone & Pires-Vanin 2006, Gallo-Junior et al. 2011). In addition, trawling fisheries are intense, they affect the sedimentation processes, lead to overfishing, and disturbance of benthic communities (Pires-Vanin 1993, Mantelatto et al. 2016). Trawling is a non-selective activity, and many species of little or none economic value are captured as bycatch (Hall et al. 2000).

To better understand the distribution and abundance of a given species it is necessary to know details on its life-history, resource use patterns, and intra- and interspecific relationships, as well as its responses to environmental conditions. Thus, the study of variation of population structure in relation to environmental conditions may contribute to elucidate the factors that regulate abundance and distribution (Begon et al. 2006).

The distribution pattern of marine organisms may vary with local environmental conditions and lead to a differential spatial distribution (Bertini et al. 2010). Characteristics such as sediment texture and organic matter content, and water temperature and salinity, are important factors regulating the distribution and ecological relationships of benthic organisms, including decapod crustaceans. These factors control local marine productivity and environmental conditions, which in turn determine species' occurrence (Buchanan & Stoner 1988, Lima et al. 2014a, Andrade et al. 2015, Bernardes et al. 2016).

The flecked box crab species *Hepatus pudibundus* (Herbst, 1785) is found in both sides of the Atlantic: on the western side, in Georgia (U.S.A.), Gulf of Mexico, Antilles, Venezuela, Guyana, and Brazil (Melo 1996), and on the eastern side, from Guinea down to South Africa. This species belongs to superfamily Aethroidea, which includes six genera (Ng et al. 2008). Two of which occurring in Brazil: *Hepatus* Latreille, 1802 and *Osachila* Stimpson, 1871 (Melo 1996). In Ubatuba, *H. pudibundus* is the second or third most abundant brachyuran crab, after portunid crabs of genus *Callinectes Stimpson,* 1860 (Mantelatto & Fransozo 2000, Braga et al. 2005, Bertini et al. 2010). Even though it is not a commercial species, it is frequently captured in the same areas of the shrimp fisheries of *Xiphopenaeus kroyeri* (Heller, 1862), *Farfantepenaeus paulensis* (Pérez-Farfante, 1967), *Farfantepenaeus brasiliensis* (Latreille, 1817), and *Penaeus schmitti* Burkenroad, 1936 (see Bertini et al. 2010 and Mantelatto et al. 2016 for details). Some authors reported that *H. pudibundus* is one of the most commonly bycatch species of fisheries targeting the shrimp *X. kroyeri* (Heller, 1862) (Fransozo et al. 2016, Mantelatto et al. 2016). However, its abundance is not affected by these fisheries because the crabs are returned to the sea while still alive.

Although *H. pudibundus* is an important species in the food webs of unconsolidated sediments along the infralittoral (Mantelatto & Petracco 1997), most previous studies focused on biological aspects, including larval and juvenile development in the laboratory and from field samplings (Rieger & Hebling 1993, Hebling & Rieger 2003, Negreiros-Fransozo et al. 2008), population structure (Mantelatto et al. 1995b, Fracasso & Branco 2005, Klôh & Di Beneditto 2010, Lima et al. 2014a), morphometry (Mantelatto & Fransozo 1992, 1994), feeding strategies (Mantelatto & Petracco 1997), reproductive biology (Reigada & Negreiros-Fransozo 1999, 2000, Lima et al. 2014b), and growth and mortality (Keunecke et al. 2007). A few studies focused on *H. pudibundus* distribution (Mantelatto et al. 1995a, Lima et al. 2014a), but none of them included areas of 20 m in depth or deeper.

Aiming to test the hypothesis of differential ocupation, the temporal and bathymetric distribution of *H. pudibundus* were evaluated and correlated with environmental factors (bottom and surface water temperature and salinity, and sediment texture and organic matter) in Ubatuba region. This region is ideal for ecological studies using benthonic populations as a baseline to understand distribution patterns and ecological relationships. Once the profile of an abundant species is known, it can be used in future monitoring and in the evaluation of possible natural/anthropic impacts, as well as serve as comparative parameter for other similar decapod populations.

Material and Methods

1. *Study area*

The northern coast of São Paulo, in the southeast of Brazil, and specially the Ubatuba region, encompasses a variety of environments such as pristine islands, bays, inlets, sandy and rocky beaches, mangroves, estuaries, and coastal rivers. Due to this spatial heterogeneity, it has a high biodiversity of decapod crustaceans (Mantelatto et al. 2018). The sediment in this region is composed by fine or very fine sand and silt and clay, given the low water movement within the area and among the regions (Mahiques et al. 1998).

This region is under the influence of three water masses: Coastal Water (CW = temperature > 20°C; salinity < 36), Tropical Water (TW = temperature > 20°C; salinity > 36), and South Atlantic Central Water (SACW = temperature < 18°C; salinity < 36) (Castro-Filho et al. 1987; Odebrecht & Castello 2001; De Léo & Pires-Vanin 2006). During late spring and early summer, the SACW penetrates the bottom layer of the coastal region and forms a thermocline over the inner shelf at depths of 10–15 m (Castro-Filho et al. 1987). During winter, the SACW retreats to the shelf break and is replaced by the CW. As a result, no stratification is present over the inner shelf during the winter months (Pires 1992; Pires-Vanin & Matsuura 1993).

2. *Data collection*

Samplings were done monthly in Ubatuba (23°26'75"S; 44°59'00"W) with a fishing boat equipped with two double rig nets, from January to December 2000. A total of nine transects (2 km each) were stablished and trawled over a 30-min period covering a sampling area of about 18000 m^2. Samplings comprised depths of 2, 5, 10, and 15 m (in the internal area of the bay), and 20, 25, 30, 35 and 40 m (in the external area) (Figure 1). An ecobathymeter coupled with a GPS was used to record depth at sampling sites. For the analysis of environmental factors, sediment and surface and bottom water samples were taken from each transect before trawling. Water was collected with a Nansen

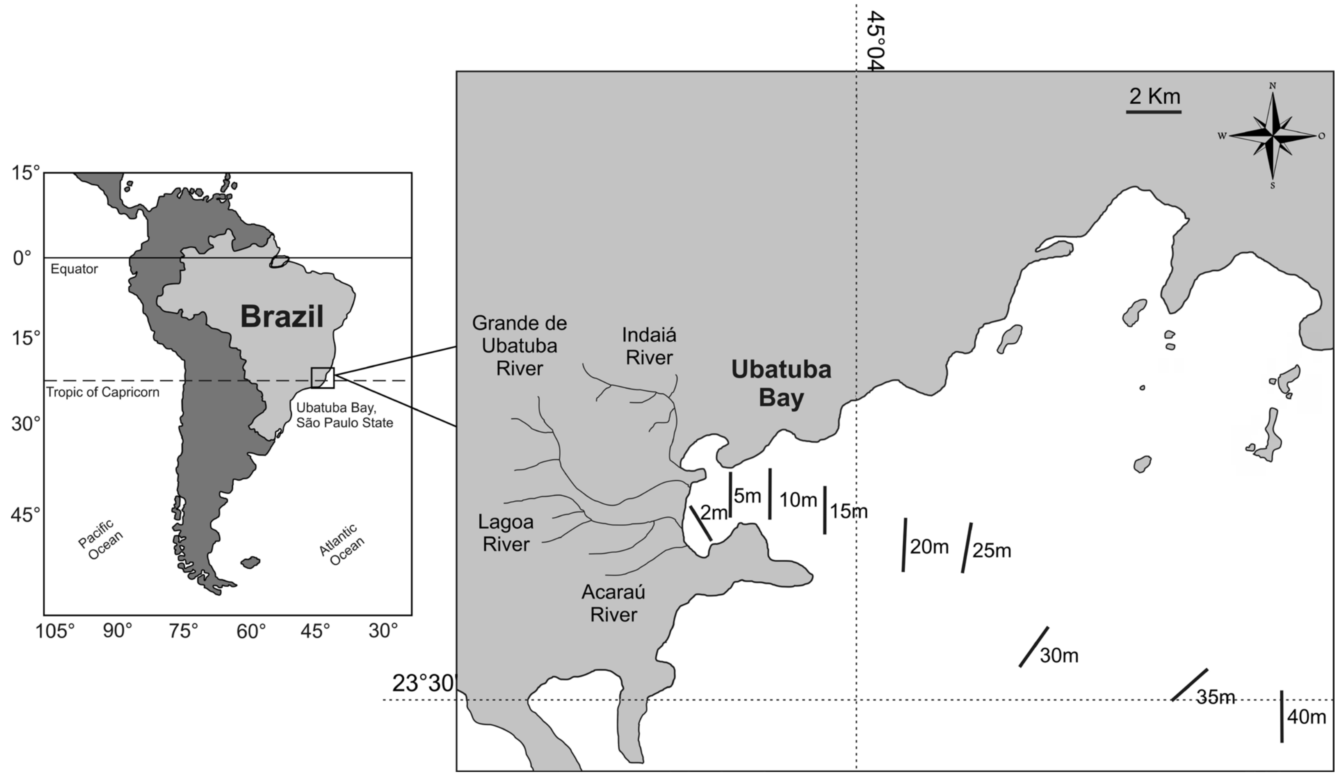

Figure 1. Map of South America. In detail, the Ubatuba coast in São Paulo, Brazil, indicating the location and depth of the transects. (Modified from Lima et al. 2014c).

bottle to obtain temperature and salinity, measurements with the ad of a mercury thermometer (ºC) and salinity and an optical refractometer (‰), respectively.

The method used to measure the texture of the sediment was similar to that described by Hakanson & Jansson (1983) and Tucker (1988). Sediment samples were collected at each transect with a Van Veen grab (0.063 m^2) for sediment grain size composition and organic matter content determination. Samples were transported to the laboratory and oven-dried at 70 °C for 72 h. To analyze the grain size distribution, two 50 g subsamples were treated with 250 ml of NaOH solution (0.2 mol l^{-1}), stirred for 5 min to release silt and clay particles, and rinsed over a 0.063 mm sieve. The grain size was classified according to the Wentworth (1922) scale: > 2 mm (gravel), 1.0–2.0 mm (very coarse sand), 0.5–1.0 mm (coarse sand), 0.25–0.5 mm (medium sand), 0.125–0.25 mm (fine sand), 0.125–0.063 mm (very fine sand). Smaller particles were classified as silt and clay.

Grain diameter was expressed in phi (φ) values (were calculated from the formula phi = -log 2 d, where d= grain diameter in mm), and the following classes were obtained: $-2 \leq \text{phi} < -1$ (gravel), $-1 \leq \text{phi} < 0$ (very coarse sand), $0 \leq \text{phi} < 1$ (coarse sand), $1 \leq \text{phi} < 2$ (medium sand), $2 \leq \text{phi} < 3$ (fine sand), $3 \leq \text{phi} < 4$ (very fine sand), and $\text{phi} \geq 4$ (silt and clay). From the cumulative distribution curves of these classes to, the 16th, 50th and 84th percentiles were extracted and the mean diameter (md) was calculated with the formula: $MD = (\text{phi}_{16} + \text{phi}_{50} + \text{phi}_{84}) / 3$ (Suguio 1973). The three most quantitatively important sediments were defined according to Magliocca & Kutner (1965): Class A corresponds to sediments in which medium sand (MS), coarse sand (CS), very coarse sand (VCS) and gravel (G); in class B, fine sand (FS) and very fine sand (VFS) and class C with silt and clay (S+C). Using these three classes, further groups were established according to the combination of granulometric fractions in several proportions: PA = (MS+CS+VCS+G) > 70%; PAB = prevalence of A over B (FS+VFS); PAC = prevalence of A over C (S+C); PB = (FS+VFS) > 70%; PBA = prevalence of B over A; PBC = prevalence of B over C; PC = (S+C) > 70%; PCA = prevalence of C over A; PCB = prevalence of C over B. For organic matter content determination, we put 10 g subsamples in porcelain containers, previously individually identified and weighed. Incinerated in an oven (500 ºC for 3 hours) and weighed again. The difference between the initial and final weigh indicated the organic matter content of each sample, which was expresses as a proportion of the initial weight (Hieri et al. 2001). The obtained data were grouped by season of the year as follows: January−March = summer, April−June = autumn, July−September = winter, and October−December = spring. The specimens of *Hepatus pudibundus* (Figure 2) were identified following Melo (1996) and sorted by sex, based on abdominal morphological features (male = triangular-shaped abdomen; female = round-shaped abdomen), and number of pleopods (males = two pairs; females = four pairs).

3. *Data analysis*

Prior the analyses the data were tested for normality (Shapiro-Wilk's test) and homoscedasticity (Levene's test) (Zar 1999). Abundance, bottom and surface temperature and bottom salinity were compared between months and transects using the Friedman test ($\alpha = 0.05$). Moreover, the phi-values were compared between transects also using the Friedman test ($\alpha = 0.05$) (Zar 1999). We used a Redundancy Analysis (RDA) to detect possible relationships between the abundance of *H. pudibundus* and the environmental variables. This analysis requires the

Figure 2. *Hepatus pudibundus* (Herbst, 1785). Adult male (carapace width 72.7 mm).

existence of two dependent variables, at least. Thus, we grouped the individuals into males (M) and females (F). The RDA produces final coordination scores that summarize the linear relationship between the explanatory and response variables. Only environmental variables with scores higher than 0.4 and lower than -0.4 were considered as biologically significant (Rakocinski et al. 1996). This analysis was performed using the Vegan (Oksanen et al. 2012) package for R (R Development Core Team, 2012).

Results

Along the year there were significant differences between surface (Friedman test Fr = 93.4; p = 0.0001) and bottom water temperature (Friedman test Fr = 51.84; p = 0.0001). Moreover, a thermocline was seen from January through April (Figure 3A, B). Bottom temperatures decreased gradually with the increase in depth (Friedman test Fr = 54.8; p = 0.0001), whereas surface temperature was homogeneous (Fr = 13.4; p = 0.0974) (Figure 3C, D). Spatially, bottom salinity was lower at 2 m (33.1‰) and higher at 40 m of depth (35.4‰) (Friedman test; Fr = 20.4; p = 0.0089) (Figure 4).

Phi-values differed between transects (Fr = 71.68; p = 0.0001). The highest proportions of sediment silt and clay occurred in association with high organic matter content in the 5−10 m deep transects. Below 10 m of depth, these proportions decreased. Most transects were characterized by a high amount of silt and clay, except the transects 30 and 35 m deep, which had lower phi-values (Figure 5), i.e., a coarser sediment grain size composition.

In total, 1808 crabs were collected. There were no significant differences in abundance between months (Friedman test; Fr = 11.4; p = 0.4102) or seasons (Friedman test; Fr = 5.9; p = 0.1166) (Table 1). There was, however, a significant variation in abundance between transects (Friedman test; Fr = 34.0; p = 0.0001) (Table 1). Higher *H. pudibundus* abundances were associated with bottom temperatures of 19−22 ºC, salinities of 38−40 ‰, sediment phi of 3.08−4.48, and organic matter content of 2.90−7.15% (Figure 6). The highest abundance was recorded in winter at 15 m of depth, and the lowest in spring/summer at 30−40 m of depth. Nonetheless, the species showed a wide distribution, being found in all seasons. In spring and summer, the crabs were more abundant in the transects 10−20 m deep where the organic matter content was higher (Figure 7). As can be seen in the RDA Axis 1 (94.4 %), the abundance of males and females was correlated with sediment phi and organic matter content, and these two factors were the most important determinants of *H. pudibundus* distribution (Table 2).

Discussion

Hepatus pudibundus showed a wide spatio-temporal distribution but its abundance varied according to variation in environmental factors. In Ubatuba there are several bays and inlets which form a semi-enclosed region water circulation difficult (Mahiques 1995). These characteristics favor the entrapment of urban sewage coming from the Northern coast of São Paulo, which increases the food availability to detritivores and filter-feeders (Negreiros-Fransozo et al. 1991). Also, environments with a high content of organic matter favor the establishment and development of a variety of organisms (Negreiros-Fransozo et al. 1991). Therefore, the establishment of this species may be due to the good conditions in this place, since this species is an opportunistic predator (Mantelatto & Petracco 1997).

According to Fransozo et al. (2012), the influx of this nutrient-rich cold-water mass may influence the composition and abundance of decapod crustaceans. Although temperature was not a significant factor, the decrease in *H. pudibundus* abundance during spring and summer may have been related to the influx of SACW, which caused a seasonal migration to shallower, warmer areas, where the organic matter increases in this period. During summer, and with the entrance of SACW, abundant salpids (Tunicata, Salpidae), among other diverse invertebrate groups, feed on phyto- and nanoplankton, and their decomposition and defecation form fecal pellets that are incorporated into the sediment as organic matter and benefit the benthos (Pires-Vanin et al. 1993).

The higher abundance in deeper areas during autumn and winter may be explained by the action of currents and high amplitude waves, which swirls the sediment and suspends the abundant detritivore and filter-feeding macrofauna (Pires et al. 1993) that are food sources for many decapods, induding *H. pudibundus*. Moreover, as in these seasons the temperature becomes more homogeneous, as well as *H. pudibundus*, other carnivorous species are more predominant in the inner platform, such as, *Callinectes ornatus* Ordway, 1863; *Achelous spinimanus* (Latrelle, 1819) and *Libinia spinosa* Guérin, 1832 (Pires-Vanin et al. 1993, Petti et al. 1996).

The low abundance seen in the very shallow transects (2 m deep) may have been caused by the freshwater influx coming from four rivers that form estuaries in Ubatuba and decrease salinity (Mantelatto et al. 1995a). The same author also found a lower abundance near estuaries in Fortaleza Bay, in the northern coast of São Paulo. Many authors consider sediment texture and organic matter content as the causes of abundance fluctuations of benthonic species (Ishikawa 1989, Fransozo et al. 1992, Costa et al. 2005). In our study, 72% of the individuals were captured between 10−25 m of depth. The sediment in these transects was composed mainly by silt and clay, and fine and very fine sand, which usually to have a higher percentage of organic matter (Castilho et al., 2008). Therefore, these areas are favorable for *H. pudibundus*, offering food and shelter for a species that buries into the sediment (Mantelatto et al. 1995a). Moreover, Mantelatto & Petracco (1997), which investigated

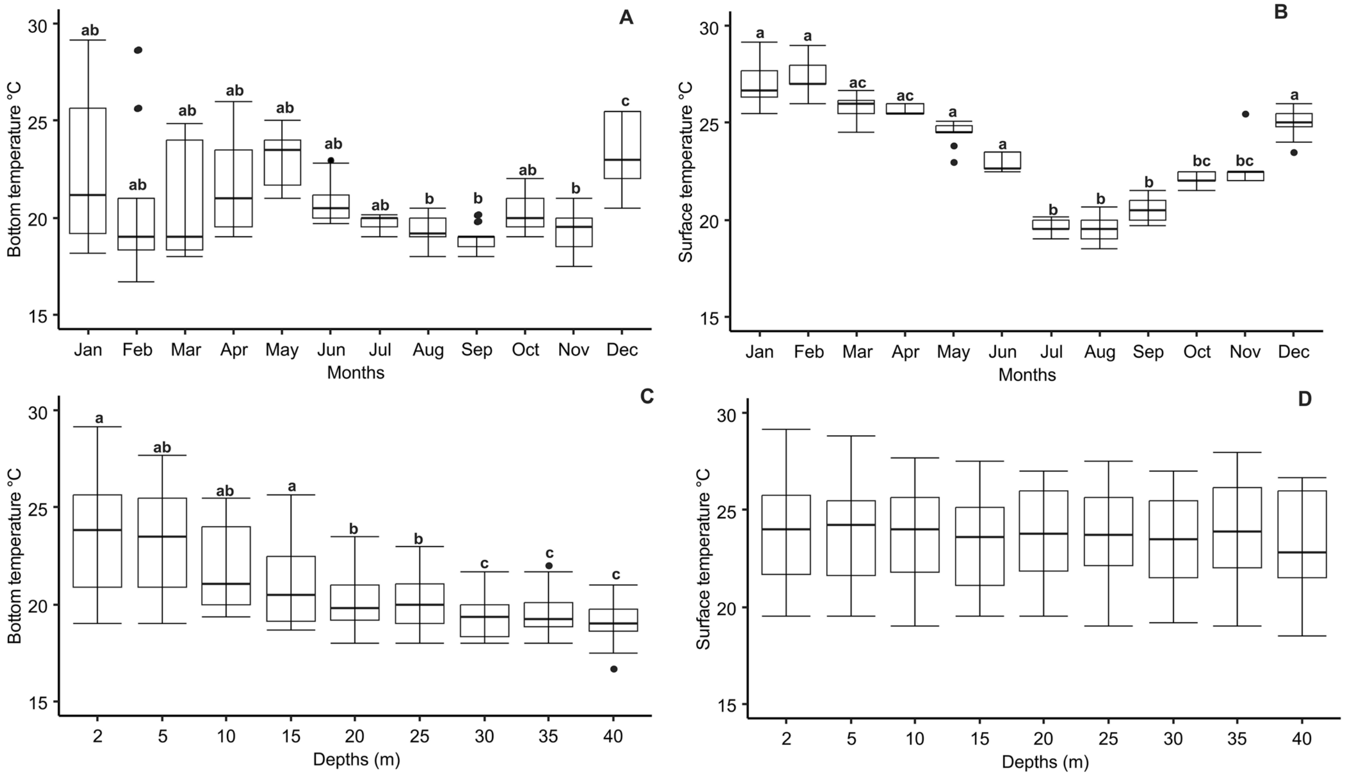

Figure 3. Boxplots of bottom (BT) and surface (ST) temperature (median, minimum and maximum) per month (A and B) and per transect (C and D) in January-December 2000, in the region of Ubatuba, São Paulo, Brazil (Friedman Test; different letters indicate a statistically significant difference).

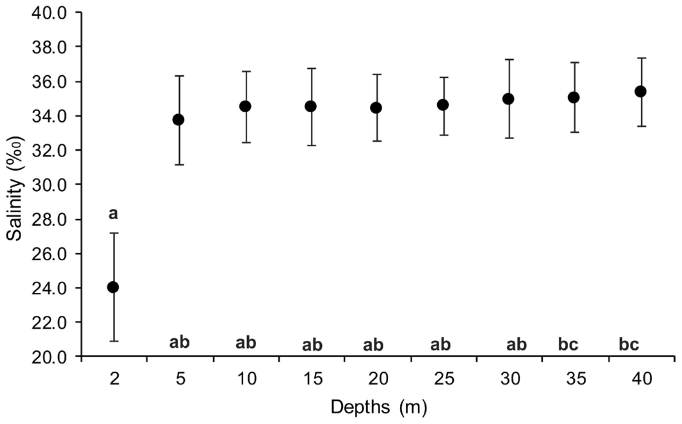

Figure 4. Variation in mean salinity according to depth, Ubatuba, 2000.

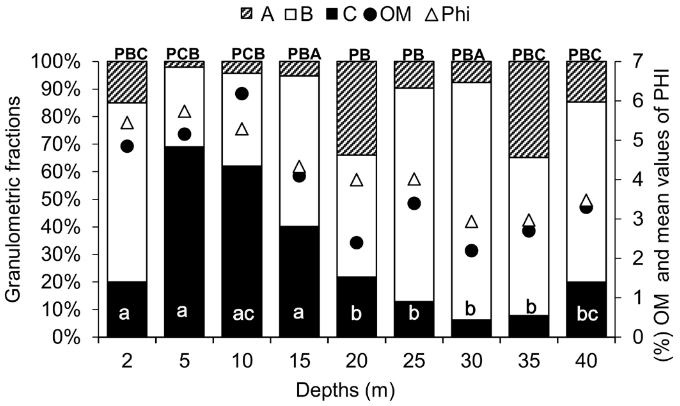

Figure 5. Granulometric fractions, organic matter content (% OM), and sediment mean grain size (Phi) of each transect. Grain-size classes (%) are: class A (sand, coarse sand, very coarse sand, and gravel), class B (fine sand and very fine sand), and class C (silt and clay).

the diet of *H. pudibundus*, concluded that this species is an opportunistic predator which has a large variety of preys, including mollusks, annelids, and foraminiferans, which are more abundant in finer sediments.

In our study there was a positive correlation between the abundance and phi (sediment texture) and organic matter content, corroborating the findings in close areas, as made by Mantelatto et al. (1995a) in Fortaleza Bay, where *H. pudibundus* was also more abundant in regions with higher organic matter content. On the other hand, Lima et al. (2014a) found no significant relationship, probably because their sampling was restricted to depths of 20 m or less. Nonetheless, they found the higher number of individuals in Mar Virado Bay, in which the silt and clay fraction predominates. Like *H. pudibundus*, other species of crabs that are buried such as *Arenaeus cribrarius* (Lamarck 1818) by Pinheiro et al. (1996), *Callinectes danae* Smith, 1869 by Chacur & Negreiros-Fransozo (2001), and *L. spinosa* by Braga et al. (2007), are also most frequently found in areas with finer sediments along the Northern coast of São Paulo.

According to McNaughton & Wolf (1970), the dominance of certain species may be explained by two non exclusive hypotheses. The first postulates that dominant species are generalist and can tolerate varied environmental conditions, and the second one says they are specialists

Table 1. *Hepatus pudibundus* (Herbst 1785). Number of individuals per month and sampling station captured from January until December 2000 (2, 5, 10, 15 (internal area), 20, 25, 30, 35 and 40 m (external area)). (Results of Friedman = Fr test, different letters indicate a statistical significant difference (p < 0.05).

Season	Months	Internal area				External area					Total	
		2m	5m	10m	15m	20m	25m	30m	35m	40m	Month	Season
Summer	Jan	0	0	47	39	7	0	0	1	0	94	367
	Feb	0	15	6	67	23	5	0	0	0	116	
	Mar	3	11	94	38	5	4	2	0	0	157	
Autumm	Apr	0	5	11	30	43	4	43	1	0	137	461
	May	0	2	7	2	2	24	26	50	90	203	
	Jun	0	4	0	78	3	1	0	35	0	121	
Winter	Jul	5	14	15	85	2	66	1	3	64	255	549
	Aug	0	34	7	23	38	65	3	7	0	177	
	Sep	15	2	8	84	0	8	0	0	0	117	
Spring	Oct	3	9	33	111	73	23	0	1	0	253	431
	Nov	24	27	4	20	13	24	4	0	0	116	
	Dec	1	4	12	17	19	8	0	1	0	62	
Total		51	127	244	594	228	232	79	99	154	1808	1808
Fr Test		(A)	(AB)	(AB)	(B)	(AB)	(AB)	(A)	(A)	(A)		

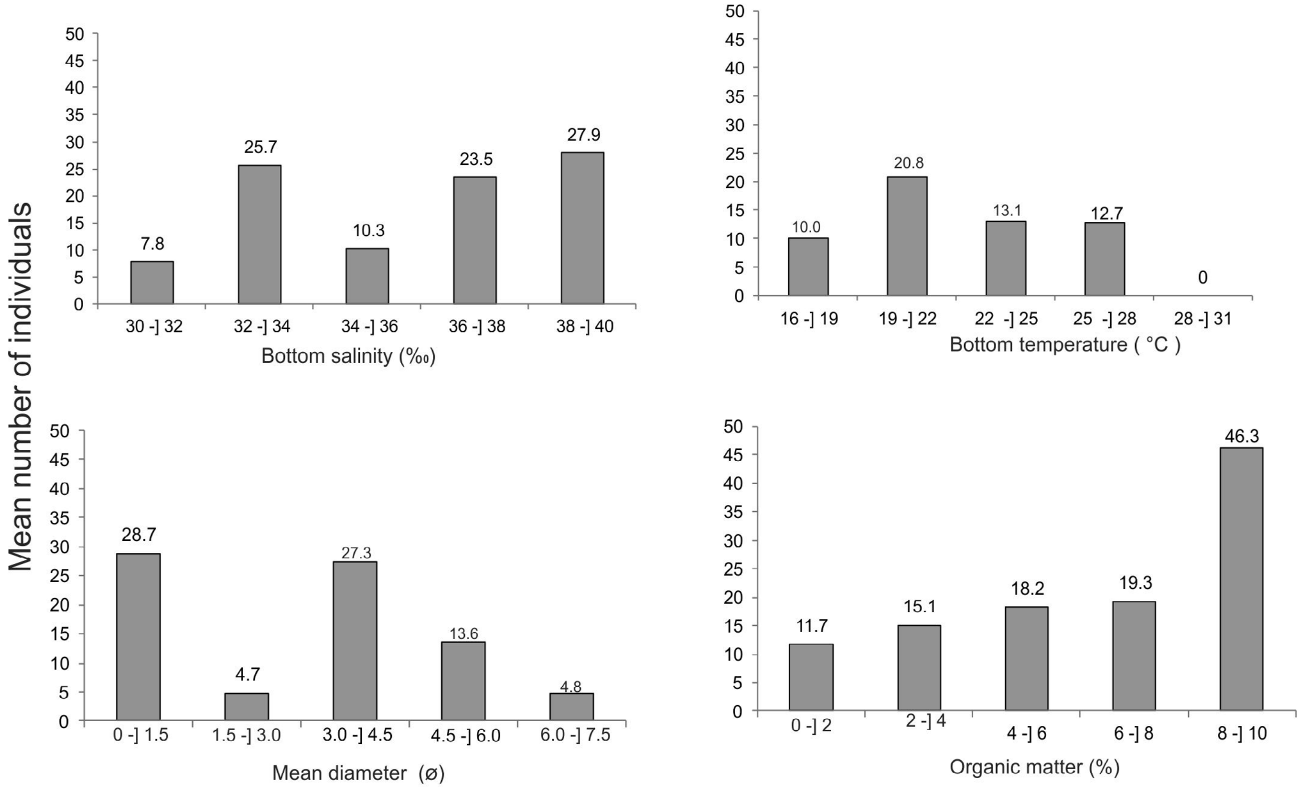

Figure 6. *Hepatus pudibundus* (Herbst, 1785). Mean number of individuals per trawl for each class of environmental factors in Ubatuba, State of São Paulo, Southeastern Brazil (January-December 2000).

and adapted to a narrower set of conditions. In general, areas deeper than 20 m in Ubatuba suffer a high influence of sea currents, resulting in more heterogeneous sediments with predominance of fine and very fine sand, and low organic matter content (Furtado & Mahiques 1990). As *H. pudibundus* is a burrowing species, coarse sandy sediments with dendritic fragments may hinder its establishment (Bertini & Fransozo 2004) thus, sheltered bays with a heterogeneous substrate may be unfavorable to this species (Lima et al. 2014a).

Our results strong support the hypothesis that the distribution of *H. pudibundus* is modulated by abiotic factors, especially those related to the sediment. It's worth mentioning that Ubatuba has been under an intense urbanization process, which will likely affect the local marine

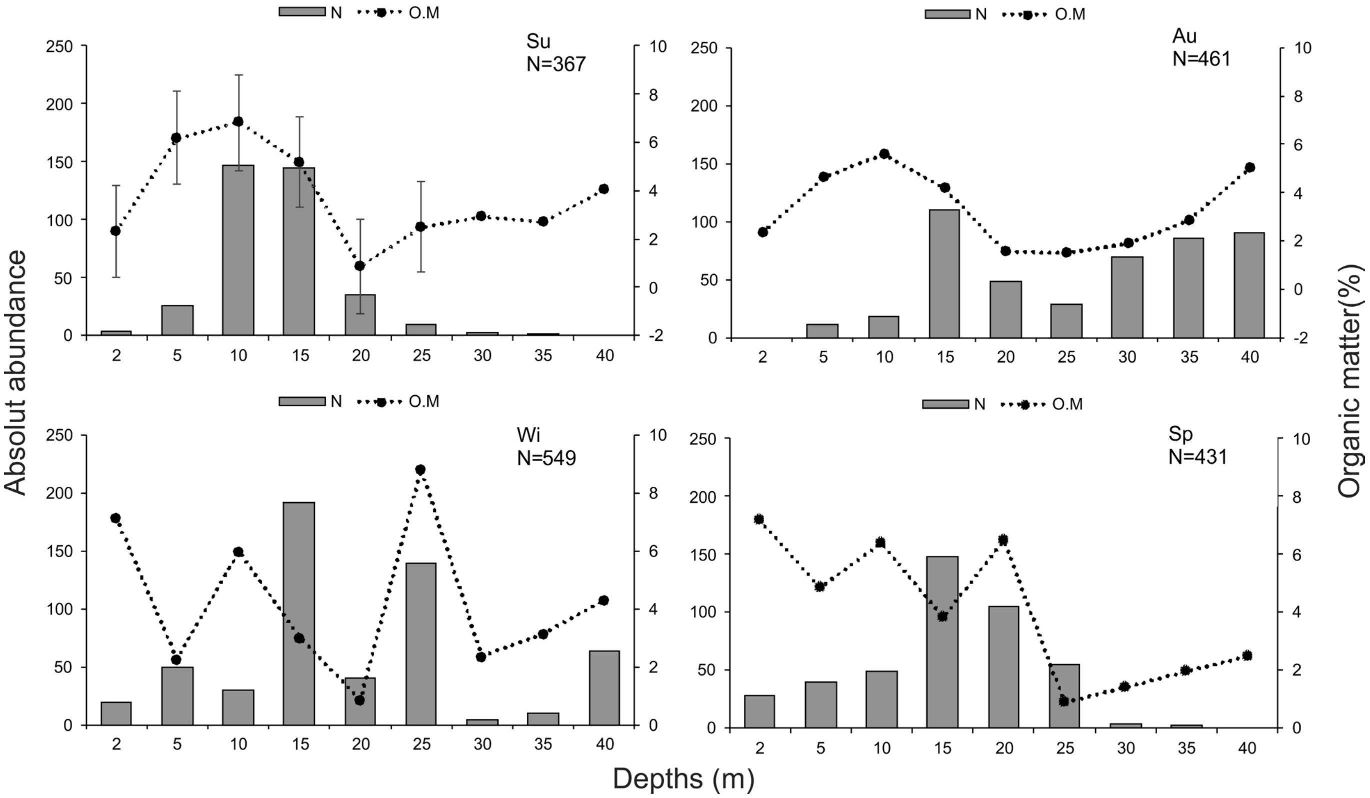

Figure 7. *Hepatus pudibundus* (Herbst, 1785). Seasonal distribution according to depth and organic matter content. Bars indicate the number of crabs and dots indicate the organic matter content (N = number of crabs, OM = Organic matter, Su = Summer, Au = Autumn, Wi = Winter, Sp = Spring).

Table 2. *Hepatus pudibundus* (Herbst 1785). Redundancy Analysis (RDA) showing the relationship between demographic groups and environmental variables, in Ubatuba Bay, Sao Paulo, Brazil.

	Axis 1	**Axis 2**
Eigenvalue	0.020	0
Proportion Explained	0.944	0.056
Cumulative Proportion	0.944	1
Abundance of individuals		
Males	-0.329	-0.091
Females	-0.376	0.079
Environmental variables		
Botton temperature (BT)	0.105	-0.132
Botton salinity (BS)	-0.213	-0.638
Organic Matter (OM)	**-0.872**	0.237
Phi	**-0.440**	-0.475

* Values in bold indicate the variables considered as biologically significant (i. e. > 0.4 and < - 0.4) Rakocinski et al. (1996).

diversity in different ways. Thus, only through comprehensive and rigorous studies on key populations and communities of commercial and/or ecological importance it will be possible to stablish conservation strategies and protected areas. Also, abundant species could be used as bioindicators, to ensure that natural resources are used in a sustainable way, and that the natural biodiversity is being protected against natural and anthropic actions.

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Author Contributions

Substantial contribution in the concept and design of the study: All authors.

Adilson Fransozo: Contribution to data collection.

Veronica Pereira Bernardes, Fernando Luis Mantelatto, Thiago Elias da Silva, Aline Nonato de Sousa, Camila Hipólito Bernardo and Adilson Fransozo: Contribution to data analysis and interpretation.

Veronica Pereira Bernardes, Fernando Luis Mantelatto, Thiago Elias da Silva, Aline Nonato de Sousa, Camila Hipólito Bernardo and Adilson Fransozo: Contribution to manuscript preparation.

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Conflicts of interest

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

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Inventory

Dragonflies and damselflies (Insecta: Odonata) from a Cerrado area at Triângulo Mineiro, Minas Gerais, Brazil

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Abstract: Odonata is considered, among the aquatic insect orders, the second largest group in number of species. Its global richness is estimated in about 6,000 described species. The Brazilian richness represents around 14% of the world's odonatofauna, however, the knowledge on Brazilian dragonflies distribution is still poor. This study purpose an inventory of the dragonflies species present in aquatic habitats from a Preserved Area according to the Brazilian Forest Code, located in the Cerrado biome at Triângulo Mineiro, Minas Gerais. In the dry season, from April to June of 2017, we collected 680 specimens belonging to 36 species and six families. Among the collected species, *Elasmothemis williamsoni* was observed by the first time in Minas Gerais State, and we also found a new species of *Tigriagrion* (Zygoptera: Coenagrionidae) which is being described by taxonomists. Considering the fast agricultural advance over natural Cerrado systems, species lists can be important to define priority conservation areas for odonate species.

Keywords: *inventory, biodiversity, vereda, Odonata.*

Libélulas e donzelinhas (Insecta: Odonata) de uma área de Cerrado no Triângulo Mineiro, Minas Gerais, Brasil

Resumo: Odonata é considerada a segunda ordem de insetos com maior número de espécies de insetos aquáticos. Sua riqueza global é estimada em cerca de 6.000 espécies descritas. A odonatofauna encontrada no Brasil representa cerca de 14% da riqueza mundial, no entanto, o conhecimento sobre a distribuição de libélulas brasileiras ainda é incipiente. Este estudo teve como objetivo um inventário das espécies de libélulas presentes em habitats aquáticos de uma Área Preservada de acordo com o Código Florestal Brasileiro, localizada no bioma Cerrado do Triângulo Mineiro, em Minas Gerais. Na estação seca, de abril a junho de 2017, foram coletados 680 espécimes pertencentes a 36 espécies e seis famílias. Entre as espécies coletadas, *Elasmothemis williamsoni* foi observada pela primeira vez no Estado de Minas Gerais, e foi encontrada também uma nova espécie de gênero *Tigriagrion* (Zygoptera: Coenagrionidae) que está sendo descrita. Considerando o rápido avanço da agricultura sobre os sistemas naturais do Cerrado, as listas de espécies podem ser importantes para definir áreas prioritárias para a conservação de espécies de Odonata.

Palavras-chave: *inventário, biodiversidade, vereda, Odonata.*

Introduction

Among the aquatic insects, Odonata (dragonflies and damselflies) is considered the second largest group in number of species (Djikstra et al 2014). Although the actual number of described species can be close to 7,000 (Kalfman et al. 2008), more recent data shows this number only near 6,000 (Djikstra et al. 2014). In the tropical regions, e.g., Neotropical region (von Ellenrieder 2009a) Odonata shows high diversity, being Brazil the country with the most known species richness: 856 species, which represents around 14% of the global fauna (Pinto 2018).

Studies involving Odonata are raising in Brazil (see Miguel et al. 2017), since odonates are charismatic insects (Corbet 1999), has a well resolved taxonomic status (Garrison et al. 2006, 2010, Lencioni 2005, 2006, 2017) and are easily observed and manipulated in the field (De Marco & Vianna 2005). Many of these studies are focused on describing new species (e.g., Guillermo-Ferreira et al. 2016, Ávila et al. 2017, Vilela et al. 2018), in species inventories (e.g. Vilela et al. 2016), and also in ecological studies (Klein et al. 2018), at different localities in Brazilian biomes, from the Pampas in the southern (e.g. Renner et al. 2017) to the Amazonic rain forest, in the northern Brazil (e.g. Monteiro-Júnior et al. 2014). Overall, these studies are adding more information about the distribution of species in different Brazilian states (Machado 1998, Costa et al. 2000, Costa & Oldrini 2005), and helping to reveal the Odonata Brazilian diversity throughout its biomes. However, even though Odonata is actually one of the most studied insect orders in Brazil, there still is a lack of knowledge about its distribution in many places in the national territory.

One reason to this bias is that many studies that aim to inventory biodiversity are concentrated near locations that offer convenient access, infrastructure, and logistics (Dennis & Thomas 2000, Hortal et al. 2007), and have historical patterns of colonization and inventorying (Bini et al. 2006, Meyer et al. 2015). In Brazil, there is an enormous gap of information about the northeast region, where only some sporadic captures were made (De Marco & Vianna 2005). In Minas Gerais state, most of studies are concentrated in the central and south regions (Souza et al. 2013, Almeida et al. 2013, Bedê et al. 2015). Little is known about the odonatofauna from Triângulo Mineiro, a region located on the Cerrado domain, in the western of the state (e.g., Vilela et al. 2016). The fact that the Triângulo Mineiro is largely occupied by agrosystems increases the priority for Odonata inventories on this area (Silva 2000, De Marco & Vianna 2005).

For instance, the Cerrado biome is a biodiversity hotspot located at the central region of Brazil (Myers et al. 2000). In the past years, many studies were made in relation to the accelerated process of deforestation of the Brazilian Cerrado (Ferreira-Peruquetti & Fonseca-Gessner 2003, Carvalho et al. 2013, Dutra & De Marco Jr. 2015) and those studies shows that Odonata community can be affected by the current environmental disturbances. Furthermore, the lack of knowledge about biodiversity data can strongly influence species distribution models, widely used for analytical and predictive tools in conservation (Peterson et al. 2011). Then, considering the current advance of agrosystems on Cerrado native vegetation and the gaps of knowledge on the distribution of Brazilian dragonfly species, information about species distribution may contribute to the elaboration of effective strategical plans for the conservation (Whittaker et al. 2005, Diniz et al 2010) of the odonates fauna. Therefore, this study aims to provide a checklist of odonate species from a Cerrado area at Triângulo Mineiro, Minas Gerais, where the principal activity is the eucalyptus forests. Additionally, this study aims to contribute with information about the distribution of Odonata at Brazilian Cerrado.

Material and methods

1. *Study area*

The study was made at an eucalyptus farm named Fazenda Nova Monte Carmelo belonging to the company Duratex©, located in five municipalities (Araguari, Estrela do Sul, Indianópolis, Nova Ponte, and Romaria) of Triângulo Mineiro region, Minas Gerais state, Brazil (18°57'S, 47°43W). The silviculture activities of Duratex started about 20 years ago, with the introduction of *Pinus* sp. in the area. After some years, the *Pinus* sp. plantation started to be replaced by *Eucalyptus* sp. and today, almost all the farming area are occupied by eucalyptus silviculture (Duratex S.A. Personal Communication). The Fazenda Monte Carmelo area (52,000 ha) is mainly covered by the eucalyptus farming (38,000 ha) and had left about 13,000 ha of natural Cerrado areas, according to the Brazilian Law nº 12.651, the Forest Code, that demands the conservation of 20% of particular rural properties natural areas, located at Cerrado biome, as a Legal Reserve and the conservation of water bodies, if there is some in the property (Brasil 2012). All the sampling areas were fragments located within the Preserved Area of Nova Monte Carmelo (PANMC) (Figure 1) that is surrounded by a matrix of eucalyptus plantations, and some other farming activities in the neighboring farms, as wheat and corn cultures. The PANMC is covered by Cerrado biotopes, as *stricto sensu* cerrado, campos de murundus and veredas (which are composed by permanently saturated soil, forming small to large ponds). The climate in the region is classified as Aw in the Köppen system, with two distinct seasons: a rainy (October-March) and a dry season (April-September) with annual mean temperature between 20°C and 22°C and rainfall of 1,450 mm (Gottsberger & Silberbauer-Gottsberger 2006, Alvarez et al. 2013).

Altogether, 13 aquatic environments were sampled (Figure 1), that could be natural vereda swamps (VE) or artificial lakes (AL) originated by modifications of the land, made to introduce the eucalyptus farm, and by opening roads. Most of the sampled points were exclusively lentic habitats, however, in three points (S3, S5, S13), we found tight channels with moving water nearby to the lentic habitats. Some of the aquatic environments had a riparian zone composed by native cerrado mature shrubs and trees, and those were classified as shaded areas (SH) whereas others were located in open areas (OP), with fields predominantly covered by grasses, with only few sparse shrubs (Soares et al. 2015). The area of each lake was measured by using *Google Earth Pro*. The area, coordinate, habitat characteristic (VE or AL), and riparian zone vegetation tipe (SH or OP) of each lake are listed on Table 1.

2. *Data collection*

We sampled adult dragonflies from April 2018 to July 2018. The sampling were made during the peak time of Odonata activities, between 10:00h and 15:00h, with the aid of entomological nets used by the two members of the team (Renner et al. 2015). Each site was sampled during three hours, by walking around marginal zones of lakes or flooded

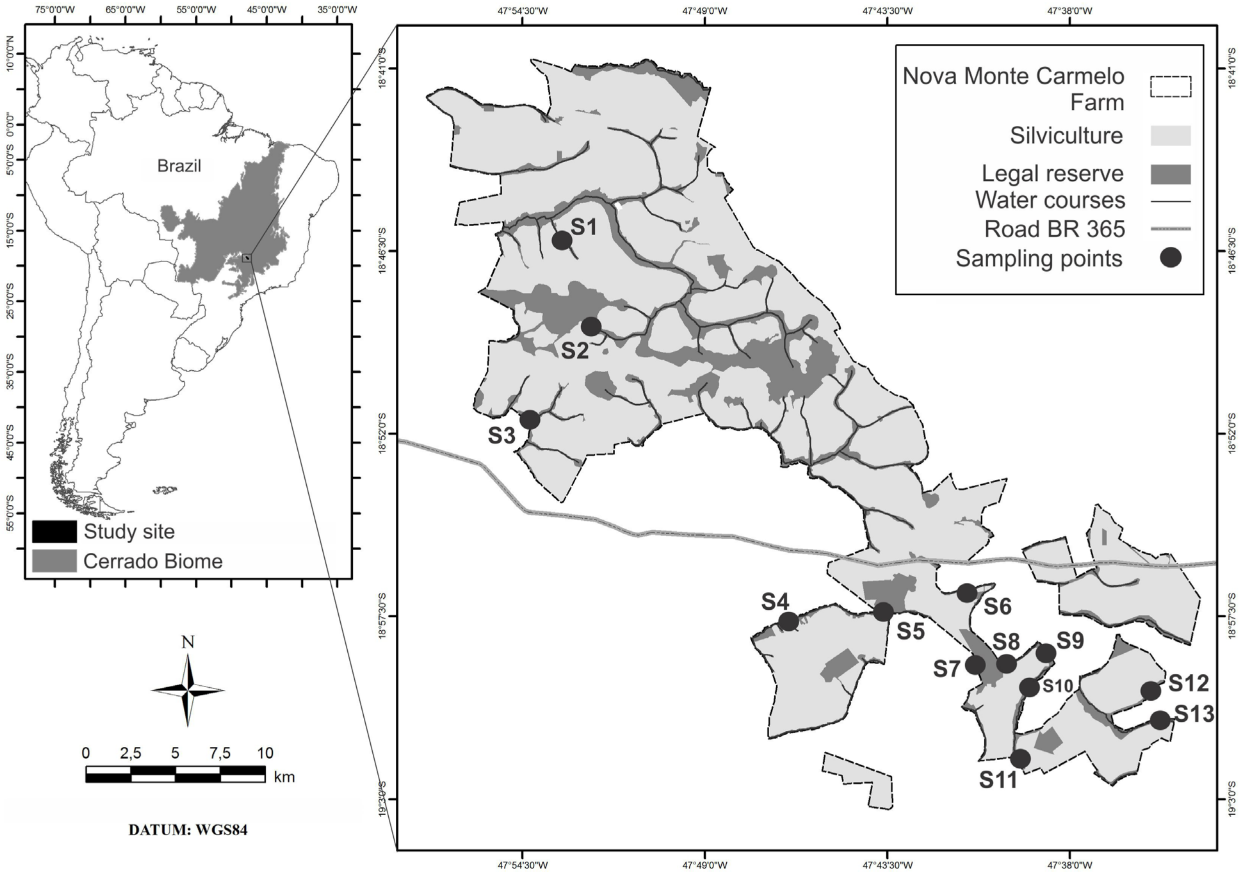

Figure 1. Map of Fazenda Nova Monte Carmelo (MG), Brazil, showing the sampling points, the eucalyptus plantation area, the Legal Reserve, watercourse, and the BR 365 highway.

Table 1. Lakes characteristics. The coordinates and the area of each lake were acquired by using Google Earth Pro. AL: Artificial lake; VE: Vereda; OP: Open area; SH: Shaded area.

Lakes	Aquatic Habitat	Vegetation Tipe	Coordinates	Area
S1	AL	SH	18°46'14"S, 47°53'12"W	4028,7m^2
S2	VE	OP	18°48'35"S, 47°52'17"W	5077,8m^2
S3	VE	SH	18°51'42"S, 47°54'12"W	5077,4m^2
S4	AL	OP	18°57'39"S, 47°46'24"W	246,1m^2
S5	VE	SH	18°57'19"S, 47°43'36"W	19124,9m^2
S6	VE	SH	18°56'42"S, 47°41'07"W	1800,0m^2
S7	AL	OP	18°58'51"S, 47°40'47"W	1309,3m^2
S8	VE	OP	18°58'56"S, 47°39'47"W	1287,7m^2
S9	AL	SH	18°58'40"S, 47°38'42"W	170758,7m^2
S10	AL	OP	18°59'39"S, 47°39'14"W	64062,0m^2
S11	AL	OP	19°01'50"S, 47°39'34"W	67012,2m^2
S12	AL	OP	18°59'48"S, 47°35'46"W	26642,9m^2
S13	AL	OP	19°00'37"S, 47°35'16"W	82613,6m^2

vereda areas. At each hour, we walked a perimeter of 100 meters around the aquatic habitat that was repeated two times. We focused on adults only, since the majority of the larvae are still unknown (Garrison et al. 2006). The collection authorization process was issued by ICMBio, under the number 28398-1.

All specimens collected were preserved in acetone from 6 to 24 hours, according to their size, and later determined to genera level according to Garrison et al. (2006, 2010), and species level according to Lencioni (2005, 2006, 2017) for Zygoptera. Additional Anisoptera identifications were made through specialized literature from each genus (e.g. Costa et al. 2002, von Ellenrieder 2009b). Species data were compared to the original species descriptions if needed. All specimens collected were deposited at the Laboratório de Ecologia – Evolução da Biodiversidade (LEEBIO), at Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.

3. *Statistical analysis*

Collection efforts and the number of sampled species were based on the richness percentage estimated by the mean of non-parametric estimator building the collector's curve by Jackknife1 to estimate the actual number of species. The Jackknife1 and a rarefaction curve were calculated using the Software Estimates (Colwell 2009), with 1000 repetitions.

Results

In total, we sampled 680 specimens belonging to 6 families, 21 genera and 36 species (Table 2). Zygoptera were collected in greatest abundance (n = 500), followed by Anisoptera (n = 180). The family Coenagrionidae was the richest in species (15), followed by Libellulidae (13), Calopterygidae (3), Lestidae (2), Aeshnidae (2) and Gomphidae (1). The most abundant family was Coenagrionidae (n = 450) followed by Libellulidae (n = 175), Lestidae (n = 42), Calopterygidae (n = 10), Gomphidae (n = 4) and Aeshnidae (n = 2). Some of the sampled species are represented in Figure 2.

The most representative specie was *Telebasis carmesina* Calvert 1909 (Coenagrionidae), with 130 individuals, followed by *Acanthagrion truncatum* Selys, 1876 (Coenagrionidae), with 121, *Oxyagrion microstigma* Selys, 1876 (Coenagrionidae) with 90, *Erythrodiplax castanea* Burmeister, 1839 (Libellulidae) with 59 and *Erythrodiplax latimaculata* Ris, 1911 (Libellulidae) with 49 specimens. Those numbers represents 66% of the total collected specimens.

Distinctly, some species were less represented in abundance of specimens. The species *Acanthagrion temporale* Selys, 1876, *Oxyagrion santosi* Martins, 1967, *Oxyagrion terminale* Selys, 1876, *Homeoura chelifera* Selys, 1876, *Tigriagrion* sp. nov., *Mnesarete pudica* Hagen in Selys, 1853, *Elasmothemis williamsoni* Ris, 1919, *Erythrodiplax ana* Guillermo-Ferreira & Vilela 2016, *Micrathyria catenata* Calvert, 1909, *Micrathyria hesperis* Ris, 1911, *Orthemis discolor* Burmeister, 1839, *Anax amazili* Burmeister 1839 and *Remartinia luteipennis* Burmeister 1839, which represents 36% of the species richness, were represented by only one specimen each (singletons) at sampling. The species *Cyanallagma nigrinuchale* Selys, 1876, *Erythrodiplax fusca* Rambur, 1842 and *Idiataphe longipes* Hagen, 1861 were represented by two specimens (doubletons) each.

Although the samples were made at lentic systems, in three sampled points we saw tight channels with moving water close to the lentic habitats. On those points, we collected *Hetaerina longipes* Hagen & Selys 1853, *Hetaerina rosea* Selys 1853, *Mnesarete pudica*, and *Argia lilacina* Selys, 1865, which are known to inhabit lotic environments, and a new species from the *Tigriagrion* genera.

The new species of *Tigriagrion* was collected in a vereda located in a shaded preserved Cerrado fragment, surrounded by high diversity of native plants, most trees and shrubs. The species are currently under description by taxonomists.

Some of the species found are widely spread in the national territory, as *Acanthagrion gracile* Rambur, 1842, *A. truncatum*, *A. amazili*, *E. castanea, E. fusca*, *E. juliana* Ris, 1911, *E. latimaculata*, *H. rosea*, *Ischnura fluviatilis* Selys, 1876, *M. hesperis*, *Oligoclada abbreviata* Rambur, 1842, and *O. discolor*. Others can be considered rare species, having more restricted distribution, as *E. williamsoni*, *E. ana* and *C. nigrinuchale*. Among the rare species, we found a new register for Minas Gerais state: *Elasmothemis williamsoni* (Libellulidae), that was previously collected in other Cerrado areas at Mato Grosso do Sul and Goiás States.

The rarefaction curve, which was based on the sampling events data, gave us a view of the sampling effectiveness of this study (Figure 3). The estimator showed that the richness found corresponds to 67% of the medium estimated richness by Jackknife 1 (Sest = 53.5 ± 3.93).

Discussion

In general, the number of species in a single assemblage (pool) varies strongly in Brazilian Cerrado habitats, from as small as 26 and 31 species (Almeida et al. 2013, Vilela et al. 2016) to as numerous as 50-80, generally at sites with more sampled areas (Calvão et al. 2013, Carvalho et al. 2013, Dutra & De Marco 2015, Ferreira-Peruquetti & Fonseca-Gessner 2013) (Table 3). The number of species recorded in our study can be considered intermediary based on the small sampling effort, and the fact that we cover only half of the preserved area, indicating that the PANMC can present a rich pool of odonates, when compared to other places in Minas Gerais (Almeida et al. 2013, Souza et al. 2013). Additionally, there are many distinct Cerrado biotopes found around the aquatic habitats in the RLNMC, e.g, cerrado *strictu sensu* and campos de murundus, creating a gradient that could increase the diversity of odonates (Bedê et al. 2015).

Coenagrionidae has the greatest species number for the suborder Zygoptera in Brazil (Lencioni 2006). For instance, in our study, *Acanthagrion lancea, A. truncatum, Oxyagrion microstigma* and, *Telebasis carmesina* (Coenagrionidae), represented 50% of all collected specimens. These species were also widely found at Cerrado area in other studies (Ferreira-Peruquetti & Fonseca-Gessner 2003, Calvão et al. 2014, Dutra & De Marco 2015, Vilela et al. 2016, Rodrigues & Roque 2017). Females of these Coenagrionidae species oviposits in aquatic plants from *Eleocharis* genera (Guillermo-Ferreira & Del-Claro 2011, Vilela et al. 2016), which were commonly found at aquatic habitats in the study area. Probably, this may explain the high abundance of those species in our results. On the other hand, some other Zygoptera species, as *Cyanallagma nigrinuchale,* and *Telebasis coccinea* were collected in lower quantity. These species were restrict to shaded riparian areas

Table 2. Inventory list of Odonata species from Fazenda Nova Monte Carmelo, Minas Gerais (MG), Brazil, showing the localities, the characteristics of aquatic habitat and vegetation type where each species were collected, and the collection identity given to the species. AL: Artificial lake; VE: Vereda; OP: Open area; SH: Shaded area.

Suborder	Family	Species	Amostrated points	Aquatic habitat	Vegetation type	Collection ID
ZYGOPTERA	Calopterygidae	*Hetaerina longipes* Hagen in Selys, 1853	S5	VE	SH	DS2017001
		Hetaerina rosea Selys, 1853	S5	VE	SH	DS2017002
		Mnesarete pudica (Hagen in Selys, 1853)	S13	AL	OP	DS2017003
	Coenagrionidae	*Acanthagrion gracile* (Rambur 1842)	S5, S6	VE	SH	DS2017004
		Acanthagrion lancea Selys, 1876	S3, S5-S13	VE, AL	OP, SH	DS2017005
		Acanthagrion temporale Selys, 1876	S9	AL	SH	DS2017006
		Acanthagrion truncatum Selys, 1876	S1-S13	VE, AL	OP, SH	DS2017007
		Argia lilacina Selys, 1865	S3, S5, S13	VE, AL	OP, SH	DS2017008
		Cyanallagma nigrinuchale (Selys, 1876)	S5	VE	SH	DS2017009
		Homeoura chelifera (Selys, 1876)	S13	AL	OP	DS2017010
		Ischnura capreolus (Hagen, 1861)	S6, S8-S10, S12, S13	VE, AL	OP, SH	DS2017011
		Ischnura fluviatilis Selys, 1876	S11, S13	AL	OP	DS2017012
		Oxyagrion microstigma Selys, 1876	S1-S10, S12, S13	VE, AL	OP, SH	DS2017013
		Oxyagrion santosi Martins, 1967	S3	VE	SH	DS2017014
		Oxyagrion terminale Selys, 1876	S3	VE	SH	DS2017015
		Telebasis carmesina Calvert, 1909	S1, S2, S5-S10, S11, S12	VE, AL	OP, SH	DS2017016
		Telebasis coccinea (Selys, 1876)	S6	VE	SH	DS2017017
		Tigriagrion sp. nov.	S3	VE	SH	DS2017018
	Lestidae	*Lestes auritus* Hagen in Selys, 1862	S2, S9, S10	VE, AL	OP, SH	DS2017019
		Lestes forficula Rambur, 1842	S2, S7, S10	VE, AL	OP, SH	DS2017020
ANISOPTERA	Aeshnidae	*Anax amazili* (Burmeister, 1839)	S1, S10	AL	OP, SH	DS2017021
		Remartinia luteipennis (Burmeister, 1839)	S10	AL	OP	DS2017022
	Gomphidae	*Cacoides latro* (Erichson in Schomburgk, 1848)	S1	AL	SH	DS2017023
	Libellulidae	*Elasmothemis williamsoni* (Ris, 1919)	S6	VE	SH	DS2017024
		Erythrodiplax ana Guillermo-Ferreira & Vilela 2016	S3	VE	SH	DS2017025
		Erythrodiplax castanea (Burmeister, 1839)	S1-S3, S5-S13	VE, AL	OP, SH	DS2017026
		Erythrodiplax fusca (Rambur, 1842)	S7	AL	OP	DS2017027
		Erythrodiplax juliana Ris, 1911	S1-S3, S6-S10, S13	VE, AL	OP, SH	DS2017028
		Erythrodiplax latimaculata Ris, 1911	S1-S3, S5-S9, S12	VE, AL	OP, SH	DS2017029
		Idiataphe amazonica (Kirby, 1889)	S2, S9, S11	VE, AL	OP	DS2017030
		Idiataphe longipes (Hagen, 1861)	S9	AL	SH	DS2017031
		Micrathyria catenata Calvert, 1909	S7	AL	OP	DS2017032
		Micrathyria hesperis Ris, 1911	S2	VE	OP	DS2017033
		Oligoclada abbreviata (Rambur, 1842)	S1, S2, S4, S7, S8	VE, AL	OP	DS2017034
		Orthemis discolor (Burmeister, 1839)	S3	VE	SH	DS2017035
		Tramea binotata (Rambur, 1842)	S1, S2, S3, S5, S8, S12	VE, AL	OP, SH	DS2017036

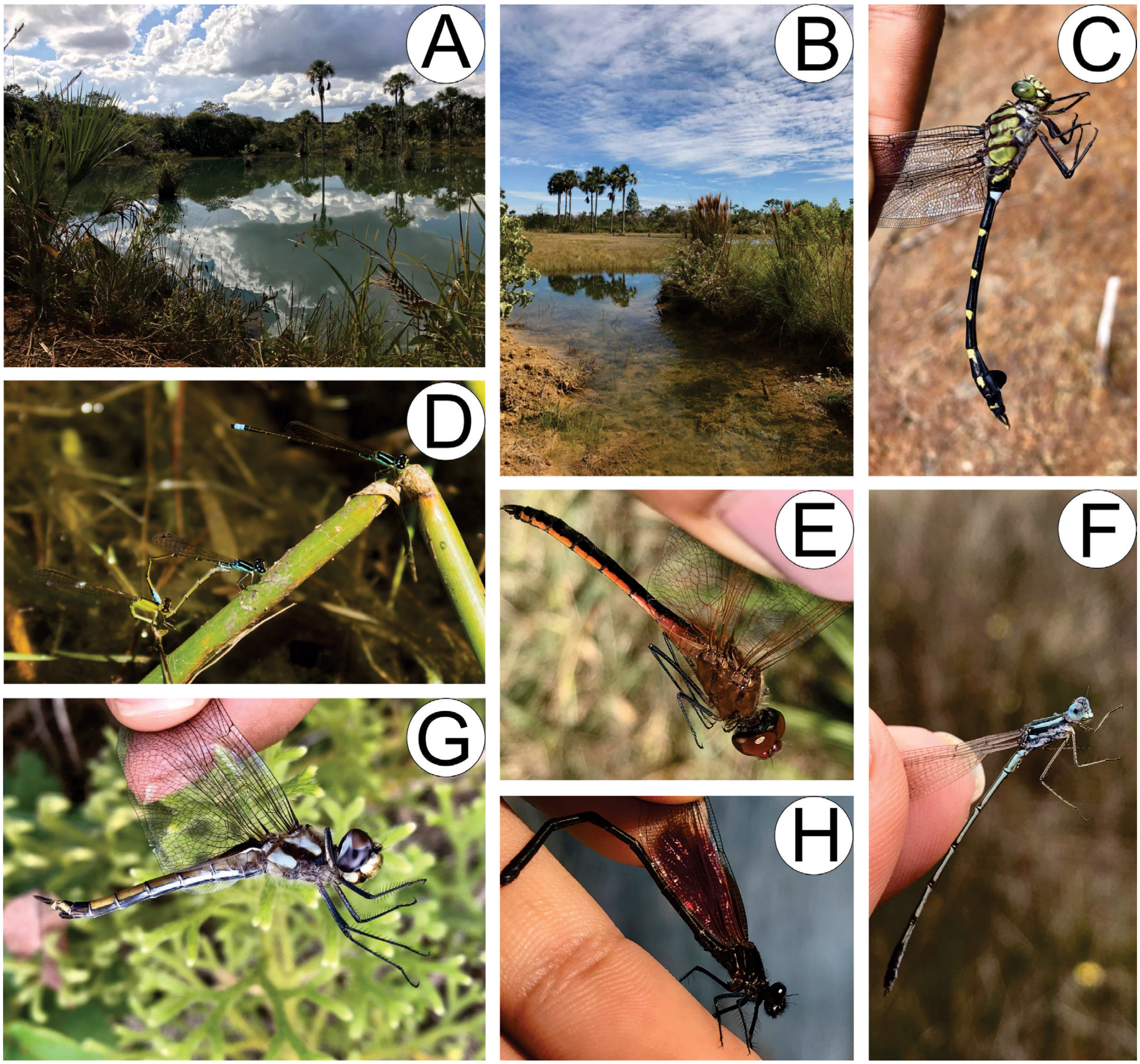

Figure 2. Two of 13 sampled sites in the Legal Reserve from Fazenda Nova Monte Carmelo, Brazil: a) a vereda surrounded by a preserved riparian zone; b) a vereda surrounded by a campo sujo fitofisionomy. Six of 36 collected species at Fazenda Nova Monte Carmelo, Minas Gerais (MG), Brazil: c) *Cacoides latro* (Gomphidae); d) *Ischnura capreolus* (Coenagrionidae); e) *Idiataphe longipes* (Libellulidae); f) *Lestes forficula* (Lestidae); g) *Tramea binotata* (female, Libellulidae); h) *Mnesarete pudica* (Calopterygidae).

and rarely are collected in surveys at Cerrado (Vilela et al. 2016, Dutra & De Marco 2015).

The anisopteran species *Erythrodiplax castanea*, *E. fusca*, *E. juliana*, *Idiataphe amazonica*, *Micrathyria hesperis*, *Oligoclada abbreviata*, and *Tramea binotata* were found predominantly at open areas. These species has a wide geographic range and predominate in open vegetation areas (Machado et al. 1991, Costa et al. 2000, Ferreira-Peruquetti & Fonseca-Gessner 2003, Souza et al. 2013, Calvão et al. 2014, Bedê et al. 2015, Dutra & De Marco 2015, Takiya et al. 2016, Rodrigues & Roque 2017). The large occurrence of these species at open areas consists mostly due to the fact that many anisopteran species can be considered fliers: tend to have larger body size and are able to produce endogenous heat, controlling their inner temperature, flying most of the time and regulating their body temperature by controlling haemolymph (Corbet 1999, Corbet & May 2008). Those characteristics can be attributed to the mosaic of open areas, that favors the occupation by the fast and agile flying dragonflies (see Machado et al. 1991). Considering the conservation perspective, those species could be less important in contrast with species found at shaded areas, which are less abundant, and have restricted distribution. Therefore, species with restricted distribution could indicate priority conservation areas for preserving Odonata species.

The low quantity of Aeshnidae, and Gomphidae species found can be associated with the fact that they are strong flyers, and species within some genera, as *Gynachantha* (Aeshnidae), presents phytotelmata and crepuscular habits (Bedê et al. 2000). Only one specimen of *Anax*

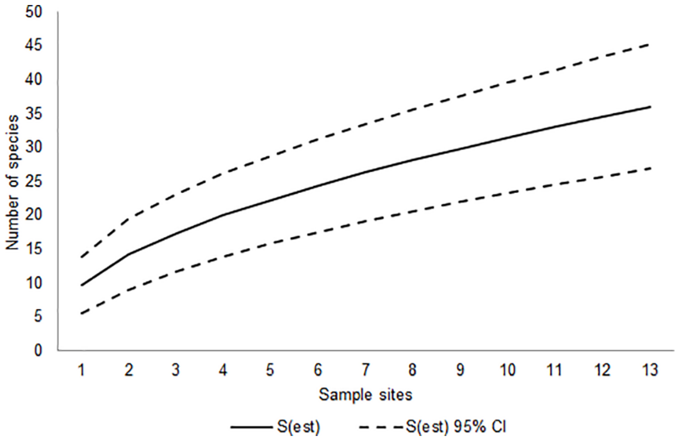

Figure 3. Rarefaction curve (Sest) and its Confidence Interval (Sest 95% CI) of Odonata species collected at the Fazenda Nova Monte Carmelo, (MG), Brazil.

Table 3. Studies with adult Odonata at Cerrado biome, showing the number of collected species, the Federative Unit (FU), the season, and the number of sampled areas.

Published studies	Richness	FU	Sampled areas
Ferreira-Peruquetti & Fonseca-Gessner 2003	85	SP	18
Almeida et al. 2013	26	MG	3
Carvalho et al. 2013	53	MT	10
Calvão et al. 2014	67	MT	9
Dutra & De Marco 2015	53	GO	58
Vilela et al. 2016	31	MG	2
Present study	**36**	**MG**	**13**

amazili was collected, despite it has been seen in majority of the sampled areas and presents wide distribution in Brazil (Teixeira 1971, Dalzochio et al. 2012, Rodrigues & Roque 2017). Almeida et al. (2013) had success at sampling Gomphidae species in Parque Nacional da Serra do Cipó, Minas Gerais state, by using malaise trap, and light sheet. Therefore, other methods can be tested for sampling species of these two families.

Only one *Erythrodiplax ana* specimen was collected in a shaded area within the Legal Reserve (Vereda). This specie was recently described, and found in another vereda within Triângulo Mineiro, located at Reserva Ecológica do Clube Caça e Pesca Itororó de Uberlândia, Minas Gerais state (Guillermo-Ferreira et al. 2016), about 44 km from the vereda where it was collected in Fazenda Nova Monte Carmelo. This specie was also collected at Parque Nacional da Chapada dos Guimarães (MT) (Guillermo-Ferreira et al. 2016), so far being restricted to Cerrado domain.

A male *Elasmothemis williamsoni* (Libellulidae) was collected in a shaded, well preserved riparian zone. As far we know, this specie was previously collected at Serra da Bodoquena in Mato Grosso do Sul state (Rodrigues & Roque 2017), and in the southeastern region of Goiás state (Klein et al. 2018), being the both sites located within Cerrado biome.

The new finding of a *Tigriagrion* species reveals the importance that conservation measures has on this Cerrado area, that is under protection by the Brazilian Forest Code, and also adds important data about its distribution. It was found in a vereda at PANMC within a shaded riparian zone, where we found both lotic and lentic systems. Some other *Tigriagrion* species, as *Tigriagrion aurantinigrum*, are known to habit lotic systems, as streams (De Marco & Vital 2008, Vilela et al. 2017).

We can see by our results that widespread and more restricted species, as the new finding *Tigriagrion* sp. nov., can inhabit aquatic habitats found in this Preserved Area. This results show the importance to continue preserving the aquatic habitats where those species occurs, and show that veredas can be an important fitofisionomy to inhabit rare Odonata species.

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Author Contributions

Lucas Rodrigues Borges: 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.

Marcela Silva Barbosa: Contribution to data collection. Contribution to data analysis and interpretation.

Marco Antônio Alves Carneiro: 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.

Diogo Silva Vilela: Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

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

Conflicts of interest

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

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Erratum

In the article “Dragonflies and damselflies (Insecta: Odonata) from a Cerrado area at Triângulo Mineiro, Minas Gerais, Brazil” with the DOI code number http://dx.doi.org/10.1590/1676-0611-bn-2018-0609, published at Biota Neotropica 19(1):e20180609:

Where it was written:

Tramea binotata (Rambur, 1842)

Should read:

Tramea calverti Muttkowski, 1910

In page 4, where it was written:

In general, the number of species in a single assemblage (pool) varies strongly in Brazilian Cerrado habitats, from as small as 26 and 31 species (Almeida et al. 2013, Vilela et al. 2016) to as numerous as 50-80, generally at sites with more sampled areas (Calvão et al. 2013, Carvalho et al. 2013, Dutra & De Marco 2015, Ferreira-Peruquetti & Fonseca-Gessner 2013) (Table 3). The number of species recorded in our study can be considered intermediary based on the small sampling effort, and the fact that we cover only half of the preserved area, indicating that the PANMC can present a rich pool of odonates, when compared to other places in Minas Gerais (Almeida et al. 2013, Souza et al. 2013). Additionally, there are many distinct Cerrado biotopes found around the aquatic habitats in the RLNMC, e.g., cerrado strictu sensu and campos de murundus, creating a gradient that could increase the diversity of odonates (Bedê et al. 2015).

Should read:

Overall, the richness in an odonate community has a great disparity in Brazilian Cerrado habitats. In some areas, species richness is low, between 26 and 31 species (Almeida et al. 2013, Vilela et al. 2016). In other areas, the richness of species is relatively high, between 50 and 80 species, probably due to the fact that these areas present more sampling points, which increases the species richness (Calvão et al. 2013, Carvalho et al. 2013, Dutra & De Marco 2015, Ferreira-Peruquetti & Fonseca-Gessner 2013) (Table 3). The richness recorded in our study fits better on an intermediary level taking in account the small sampling effort, and the fact that we cover only half of the preserved area. These results indicate that the PANMC has the potential to hold a rich pool of odonates, in comparison to other places in Minas Gerais (Almeida et al. 2013, Souza et al. 2013). Moreover, around the aquatic habitats in the LRNMC, there are other different Cerrado phytophysiognomies, such as, cerrado strictu sensu and campos de murundus. These habitats can build a slope of environmental heterogeneity, which can boost the odonate diversity (Bedê et al. 2015, Souza et al. 2017).

In page 9, where it was written:

SOARES, D.M., NASCIMENTO, A.R.T., SILVA, L.C. & DE-PINHO-JÚNIOR, G.V. 2015. Natural Regeneration and Biological Invasion by Pinus caribaea Morelet in Two Vereda Sites: Woody Vegetation Response. Am. J. Plant Sci. 6(17): 2708–2717.

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Thematic Review

Insect galls on *Myrtaceae*: richness and distribution in brazilian restingas

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Abstract: Inventories in Brazilian restingas have been indicating that Myrtaceae are the plant family with the greatest richness of insect galls. A compilation of published data plus new records was elaborated with the aim of stablishing the number of gall morphotypes on this family in this physiognomy of the Atlantic Forest, producing a list of galled species, pointing out the predominant gall features, evaluating the taxonomical knowledge of the gallers, listing the associated fauna, and based on host plant endemisms and monophagy proposing the endemism of some galling species. Myrtaceae harbor 111 morphotypes of insect gall (about 75% induced by Cecidomyiidae, Diptera) on 25 host plant species, 15 endemic. *Eugenia* L. highlights as the plant genus with the highest number of galled species and gall richness. Leaves are the most galled organ. There is a predominance of globoid and fusiform shapes, green color, glabrous surface and a single internal chamber. The taxonomical data on gallers is deficient as many records have been presented at supraspecific levels. The associated fauna is rich and includes parasitoids, inquilines and predators. Twelve species of Cecidomyiidae, a single species of Curculionidae (Coleoptera) and one species of Eriococcidae (Hemiptera) have been associated exclusively with endemic hosts and then are proposed in the present study as endemic too. The geographical distribution of many galls and respective gallers are restricted to the State of Rio de Janeiro, where most inventories have been carried out. For the first time, *Eugeniamyia dispar*, previously known from a rural area of Rio Grande do Sul and restinga areas of São Paulo, is recorded in the State of Rio de Janeiro.

Keywords: *Atlantic Forest, diversity, endemism, plant-insect interaction.*

Galhas de insetos em *Myrtaceae*: riqueza e distribuição nas restingas brasileiras

Resumo: Inventários em restingas brasileiras indicam as Myrtaceae como a família de planta com maior riqueza de galhas de insetos. Uma compilação das informações publicadas acrescida de novos registros foi elaborada com o objetivo de estabelecer o número de morfotipos de galhas, produzir uma lista das espécies botânicas com galhas, indicar as características morfológicas predominantes das galhas, avaliar o conhecimento taxonômico dos galhadores, relacionar a fauna associada, e com base no endemismo das plantas hospedeiras e monofagia, propor o endemismo de algumas espécies galhadoras. As Myrtaceae hospedam 111 morfotipos de galhas de insetos (a maioria induzida por Cecidomyiidae, Diptera) em 25 espécies de plantas, 15 endêmicas. *Eugenia* L. destaca-se como o gênero botânico com o maior número de espécies hospedeiras e riqueza de galhas. A folha é o principal órgão hospedeiro. Há predomínio das formas globoides e fusiformes, coloração verde, superfície glabra e uma única câmara interna. O conhecimento taxonômico dos galhadores ainda é deficiente com muitos registros em categorias supraespecíficas. A fauna associada é rica e inclui parasitoides, inquilinos e predadores. Doze espécies de Cecidomyiidae, uma espécie de Curculionidae (Coleoptera) e uma espécie de Eriococcidae (Hemiptera) estão associadas exclusivamente a hospedeiros endêmicos e são propostas neste estudo como endêmicas também. A distribuição geográfica de diversas galhas e seus respectivos galhadores está restrita ao estado do Rio de Janeiro, onde a maioria dos inventários foi realizada. Pela primeira vez, *Eugeniamyia díspar* (Cecidomyiidae), previamente conhecida de uma área rural do Rio Grande do Sul e de áreas de restingas de São Paulo, é registrada no estado do Rio de Janeiro.

Palavras-chave: *Mata Atlântica, diversidade, endemismo, interação inseto-planta.*

Introduction

Restingas are one of the Atlantic Forest phytophysiognomies, which are defined as long strips of marine sandy depositions, dated of Quaternary (Araújo 1992). In the past, they occupied about 80% of the Brazilian coast (Lacerda et al. 1993), but due to human activities, especially property speculation, this physiognomy has been suffering a great loss of area. For this reason, restingas are considered one of the most threatened Atlantic Forest physiognomies. Tthe main formations are found from Bahia (Northeast region) to São Paulo (Southeast region) (Neiman 1989).

Restinga vegetation is adapted to extreme environmental conditions, such as high salinity, low water availability and strong sun radiation (Scarano et al. 2001). Nevertheless, this phytophysiognomy harbors a considerable biological diversity and a great variety of unique vegetal communities (Lacerda et al. 1982, Rizzini 1992) due to the topographic diversity of the environmental conditions (Araújo & Henriques 1984).

Myrtaceae are one of the most species-rich woody plant families in Brazilian restingas (Araújo 2000, Souza & Morin 2008, Lourenço & Barbosa 2012), the second most speciose in the Atlantic Forest biome (Stehman et al. 2009), and the fourth in Brazil (Forzza et al. 2010). In fact, this family is highly representative of a variety of biomes of the Neotropics.

The most speciose taxa of Myrtaceae in Brazilian restingas are *Eugenia* L. and *Myrcia* DC. (Giaretta & Peixoto 2015, Martins et al. 2008, Rosario et al. 2005). Both have been also pointed out as the most species-rich genera of Myrtaceae in Brazil with 350 and 260 species, respectively (Landrum & Kawasaki 1997, Sobral et al. 2018) and in the world with 1,038 and 753 species, respectively (Govaerts et al., 2014).

Several insect gall inventories have been conducted in restinga areas, all in the Southeast region of Brazil, mainly in the state of Rio de Janeiro (Angra dos Reis - Maia & Oliveira (2010), Mangaratiba - Rodrigues et al. (2014), Rio de Janeiro - Oliveira & Maia (2005) and Silva & Maia (2016), Maricá - Maia (2001a), Carapebus - Maia (2001a), Parque Nacional da Restinga de Jurubatiba - Monteiro et al. (2004), Parque Estadual da Costa do Sol - Carvalho-Fernandes et al. (2016), Arraial do Cabo - Monteiro et al. (1994), São João da Barra - Carvalho-Fernandes et al. (2016)), but also in the State of São Paulo (Bertioga - Maia et al. 2008) and Espírito Santo (Guarapari - Bregonci et al. (2010). Besides, additional records were provided by Maia et. al (2002), Silva & Rodrigues (2011) and Carvalho-Fernandes & Maia (2011). These inventories have been showing considerable insect gall diversity and highlighting Myrtaceae as the plant family with the greatest number of galled species and the highest gall richness (Table 1). Such richness probably is related to the hygrothermic stress caused by daily temperature contrasts, humity, wind gusts, strong sun radiation and sandy soil (Monteiro et al. 1994, Maia 2001a, Monteiro et al. 2004).

As the available information about insect galls on Myrtaceae are scattered in several publications, the current knowledge is not consolidated. The present review aims to compile all published data on them, add new records and answer the following questions: 1) How many insect galls have been recorded on Myrtaceae in restingas?, 2) How many species of Myrtaceae are galled in this physiognomy?, 3) Which are the galled species?, 4) How is geographic distribution of these plant species and what is their origin and conservational status?, 5) Which are the galled plant organs?, 6) Which are the most frequent gall features (shape, colour, presence or absence of trichomes, number of internal chamber)?, 7) Which are the galling taxa?, 8) How is the geographic distribution of the galling species?, 9) is there any endangered galler?, and 10) How is the composition of predaceous, inquilinous and parasitoids guilds?.

Material and Methods

A bibliographic survey was done to elaborate a list of insect galls on Myrtaceae in restingas. It was carried out by consulting the database Web of Science using “insect gall“ and “restinga“ as keywords. It included ten published inventories (cited in the Introduction), an insect gall compilation (Maia 2013), scattered records (Maia et al. 2002, Carvalho-Fernandes & Maia 2011, Silva & Rodrigues 2011) and two catalogues of Cecidomyiidae (Diptera) of Rio de Janeiro (Maia 2005, Maia & Barros 2009). Only host plants identified at species level were considered.

Additionally, new records of insect galls are provided. They were obtained from surveys carried out in the Parque Natural Municipal Chico Mendes (Rio de Janeiro, RJ), in February, 2016. The park has 10 paths totaling five quilometers. These paths were pursued in the search of galls for two hours by two persons. The hiking method was adopted to allow the observation of a large number of specimens. Galls were photographed as voucher material.

Botanic names were updated and conservational status of all plant species as well as data on plant endemisms were verified, using the site Flora do Brasil (2020). Based on the high specificity of the gallers in relation to the host plants, galling species associated exclusively with endemic plants were proposed as endemic. Similarly, gallers associated exclusively with threatened plants were proposed as threatened too.

Unfortunately, the morphological gall characterization is not standardized in the literature. The fullest descriptions include galled plant organ, gall shape, color, presence or absence of trichomes, and number of internal chamber, but these features were not equally contemplated in the analysed inventories. Some of them offer very parsimoniosy descriptions. Part of the missing features was retrieved based on gall pictures whenever possible. Besides, different authors adopt different terms to refer to the same gall shape. They are listed in the present study. With regard to the gall morphology, published data are not standardized, resulting in not comparable descriptions as well as in information gaps. Furthermore, different terms have been used to describe the same gall shape. A total of 33 terms were retrieved from literature, but several correspond to the same shape: 1) conical = drop-shaped = triangular, 2) lenticular = discoid = epidermic = parenchymal, 3) globoid = globose = globular = spherical = spheroid, 4) cylindrical = tubular, 5) fusiform = elliptical, 6) edge leaf roll = marginal leaf roll, 7) leaf roll = total leaf roll = entire leaf roll, 8) rosette = leaves rosette. In the present study, the following terms were adopted: conical, lenticular, globoid, cylindrical, fusiform, marginal leaf roll, leaf roll, and rosette. Other recorded shapes were claviform, spiraled, ovoid, bivalve, star-shaped, pineapple-shaped, biconical, and leaf blade fold. These last terms were retained in this publication.

Table 1. Plant families with the highest number of galled species and greatest gall richness in Brazilian restingas.

Localities (municipality/state)	Family with the highest number of galled species	Ratio between galled species on this family and total number of galled species in the locality	Family with the greatest gall richness	Ratio between gall morphotypes on this family and total number of gall morphotypes in the locality	References
Angra dos Reis/RJ	Myrtaceae	4/22 (18.18%)	Myrtaceae	9/36 (28.12%)	Maia & Oliveira (2010)
Mangaratiba/RJ	Myrtaceae	9/70 (12.86%)	Myrtaceae	20/147 (13.60%)	Rodrigues et al. (2014)
Rio de Janeiro/RJ	Myrtaceae	4/25 (16.00%)	Myrtaceae	7/43 (16.28%)	Oliveira & Maia (2005)
Rio de Janeiro/RJ	Fabaceae	4/24 (16.67%)	Fabaceae	6/31 (19.35%)	Silva & Maia (2016)
Maricá/RJ	Myrtaceae	6/41 (14.63%)	Myrtaceae	17/68 (25.00%)	Maia (2001a)
Carapebus/RJ	Myrtaceae	6/30 (20.00%)	Myrtaceae	17/61 (27.87%)	Maia (2001a)
Parque Nacional da Restinga de Jurubatiba/RJ	Myrtaceae	7/40 (17.50%)	Myrtaceae	25/72 (34.72%)	Monteiro et al. (2004)
Parque Estadual da Costa do Sol/RJ	Myrtaceae	9/68 (11.54%)	Myrtaceae	33/124 (26.62%)	Carvalho-Fernandes et al. (2016)
Arraial do Cabo/RJ	Myrtaceae	6/35 (17.14%)	Myrtaceae	10/51 (19.61%)	Monteiro et al. (1994)
São João da Barra/RJ	Myrtaceae	6/52 (11.54%)	Myrtaceae	9/66 (13.64%)	Carvalho-Fernandes et al. (2016)
Bertioga/SP	Myrtaceae	14/123 (11.38%)	Myrtaceae	31/236 (13.13%)	Maia et al. (2008)
Guarapari/ES	Nyctaginaceae	3/21 (14.28%)	Nyctaginaceae	5/38 (13.16%)	Bregonci et al. (2010)

Results

A total of 111 insect gall morphotypes on 25 plant species of nine genera were accounted (Table 2). This value represents 90.2% of the gall records on Myrtaceae for restinga, as 12 morphotypes were not included as they were associated with six undetermined plant species. *Eugenia* L. and *Myrcia* DC. showed the highest number of galled species (ten each) and the greatest gall richness (47 and 34 morphotypes, respectively). The other genera included a single galled species and from one to ten gall morphotypes. *Eugenia* and *Myrcia* together harbored about 73.0% of the gall richness.

All host plant species are native, being 15 endemic in Brazil. Among the last, 11 occur exclusively in the Atlantic Forest (Table 2). Concerning the conservational status, six plant species are less concerning, 18 have not been yet evaluated, and one is data deficient (Table 2).

Leaves were the most galled plant organ (about 63.0%), but galls on stems, buds, flowers (flower buds and flower peduncles) and fruits were also recorded (Table 3). The richness of bud and stem galls were similar, about 17.5% and 14.0%, respectively. Fruit and flower galls were rare (each with less than 3%).

The most frequent shapes were conical and globoid (both with 18 morphotypes), fusiform and lenticular (both with 13 morphotypes), and marginal leaf roll (10 morphotypes). The other shapes included from seven to a single morphotype. Green galls predominated (n=55, about 49.5%), but brown, yellowish, reddish, whitish and black galls were also found. Most galls exhibited a single color, whereas few varied in color. Most galls were glabrous (n=85, about 76.6%) and a single morphotype was hairy (0.90%). There is no information about the others. Seventy-four morphotypes were one-chambered (66.67%), eight were multichambered (7.21%) and a single one showed from one to three chambers (0.90%) (Table 3).

Most gallers were determined (n=80, about 67.0%), but only 21 (about 19.0%) were identified in species and ten (about 9.0%) in genus. Records in suprageneric categories predominated, especially at family (Table 3). The gallers were distributed in five orders: Diptera (Cecidomyiidae), Thysanoptera, Hymenoptera, Coleoptera, and Hemiptera. Cecidomyiids induced most galls (n=69, about 57.0%), while the others induced 4 (3.5%), 3 (2.7%), 2 (1.8%) and 2 (1.8%), respectively. Lepidopteran galls were not found (Table 3).

Twelve species of gall midges were identified, being distributed in ten genera, *Clinodiplosis* Kieffer, 1894, *Dasineura* Rondani, 1840, *Neolasioptera* Felt, 1908, *Bruggmannia* Tavares, 1906, *Eugeniamyia* Maia, Mendonça & Romanovski, 1997, *Jorgenseniella* Maia, 2005, *Myrciamyia* Maia, 1995, *Myrciariamyia* Maia, 1994, *Neomitranthella* Maia, 1996, and *Stephomyia* Rondani, 1840. *Dasineura* and *Stephomyia* were the most speciose genera associated with Myrtaceae in Brazilian restingas. *Pacholenus pelliceus* Boheman, 1836 (Coleoptera) and *Tectococcus ovatus* Hempel, 1900 (Hemiptera) were the other identified galling species.

Concerning the geographic distribution, 12 galling species have been recorded, until this moment, only in restingas of Rio de Janeiro State (Table 4). The other species have a less restricted distribution. *Eugeniamyia dispar,* previously known from a rural area of Rio Grande do Sul and restinga areas of São Paulo, is recorded for the first time in the State of Rio de Janeiro. The distribution of *Neolasioptera eugeniae* Maia, 1993 includes restinga areas of RJ and an ombrophilous Forest area of Minas Gerais. *Dasineura gigantea* Angelo & Maia, 1999 was described from Forest areas of Paraná and Santa Catarina, and later this species was reported in restinga areas of São Paulo. *Dasineura myrciariae* Maia, 1993 occurs in the states of Rio de Janeiro and Espírito Santo. *Jorgenseniella eugeniae* Maia, 2005 and *Pacholenus*

Table 2. Galled species of Myrtaceae, their synonyms, common names, origin, distribution in Brazilian phytogeographic domains, conservational status and number of gall morphotypes in restingas. DD – deficient data, LC – less concerning, NE – not evaluated, VU – vulnerable.

Plant species/Synonyms/Common names	**Origin**	**Phytogeographic domain**	**Conservational status**	**Nr. gall morphotypes (n=111)**
Blepharocalyx salicifolius (Kunth) O. Berg /-/ "maria-preta, murteira, murtinha"	Native	Atlantic Forest Caatinga Cerrado Pampa	LC	1
Calyptranthes brasiliensis Spreng. /-/ "guamirim-branco"	Native	Atlantic Forest Cerrado	NE	2
Campomanesia guaviroba (DC.) Kiaersk. /-/ "guabirobão, guabirobeira"	Native	Atlantic Forest Cerrado	NE	1
Eugenia astringens Cambess./= *Eugenia rotundifolia* Casar., *Eugenia umbelliflora* O. Berg, *Eugenia adstringens* (mispelling error)/ "jabuticaba-do-mangue, aperta-goela, apê-açu"	Endemic	Atlantic Forest	NE	8
Eugenia copacabanensis Kiaersk. /-/ "princesinha-de-Copacabana	Endemic	Atlantic Forest	LC	10
Eugenia hiemalis Cambess. /= E. *multiflora* (Lam.) DC./ "guamirim-burro"	Native	Atlantic Forest Cerrado	LC	6
Eugenia monosperma Vell. /-/ "araçarana"	Endemic	Atlantic Forest	NE	2
Eugenia punicifolia (Kunt) DC. / = *Eugenia ovalifolia* Cambess. / "pitanga do campo, murta vermelha"	Endemic	Amazon Atlantic Forest Caatinga Cerrado	NE	8
Eugenia selloi B. D.Jacks. /-/ "pitangatuba"	Endemic	Atlantic Forest	NE	1
Eugenia speciosa Cambess. /-/ "laranjinha-do-mato, guamirim"	Native	Atlantic Forest	NE	2
Eugenia sulcata Spring ex Mart. /-/ "pitanga-preta"	Endemic	Atlantic Forest	NE	2
Eugenia uniflora L. /-/ "pitangueira, pitanga, pitanga-vermelha"	Native	Atlantic Forest Cerrado Pampa	NE	8
Myrcia brasiliensis Kiaersk. / = *Gomidesia schaueriana* O. Berg / "batinga, guamirim-araçá, guamirim"	Endemic	Atlantic Forest	NE	3
Myrcia ilheosensis Kiaersk. / = *Gomidesia fenzliana* O. Berg/ "papa-güela"	Endemic	Atlantic Forest	NE	1
Myrcia multiflora (Lam.) DC. /-/ "cambuí, camboim"	Native	Amazon Atlantic Forest Caatinga Cerrado	NE	1
Myrcia lundiana Kiaersk. /-/ "araçá caiçara"	Endemic	Atlantic Forest	NE	6
Myrcia ovata Cambess. /-/ "guabijueiro"	Endemic	Atlantic Forest	LC	8
Myrcia palustris DC. /-/ "pitangueira-do-mato"	Native	Atlantic Forest Caatinga	NE	2
Myrcia racemosa O. Berg /= *Myrcia acuminatissima* O. Berg /-/	Endemic	Atlantic Forest Cerrado	NE	1
Myrcia splendens (SW.) DC. /= *Myrcia fallax* (Rich.) DC./ "guamirim-de-folha-fina, guamirim-miúdo"	Endemic	Amazon Atlantic Forest Caatinga Cerrado Pantanal	NE	11
Myrcia tenella (DC.) O. Berg /-/ "cambuí"	Native	Amazon Atlantic Forest Caatinga Cerrado	DD	1
Myrciaria floribunda (West ex Willdenow) Berg /-/ "uvaia, cambuizino, cambuí"	Native	Amazon Atlantic Forest Caatinga Cerrado	LC	10
Neomitranthes obscura (DC.) N. J. E. Silveira /-/ "guapeí-una, batinga preta, araçá preto, pitanga de cachorro pitanga da restinga"	Endemic	Atlantic Forest	LC	6
Plinia cauliflora (Mart.) Kausel Berg / =*Myrciaria jaboticaba* (Vell.) Berg/ "jabuticaba"	Endemic	Atlantic Forest	NE	2
Psidium cattleianum Sabine /-/ "araçá-vermelho, araçá-amarelo, araçá-yaci, araçá-branco, araçá"	Endemic	Atlantic Forest Caatinga Cerrado	NE	8

Table 3. Gall features on Myrtaceae in Brazilian restingas (shape, color, presence or absence of trichomes, number of internal chamber, galled plant organ, galling order and gallers' taxonomical categories). The total number of gall morphotypes varies due to the available information in the literature. The number of the gall morphotypes by host organ is higher than 111, because some galls were recorded in two different plant organs.

Gall features	**Number of morphotypes**	
Galled organ		
Leaf	72	64.86%
Bud	20	18.02%
Stem	16	14.41%
Flower	3	2.70%
Fruit	3	2.70%
Shape		
Conical	18	16.22%
Globoid	18	16.22%
Fusiform	13	11.71%
Lenticular	13	11.71%
Marginal leaf roll	10	9.01%
Cylindrical	7	6.31%
Leaf roll	6	5.40%
Ovoid	5	4.50%
Claviform	3	2.70%
Rosette	3	2.70%
Spiraled	1	0.90%
Bivalve	1	0.90%
Star-shaped	1	0.90%
Pineapple-shaped	1	0.90%
Biconical	1	0.90%
Leaf blade fold	1	0.90%
Color		
Green	55	49.54%
Brown	14	12.61%
Yellowish	12	10.81%
Reddish	12	10.81%
Whittish	1	0.90%
Black	1	0.90%
Trichomes		
Absent	85	76.60%
Present	1	0.90%
Number of internal chamber		
One-chambered	74	66.67%
Multichambered	8	7.21%
From one to 3	1	0.90%
Galling order		
Diptera	69	86.25%
Thysanoptera	4	5.00%
Hymenoptera	3	3.75%
Coleoptera	2	2.50%
Hemiptera	2	2.50%
Lepidoptera	0	0.00%
Gallers' taxonomical category		
Order	8	7.20%
Family	58	52.25%
Genus	10	9.01%
Species	21	18.92%
Others	7	6.31%

Table 4. Geographical distribution of galling species associated with Myrtaceae in Brazil.

Galling species	Geographical distribution
Bruggmannia sp.	RJ (Arraial do Cabo)
Clinodiplosis profusa Maia 2001	RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari; Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; Carapebus; Parque Nacional da Restinga de Jurubatiba; São João da Barra)
Clinodiplosis sp.	SP (Bertioga)
Dasineura copacabanensis Maia, 1993	RJ (Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; São João da Barra)
Dasineura gigantea Angelo & Maia, 1999	SP (Bertioga) PR (Piraquara, Pontal do Paraná) SC (Itapoá)
Dasineura globosa Maia, 1995	RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari, Niterói; Maricá; Saquarema; Carapebus; Araruama; Cabo Frio; Arraial do Cabo; Parque Nacional da Restinga de Jurubatiba, São João da Barra)
Dasineura marginalis Maia, 2005	RJ (Mangaratiba: Ilha da Marambaia, Rio de Janeiro: Grumari; Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; Carapebus; Parque Nacional da Restinga de Jurubatiba)
Dasineura myrciariae Maia, 1995	RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes - new record; Maricá; Carapebus; Parque Nacional da Restinga de Jurubatiba) ES (Guarapari)
Dasineura sp. 1	RJ (Maricá)
Dasineura sp. 2	RJ (Maricá)
Dasineura sp. 3	RJ (Maricá, Arraial do Cabo)
Dasineura sp. 4	RJ (Araruama, Arraial do Cabo)
Eugeniamyia dispar Maia, Mendonça & Romanovski, 1997	RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes - new record) SP (Bertioga) RS (Porto Alegre)
Eugeniamyia triangularis Maia, 2011	RJ (Maricá)
Jorgenseniella eugeniae Maia, 2005	RJ (Cabo Frio; Macaé) SP (Bertioga)
Myrciamyia maricaensis Maia 1995	RJ (Maricá; Arraial do Cabo; Carapebus; Parque Nacional da Restinga de Jurubatiba)
Myrciariamyia bivalva Maia, 1994	RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes - new record; Maricá; Carapebus; Arraial do Cabo; Parque Nacional da Restinga de Jurubatiba)
Neolasioptera eugeniae Maia 1993	RJ (Paraty; Angra dos Reis: Ilha Grande; Rio de Janeiro: Parque Natural Municipal Chico Mendes - new record, Grumari; Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; São João da Barra) MG (Itamonte)
Neomitranthella robusta Maia, 1995	RJ (Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; Carapebus; Parque Nacional da Restinga de Jurubatiba)
Pacholenus pelliceus Boheman, 1836	RJ (Maricá) SP (Bertioga)
Stephomyia clavata (Tavares 1920)	RJ (Carapebus; Parque Nacional da Restinga de Jurubatiba; Araruama; Arraial do Cabo; São João da Barra) BA (Madre de Deus)
Stephomyia espiralis Maia, 1993	RJ (Mangaratiba: Ilha da Marambaia; Maricá; Araruama; Cabo Frio; Arraial do Cabo)
Stephomyia mina Maia, 1993	RJ (Maricá; Araruama; Arraial do Cabo; Carapebus)
Stephomyia rotundifoliorum Maia, 1993	RJ (Maricá; Saquarema; Araruama; Cabo Frio; Arraial do Cabo; Carapebus; Parque Nacional da Restinga de Jurubatiba; São João da Barra)
Stephomyia tetraloba Maia, 1993	RJ (Maricá; Arraial do Cabo)
Stephomyia sp.1	RJ (Carapebus)
Stephomyia sp.2	RJ (Mangaratiba: Ilha da Marambaia)
Tectococcus ovatus Hempel, 1900	Wide spread (Brazil, U.S.A. and Japan)

pelliceus Boheman, 1836 are known from records in restingas of RJ and SP. *Tectococcus ovatus* Hempel, 1900 has the widest geographical distribution, including U.S.A. (Florida and Hawaii) and Japan, due to the ornamental use of its host plant, *Psidium cattleianum*, which is endemic from Brazil and has been introduced in other countries to be used in garden landscaping (Vitorino et al. 2000).

In relation to their origin, 77 galling species are proposed as endemic in Brazil. Among them, 51 are considered endemic in the Atlantic Forest, 14 were identified in species - *Dasineura copacabanensis* Maia, 1993; *D. gigantea* Angelo & Maia, 1999; *D. globosa* Maia, 1993; *D. marginalis* Maia, 2005; *Jorgenseniella eugeniae* Maia, 2005; *Myrciamyia maricaensis* Maia, 1995; *Neomitranthella robusta* Maia, 1996; *Stephomyia clavata* (Tavares, 1920); *S. espiralis* Maia, 1993; *S. mina* Maia, 1993; *S. rotundifoliorum* 1993; and *S. tetraloba* 1993 (Diptera, Cecidomyiidae); *Pacholenus pelliceus* Boheman, 1836 (Coleoptera, Curculionidae) and *Tectococcus ovatus* Hempel, 1900 (Hemiptera, Eriococcidae). Concerning the conservational status, data are not sufficient to enable an evaluation.

Besides the inducers, other dwellers have been recorded as parasitoids, inquilines and predators (Table 5). Parasitoids were recorded in only 26 morphotypes (about 24%), all induced by Cecidomyiidae. They are represented by seven families of Hymenoptera: Eulophidae (14 gall morphotypes), Eurytomidae (7), Platygastridae (7), Eupelmidae (4), Torymidae (4), Scelionidae (1) and Braconidae (1). None parasitoid was identified in species. Five parasitoid records were presented in genus, four in subfamily, 26 in family and nine in order. The number of records is greater than the number of parasited gall morphotypes due to multiparasitism. *Rileya* Ashmead, 1888 (Eurytomidae), *Donquickeia* Marsh, 1993 (Braconidae), *Chrysonotomia* Ashmead, 1904 (Eulophidae) and *Proacrias* Ihering, 1914 (Eulophidae) were the reported genera, all found in a single gall morphotype, except the first, found in two morphotypes. Multiparasitisme have been recorded in nine gall morphotypes.

Inquilines were recorded in 17 gall morphotypes. They were represented by Diptera, Lepidoptera, Hymenoptera, Coleoptera, Hemiptera, and Thysanoptera. All morphotypes hosted a single inquilinous order, except two of them (1. bud or leaf vein gall on *Myrcia splendes*, and 2) marginal roll on *Neomitranthes obscura*). Diptera were found in five gall morphotypes, Lepidoptera and Hymenoptera in three each, Coleoptera and Hemiptera in two each, and Thysanoptera in one. Diptera were represented by Cecidomyiidae (*Dasineura tavaresi* Maia, 1995, *Resseliella* sp. and *Trotteria* sp.) and Sciaridae. Lepidoptera included *Stenoma annosa* (Butler, 1877) (Depressidae) found in two gall morphotypes on *Neomitranthes obscura*. *Stenoma annosa* is a free-living species, whose caterpillar feeds on leaves of several Myrtaceae. Eventually, it feeds on galls, causing their complete destruction, which results in the death of the galling species. Besides, other record, but in order category, is known on *Psidium cattleianum*. Hymenoptera included Eulophidae (*Aprostocetus* sp. and Tetrastichinae sp.), Formicidae (*Leptothorax* sp.) and a record n order category. Coleoptera were represented only by Curculionidae, Hemiptera included Coccidae and Membracidae, and Thysanoptera *Gynaikothrips uzeli* Zimmermann, 1900.

Predators were recorded in five gall morphotypes and they included *Lestodiplosis* sp. (Cecidomyiidae) found in four gall morphotypes, and *Novohorus* sp. (Olpiidae), a pseudoscorpion found in a single morphotype.

Additionally, Bregonci et al. (2010) reported Thysanoptera and Formicidae (Hymenoptera) in galls of Cecidomyiidae on *Myrciaria floribunda,* but their habits were not informed.

Three gall morphotypes are described for the first time on *Myrciaria floribunda*: lenticular, globoid and rosette galls, all found in the Parque Municipal Chico Mendes (Rio de Janeiro, RJ). New occurrences of gall midges species are provided herein: *Eugeniamyia dispar* in the State of Rio de Janeiro, *Dasineura myrciariae* and *Myrciariamyia bivalva*

Table 5. Parasitoids, inquilines and predators recorded in insect galls on Myrtaceae in Brazilian restingas.

Dwellers habit	Host Plant	Gall morphotype	Galling insect
Parasitoids			
Order:			
Hymenoptera	*Eugenia astringens* Cambess.	Lenticular	*Dasineura globosa* Maia, 1995 (Cecidomyiidae)
	Eugenia hiemalis Cambess.	Marginal roll	*Stephomyia cfr. clavata* (Tavares, 1920) (Cecidomyiidae)
	Eugenia punicifolia (Kunt) DC.	Cylindrical	*Stephomyia* sp.
		Globoid	Undetermined
	Myrcia splendens (SW.) DC.	Conical	Undetermined
		Globoid	Undetermined
		Fusiform	Cecidomyiidae
	Myrciaria floribunda (West ex Willdenow) Berg	Star-shaped	Cecidomyiidae
	Neomitranthes obscura (DC.) N. J. E. Silveira	Fusiform	*Stephomyia mina* Maia, 1993 (Cecidomyiidae)

Continuation Table 1.

Dwellers habit	Host Plant	Gall morphotype	Galling insect
Family:			
Eulophidae	*Eugenia astringens*	Claviform	*Stephomyia clavata*
		Cylindrical	*Stephomyia rotundifoliorum* Maia, 1993 (Cecidomyiidae)
		Marginal roll	*Dasineura marginalis* Maia, 2005 (Cecidomyiidae, Diptera)
	Eugenia copacabanensis Kiaersk.	Conical	*Dasineura copacabanensis* Maia, 1993 (Cecidomyiidae)
		Spiraled	*Stephomyia espiralis* Maia, 1993 (Cecidomyiidae)
	Eugenia hiemalis	Claviform	*Stephomyia cfr. clavata*
		Conical	Cecidomyiidae
		Marginal roll	Cecidomyiidae
	Eugenia uniflora L.	Conical	*Clinodiplosis profusa* Maia, 2001 (Cecidomyiidae)
		Lenticular	*Neolasioptera eugeniae* Maia, 1993 (Cecidomyiidae)
	Myrcia ovata Cambess.	Globoid	*Dasineura* sp. (Cecidomyiidae)
		Ovoid	*Myrciamyia maricaensis* Maia, 1995 (Cecidomyiidae)
	Myrciaria floribunda	Marginal roll	*Dasineura myrciariae* Maia, 1995 (Cecidomyiidae)
	Neomitranthes obscura	Pineapple-shaped	*Neomitranthella robusta* Maia, 1995 (Cecidomyiidae)
Eupelmidae	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*
	Eugenia hiemalis	Claviform	*Stephomyia cfr. clavata*
		Conical	Cecidomyiidae
		Marginal roll	Cecidomyiidae
Eurytomidae	*Eugenia astringens*	Claviform	*Stephomyia clavata*
		Cylindrical	*Stephomyia rotundifoliorum*
	Eugenia copacabanensis	Conical	*Dasineura copacabanensis*
		Spiraled	*Stephomyia espiralis*
	Eugenia uniflora	Conical	*Clinodiplosis profusa*
		Globoid	*Eugeniamyia dispar* Maia, Mendonça & Romanovski, 1996 (Cecidomyiidae)
	Myrciaria floribunda	Globoid	Cecidomyiidae
Platygastridae	*Eugenia hiemalis*	Claviform	*Stephomyia cfr. clavata*
		Cylindrical	*Stephomyia* sp.
Scelionidae	*Eugenia speciosa* Cambess.	Claviform	Schizomyiina (Cecidomyiidae)
	Eugenia uniflora	Conical	*Clinodiplosis profusa*
	Myrcia ovata	Ovoid	*Myrciamyia maricaensis*
	Myrcia splendens	Globoid	Undetermined
		Ovoid	Oligotrophini (Cecidomyiidae)
Torymidae	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*
		Marginal roll	*Dasineura marginalis*
	Eugenia copacabanensis	Rosette	Undetermined
	Myrcia splendens	Bud	Lasiopteridi (Cecidomyiidae)
Subfamily:			
Tetrastichinae	*Eugenia astringens*	Lenticular	*Dasineura globosa*

Continuation Table 1.

Dwellers habit	Host Plant	Gall morphotype	Galling insect
Tetrastichinae	*Eugenia copacabanensis*	Spiraled	*Stephomyia espiralis*
	Eugenia uniflora	Conical	*Clinodiplosis profusa*
	Neomitranthes obscura	Pineapple-shaped	*Neomitranthella robusta*
Genus:			
Chrysonotomia Ashmead, 1904 (Eulophidae)	*Eugenia uniflora*	Conical	*Clinodiplosis profusa*
Donquickeia Marsh, 1993 (Braconidae)	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*
Proacrias Ihering, 1914 (Eulophidae)	*Myrciaria floribunda*	Marginal roll	*Dasineura myrciariae*
Rileya Ashmead, 1888 (Eurytomidae)	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*
	Eugenia copacabanensis	Spiraled	*Stephomyia espiralis*
Inquilines			
Order:			
Lepidoptera	*Psidium cattleianum* Sabine	Globoid	Undetermined
Family:			
Curculionidae (Coleoptera)	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*
	Neomitranthes obscura	Pineapple-shaped	*Neomitranthella robusta*
Coccidae?	*Myrcia splendens*	Bud or leaf vein	Lasiopteridi (Cecidomyiidae)
Membracidae (Hemiptera)	*Campomanesia guaviroba* (DC.) Kiaersk.	Globoid	Cecidomyiidae
Sciaridae (Diptera)	*Eugenia speciosa* Cambess.	Claviform	Schizomyiina (Cecidomyiidae)
Subfamily:			
Tetrastichinae (Hymenoptera)	*Myrcia ovata*	Globoid	*Dasineura* sp. (Cecidomyiidae)
Genus:			
Aprostocetus sp. (Eulophidae, Hymenoptera)	*Myrcia ovata*	Ovoid	*Myrciamyia maricaensis*
Leptothorax sp. (Formicidae, Hymenoptera)	*Eugenia hiemalis*	Cylindrical	*Stephomyia* sp. (Cecidomyiidae)
Resseliella sp. (Cecidomyiidae)	*Eugenia astringens*	Lenticular	*Dasineura globosa*
Trotteria sp. (Cecidomyiidae)	*Eugenia astringens*	Lenticular	*Jorgenseniella eugeniae* Maia, 2005 (Cecidomyiidae)
	Myrcia splendens	Bud or leaf vein	Lasiopteridi (Cecidomyiidae)
Species:			
Dasineura tavaresi Maia, 1995 (Cecidomyiidae)	*Neomitranthes obscura*	Marginal roll	*Clinodiplosis* sp. (Cecidomyiidae)
Gynaikothrips uzeli (Zimerman, 1909) (Thysanoptera, Phlaeothripidae)	*Eugenia uniflora*	Conical	*Clinodiplosis profusa*
Stenoma annosa (Butler, 1877) (Depressariidae, Lepidoptera)	*Neomitranthes obscura*	Marginal roll	*Clinodiplosis* sp.
	Neomitranthes obscura	Pineapple-shaped	*Neomitranthella robusta*
Predators:			
Genus:			
Lestodiplosis sp. (Cecidomyiidae)	*Eugenia astringens*	Marginal roll	*Dasineura marginalis*
Lestodiplosis sp.	*Myrcia multiflora*	Marginal roll	Thysanoptera
Lestodiplosis sp.	*Myrcia splendens*	Bud gall	Undetermined
Lestodiplosis sp.	*Myrciaria floribunda*	Marginal roll	*Dasineura myrciariae*
	Myrcia multiflora (Lam.) DC.	Marginal roll	Thysanoptera
Species:			
Novohorus sp. (Olpiidae, Pseudoscorpiones)	*Eugenia astringens*	Cylindrical	*Stephomyia rotundifoliorum*

in the municipality of Rio de Janeiro, and *Neolasioptera eugeniae* in the Parque Municipal Chico Mendes.

Furthermore, the globoid gall of stems of *Eugenia uniflora* is reported for the first time in the State of Rio de Janeiro (it was previously known only in Espírito Santo and the galler is unknown). And finally, the records of star-shaped galls (induced by Cecidomyiidae) and leaf rolls (induced by Thysanoptera) on *Myrciaria floribunda* are new in the municipality of Rio de Janeiro.

The morphological characterization of galls on Myrtaceae in Brazilian restingas is compiled and presented below in alphabetical order of the host plant species. Data on galled organ, gall shape, color, indumentum, number of internal chamber, galler and other dwellers are presented whenever available. Informations about their geographical distribution in Brazilian restingas are added.

Blepharocalyx salicifolius (Kunth) O. Berg.

1. Gall on leaf vein, woody, fusiform. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Calyptranthes brasiliensis Spreng.

1. Gall on bud, conical, green or brown, glabrous, multichambered. Galler: undetermined. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
2. Gall on stem. Galler: undetermined. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).

Campomanesia guaviroba (DC.) Kiaersk.

1. Gall on leaf vein, globoid, one-chambered. Galler: *Clinodiplosis* sp. (Cecidomyiidae). Locality: SP (Bertioga). Other dwellers: Membracidae (Hemiptera) – inquiline. Reference: Maia et al. (2008).

Eugenia astringens Cambess.

1. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: *Dasineura marginalis* Maia, 2005 (Cecidomyiidae, Diptera). Other dwellers: *Lestodiplosis* sp. (Cecidomyiidae) – predator; Tetrastichinae (Eulophidae), Torymidae (Hymenoptera) – parasitoids. Localities: RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari; Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba. References: Maia (2001a), Maia et al. (2005); Monteiro et al. (1994), Monteiro et al. (2004), Oliveira & Maia (2005), Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
2. Gall on bud, cylindrical, brown, glabrous, one-chambered. Galler: *Stephomyia rotundifoliorum* Maia, 1993 (Cecidomyiidae). Other dwellers: *Rileya* sp. (Eurytomidae), *Donquickeia* (Braconidae), Eupelmidae, Eulophidae, Scelionidae, Torymidae (Hymenoptera) – parasitoids; *Novohorus* sp. (Pseudoscorpiones: Olpiidae) – predators. Localities: RJ (Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba, São João da Barra). References: Maia (1993b, 2001a), Monteiro et al. (1994), Carvalho-Fernandes et al. (2016).
3. Gall on leaf, lenticular, yellow, glabrous, one-chambered. Galler: *Dasineura globosa* Maia, 1995 (Cecidomyiidae). Other dwellers: *Resseliella* sp. (Cecidomyiidae) – inquiline; Hymenoptera – parasitoids. Localities: RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari; Maricá, Saquarema, Carapebus, Araruama, Cabo Frio, Arraial do Cabo, Parque Nacional da Restinga de Jurubatiba, São João da Barra). References: Maia (1995, 2001a), Monteiro et al. (2004); Oliveira & Maia (2005); Rodrigues et al. (2014), Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
4. Gall on leaf, conical. Galler: Cecidomyiidae. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
5. Gall on leaf, conical. Galler: Cecidomyiidae. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
6. Gall on leaf, claviform, reddish, glabrous, one-chambered. Galler: *Stephomyia clavata* (Tavares 1920) (Cecidomyiidae). Other dwellers: Eulophidae, Eurytomidae (Hymenoptera) – parasitoids. Localities: RJ (Rio de Janeiro, Carapebus, Parque Nacional da Restinga de Jurubatiba, Araruama, Arraial do Cabo, São João da Barra. References: Maia (2001a), Monteiro et al. (2004); Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
7. Gall on stem, fusiform. Galler: undetermined. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
8. Gall on leaf, globoid, green, glabrous, one-chambered. Galler: undetermined. Localities: RJ (Araruama, Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
9. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: *Jorgenseniella eugeniae* Maia, 2005 (Cecidomyiidae). Other dwellers: *Trotteria* sp. (Cecidomyiidae) – inquilines. Localities: SP (Bertioga), RJ (Cabo Frio, Macaé). References: Maia et al. (2005, 2008).

Eugenia copacabanensis Kiaersk.

1. Gall on leaf, spiraled, reddish, glabrous, one-chambered. Galler: *Stephomyia espiralis* Maia, 1993 (Cecidomyiidae). Other dwellers: *Rileya* sp. (Eurytomidae), Tetrastichinae (Eulophidae) (Hymenoptera) – parasitoids. Localities: RJ (Mangaratiba: Ilha da Marambaia; Maricá, Araruama, Cabo Frio, Arraial do Cabo. References: Maia (1993b), Maia (2001a), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).
2. Gall on bud, conical, green, glabrous, pedunculated. Galler: *Dasineura copacabanensis* Maia, 1993 (Cecidomyiidae). Localities: RJ (Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, São João da Barra). References: Maia (1993a), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).
3. Gall on young leaf, leaf roll, reddish, glabrous, one-chambered. Galler: *Dasineura* sp. (Cecidomyiidae). Locality: RJ (Maricá). Reference: Maia et al. (2002).
4. Gall on leaf. Galler: Cecidomyiidae. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
5. Gall on stem. Galler: Cecidomyiidae. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
6. Gall on stem. Galler: Hymenoptera. Locality: RJ (Arraial do Cabo). Reference: Monteiro et al. (1994).

7. Gall on leaf, fusiform, reddish, glabrous, one-chambered. Galler: *Stephomyia tetraloba* Maia, 1993 (Cecidomyiidae). Localities: RJ (Maricá, Arraial do Cabo). References: Maia (2001a), Carvalho-Fernandes et al. (2016).
8. Gall on leaf, conical, reddish, glabrous, one-chambered. Galler: *Bruggmannia* sp. (Cecidomyiidae). Locality: RJ (Arraial do Cabo). Other dwellers: Eulophidae, Eurytomidae (Hymenoptera) – parasitoids. Reference: Carvalho-Fernandes et al. (2016).
9. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: undetermined. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Carvalho-Fernandes et al. (2016).
10. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: undetermined. Localities: RJ (Araruama, Cabo Frio, Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
11. Gall on leaf vein, globoid, green, glabrous, one-chambered. Galler: Hymenoptera. Locality: RJ (Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
12. Gall on bud, rosette, green, glabrous, one-chambered. Galler: undetermined. Other dwellers: Torymidae (Hymenoptera) – parasitoids. Locality: RJ (Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).

Eugenia hiemalis Cambess.

1. Gall on bud, cylindrical, brown, glabrous, one-chambered. Galler: *Stephomyia* sp. (Cecidomyiidae). Other dwellers: *Leptothorax* sp. (Formicidae, Hymenoptera) – inquiline; Eupelmidae, Platygastridae (Hymenoptera) – parasitoids. Locality: RJ (Carapebus). Reference: Maia (2001a).
2. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: Lasiopteridi (Cecidomyiidae). Other dwellers: Hymenoptera – parasitoids. Locality: RJ (Carapebus). Reference: Maia (2001a).
3. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: Cecidomyiidae. Other dwellers: Eulophidae (Hymenoptera) – parasitoids. Locality: RJ (Carapebus). Reference: Maia (2001a).
4. Gall on leaf, conical, yellow, glabrous, one-chambered. Galler: Cecidomyiidae. Other dwellers: Eupelmidae, Eulophidae (Hymenoptera) – parasitoids. Locality: RJ (Carapebus). Reference: Maia (2001a).
5. Gall on leaf, claviform, green or reddish, glabrous, one-chambered. Galler: *Stephomyia cfr. clavata* (Tavares, 1920) (Cecidomyiidae). Other dwellers: Eulophidae, Eupelmidae, Platygastridae (Hymenoptera) – parasitoids. Locality: RJ (Carapebus). Reference: Maia (2001a).
6. Gall on leaf, conical, yellow, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Carapebus). Reference: Maia (2001a).

Eugenia monosperma Vell.

1. Gall on leaf, globoid, yellow, glabrous, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).
2. Gall on leaf, lenticular, glabrous, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Eugenia punicifolia (Kunt) DC.

1. Gall on leaf, cylindrical, green or reddish, glabrous, one-chambered. Galler: *Stephomyia* sp. (Cecidomyiidae). Other dwellers: Hymenoptera – parasitoids. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
2. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
3. Gall on leaf. Galler: Cecidomyiidae. Locality: RJ (Arraial do Cabo). Reference: Monteiro et al. (1994).
4. Gall on stem, fusiform, brown, glabrous, multichambered. Galler: Lasiopteridi (Diptera, Cecidomyiidae). Other dwellers: Hymenoptera – parasitoids. Localities: RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari; Saquarema, Cabo Frio). References: Oliveira & Maia (2005), Rodrigues et al. (2014), Carvalho-Fernandes et al. (2016).
5. Gall on stem. Galler: Hymenoptera. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
6. Gall on stem. Galler: undetermined. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
7. Gall on fruit, globoid, green or yellow, glabrous, multichambered. Galler: undetermined. Other dwellers: Curculionidae (Coleoptera) – inquiline; Hymenoptera – parasitoids. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
8. Gall on fruit. Galler: Cecidomyiidae. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).

Eugenia selloi (O. Berg.) B. D. Jacks.

1. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: Cecidomyiidae. Localities: RJ (Saquarema, Araruama, Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).

Eugenia speciosa Cambess.

1. Gall on leaf, conical, yellow, glabrous, one-chambered. Galler: Schizomyiina (Cecidomyiidae). Other insects: Sciaridae – inquiline. Locality: SP (Bertioga). Reference: Maia et al. (2008).
2. Gall on leaf, claviform, green, glabrous, one-chambered. Galler: Schizomyiina (Cecidomyiidae). Other dwellers: Platygastridae (Hymenoptera) – parasitoids. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Eugenia sulcata Spring ex Mart.

1. Gall on bud, cylindrical, reddish, glabrous, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008)
2. Gall on bud, ovoid, green, glabrous. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Eugenia uniflora L.

1. Gall on leaf, globoid, spongy, whitish, glabrous, one-chambered. Galler: *Eugeniamyia dispar* Maia, Mendonça & Romanovski, 1996 (Cecidomyiidae). Other dwellers: Eurytomidae (Hymenoptera) – parasitoids. Localities: RJ (Parque Natural Municipal Chico Mendes – new record), SP (Bertioga). Reference: Maia et al. (2008).

2. Gall on leaf, conical, green or reddish, glabrous, one-chambered. Galler: *Clinodiplosis profusa* Maia 2001 (Cecidomyiidae). Other dwellers: *Chrysonotomyia* sp. (Eulophidae), Tetrastichinae (Eulophidae), Platygastridae (Hymenoptera) – parasitoids; *Gynaikothrips uzeli* (Zimerman, 1909) (Thysanoptera, Phlaeothripidae) – inquilines. Localities: RJ (Mangaratiba: Ilha da Marambaia; Rio de Janeiro: Grumari; Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba, São João da Barra). References: Maia (2001a,b); Monteiro et al. (1994), Monteiro et al. (2004), Oliveira & Maia (2005), Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
3. Gall on leaf, lenticular, green, yellowish or black, glabrous, one-chambered. Galler: *Neolasioptera eugeniae* Maia 1993 (Cecidomyiidae). Other dwellers: Eulophidae (Cecidomyiidae) – parasitoids. Localities: RJ (Paraty, Angra dos Reis: Ilha Grande; Rio de Janeiro: Grumari, Parque Natural Municipal Chico Mendes – new record; Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, São João da Barra. References: Maia (1993a, 2001a), Maia & Oliveira (2010), Monteiro et al. (1994), Oliveira & Maia (2005), Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
4. Gall on fruit, conical, reddish, glabrous, one-chambered. Galler: Cecidomyiidae. Other dwellers: Eurytomidae (Hymenoptera) – parasitoids. Localities: RJ (Maricá, Cabo Frio, São João da Barra). References: Monteiro et al. (1994), Carvalho-Fernandes et al. (2016).
5. Gall on leaf, conical, green, glabrous, one-chambered. Galler: *Eugeniamyia triangularis* Maia, 2011 (Cecidomyiidae). Locality: RJ (Maricá). Reference: Maia & Nava (2011).
6. Gall on bud flower, conical, reddish, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Rio de Janeiro: Grumari). Reference: Oliveira & Maia (2005).
7. Gall on stem, fusiform, brown, glabrous, one-chambered. Galler: undetermined. Localities: RJ (Angra dos Reis: Ilha Grande; Mangaratiba: Ilha da Marambaia). References: Maia & Oliveira (2010), Rodrigues et al. (2014).
8. Gall on leaf, fold, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).

Myrcia brasiliensis Kiaersk.

1. Gall on stem, fusiform, brown, glabrous, one-chambered. Galler: *Pacholenus pelliceus* Boheman, 1836 (Coleoptera: Curculionidae). Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
2. Gall on bud, ovoid, multichambered, glabrous. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).
3. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: *Bruggmanniella* cfr. (Cecidomyiidae). Locality: SP (Bertioga). Reference: Maia et al. (2008).

Myrcia ilheosensis Kiaersk.

1. Gall on stem, fusiform, brown, glabrous, one-chambered. Galler: *Pacholenus pelliceus* Boheman, 1836 (Coleoptera: Curculionidae). Localities: SP (Bertioga), RJ (Maricá). References: Monteiro et al. (1994), Vanin (2008), Maia et al. (2008).

Myrcia lundiana Kiaersk.

1. Gall on leaf, globoid. Galler: *Dasineura* sp. (Cecidomyiidae). Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
2. Gall on leaf. Galler: undetermined. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
3. Gall on bud, ovoid, grooved. Galler: *Myrciamyia maricaensis* Maia, 1995 (Cecidomyiidae). Locality: RJ (Parque Nacional da Restinga de Jurubatiba). References: Maia (1996), Monteiro et al. (2004).
4. Gall on peduncle flower, fusiform. Galler: Cecidomyiidae. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
5. Gall on leaf, lenticular. Galler: undetermined. Locality: RJ (Parque Nacional da Restinga de Jurubatiba). Reference: Monteiro et al. (2004).
6. Gall on leaf vein. Galler: undetermined. Locality: RJ (Parque Nacional da Restinga de Jurubatiba. Reference: Monteiro et al. (2004).

Myrcia multiflora (Lam.) DC.

1. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: Thysanoptera. Other insects associados: *Lestodiplosis* sp. – predator: Locality: SP (Bertioga). References: Maia et al. (2008).

Myrcia ovata Camb.

1. Gall on leaf, globoid, yellow, glabrous, one-chambered. Galler: *Dasineura* sp. (Cecidomyiidae). Other dwellers: Tetrastichinae (Eulophidae, Hymenoptera) – inquiline. Localities: RJ (Maricá, Arraial do Cabo). References: Maia (2001a), Maia et al. (2002), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).
2. Gall on leaf. Galler: undetermined. Locality: RJ (Maricá). Reference: Monteiro et al. (1994).
3. Gall on bud, ovoid, green, grooved, glabrous, one-chambered. Galler: *Myrciamyia maricaensis* Maia, 1995 (Cecidomyiidae). Other dwellers: Platygastridae (Hymenoptera) – parasitoids; *Aprostocetus* sp. (Eulophidae, Hymenoptera) – inquiline. Localities: RJ (Maricá, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba). References: Maia (1996, 2001a), Monteiro et al. (2004).
4. Gall on peduncle flower, fusiform, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Maricá). References: Maia (2001a), Monteiro et al. (2004).
5. Gall on leaf vein, conical, green, glabrous, one-chambered. Galler: *Dasineura* sp. (Cecidomyiidae). Localities: RJ (Araruama, Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
6. Gall on leaf, conical, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
7. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: Thysanoptera. Locality: RJ (Cabo Frio). Reference: Carvalho-Fernandes et al. (2016).

8. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: undetermined. Locality: RJ (Cabo Frio). Reference: Carvalho-Fernandes et al. (2016).

Myrcia palustris DC.

1. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).
2. Gall on bud, ovoid, brown, glabrous, multichambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Myrcia racemosa (O. Berg.) Kiaersk.

1. Gall on stem, globoid, brown, glabrous, multichambered. Galler: undetermined. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).

Myrcia splendens (SW.) DC.

1. Gall on leaf, globoid, green, glabrous, one-chambered. Galler: undetermined. Other dwellers: Hymenoptera – parasitoids. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
2. Gall on leaf, conical, yellow or green, glabrous, one-chambered. Galler: undetermined. Other dwellers: Hymenoptera – parasitoids. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
3. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: undetermined. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
4. Gall on leaf, leaf roll, reddish, glabrous, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Reference: Maia et al. (2008).
5. Gall on bud, globoid, green or brown, glabrous, multichambered. Galler: undetermined. Locality: RJ (Mangaratiba: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
6. Gall on stem, globoid, brown, glabrous. Galler: Lasiopteridi (Cecidomyiidae). Other dwellers: Platygastridae (Hymenoptera) – parasitoids. Locality: SP (Bertioga). Reference: Maia et al. (2008).
7. Gall on vein leaf, globoid, brown, glabrous, one-chambered. Galler: Lasiopteridi (Cecidomyiidae). Other dwellers: Platygastridae (Hymenoptera) – parasitoids. Locality: SP (Bertioga). Reference: Maia et al. (2008).
8. Gall on bud. Galler: Lasiopteridi (Cecidomyiidae). Other dwellers: Torymidae (Hymenoptera) – parasitoids. Locality: SP (Bertioga). Reference: Maia et al. (2008).
9. Gall on stem, fusiform, multichambered. Galler: Cecidomyiidae. Locality: SP (Bertioga). Reference: Maia et al. (2008).
10. Gall on stem or leaf vein. Galler: Lasiopteridi (Cecidomyiidae). Other dwellers: Hemiptera (Coccidae?), *Trotteria* sp. (Cecidomyiidae) – inquilines. Locality: SP (Bertioga). Reference: Maia et al. (2008).
11. Gall on bud, conical. Galler: undetermined. Other dwellers: *Lestodiplosis* sp.(Cecidomyiidae) - predator. Locality: SP (Bertioga). Reference: Maia et al. (2008).

Myrciaria floribunda Miq.

1. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: *Dasineura myrciariae* Maia, 1995 (Cecidomyiidae). Other dwellers: *Lestodiplosis* sp. (Cecidomyiidae) – predator; *Proacrias* sp. (Eulophidae, Hymenoptera) – parasitoids. Localities: RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record; Maricá, Carapebus, Parque Nacional da Restinga de Jurubatiba), ES (Guarapari). References: Maia (1995), Monteiro et al. (1994), Monteiro et al. (2004), Bregonci et al. (2010).
2. Gall on leaf, leaf roll, green, glabrous, one-chambered. Galler: Thysanoptera. Localities: RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record; Maricá, Arraial do Cabo). References: Monteiro et al. (1994), Carvalho-Fernandes et al. (2016).
3. Gall on bud, bivalve, yellow, glabrous, one-chambered. Galler: *Myrciariamyia bivalva* Maia, 1994 (Cecidomyiidae). Localities: RJ (Rio de Janeiro: Parque Natural Municipal Chico – new record; Maricá, Carapebus, Arraial do Cabo, Jurubatiba). References: Maia (1994, 2001a), Monteiro et al. (1994), Monteiro et al. (2004).
4. Gall on leaf, star-shaped, yellow, one-chambered. Galler: Cecidomyiidae. Other dwellers: Hymenoptera – parasitoids. Localities: RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record; Maricá, Carapebus). Reference: Maia (2001a).
5. Gall on leaf, lenticular, green, glabrous, one-chambered. Galler: undetermined. Locality: RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record).
6. Gall on leaf, globoid, glabrous, one-chambered. Galler: *Eugeniamyia dispar* Maia, Mendonça & Romanovski, 1996 (Cecidomyiidae). Locality: (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record).
7. Gall on stem, globoid, green, glabrous, one-chambered. Galler: undetermined. Localities: RJ (Rio de Janeiro: Parque Natural Municipal Chico Mendes – new record), ES (Guarapari). Reference: Bregonci et al. (2010).
8. Gall on leaf, globoid, green, hairy, one-, bi- or three-chambered. Galler: Cecidomyiidae. Other dwellers: *Prodecatoma* (Eurytomidae, Hymenoptera) –parasitoids, Thysanoptera and Formicidae (no data on habits). Locality: ES (Guarapari). Reference: Bregonci et al. (2010).
9. Gall on leaf, cylindrical, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Arraial do Cabo). Reference: Carvalho-Fernandes et al. (2016).
10. Gall on bud, rosette, green, glabrous, one-chambered. Galler: undetermined. Locality: RJ (Parque Natural Municipal Chico Mendes – new record).

Myrciaria tenella (DC.) O. Berg

1. Gall on young leaf, leaf fold, green, glabrous, one-chambered. Galler: Hemiptera. Localities: RJ (Araruama, São João da Barra). Reference: Carvalho-Fernandes et al. (2016).

Neomitranthes obscura (DC.) Silveira

1. Gall on leaf, conical, green, glabrous, one-chambered. Galler: Cecidomyiidae. Localities: RJ (Maricá, Arraial do Cabo, Carapebus e Parque Nacional da Restinga de Jurubatiba). References: Maia & Couri (1997), Maia (2001a), Monteiro et al. (2004).
2. Gall on leaf, leaf roll, green, glabrous, one-chambered Galler: Thysanoptera. Localities: RJ (Maricá, Araruama, Arraial

do Cabo). References: Maia 2006, Monteiro et al. (1994), Carvalho-Fernandes et al. (2016).

3. Gall on leaf, fusiform, green, glabrous, one-chambered. Galler: *Stephomyia mina* Maia, 1993 (Cecidomyiidae). Other dwellers: Hymenoptera – parasitoids. Localities: RJ (Maricá, Araruama, Arraial do Cabo, Carapebus). References: Maia (1993b, 2001a), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).
4. Gall on leaf, roll marginal, green, glabrous, one-chambered. Galler: *Clinodiplosis* sp. (Cecidomyiidae). Other dwellers: *Dasineura tavaresi* Maia, 1995 (Cecidomyiidae), *Stenoma annosa* Butler, 1877 (Lepidoptera) – inquilines. Localities: RJ (Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba), ES (Guarapari). References: Maia (1995, 1996, 2001a), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016), Silva & Maia (2016).
5. Gall on bud, pineapple-shaped, green, glabrous. Galler: *Neomitranthella robusta* Maia, 1995 (Cecidomyiidae). Other dwellers: inquilines – *Stenoma annosa* (Lepidoptera), parasitoids – Tetrastichinae (Eulophidae, Hymenoptera). Localities: RJ (Maricá, Saquarema, Araruama, Cabo Frio, Arraial do Cabo, Carapebus, Parque Nacional da Restinga de Jurubatiba). References: Maia (1996, 2001a), Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).
6. Gall on stem, fusiform, brown, glabrous, one-chambered. Galler: undetermined. Localities: RJ (Saquarema, Araruama, Arraial do Cabo, Parque Nacional da Restinga de Jurubatiba. References: Monteiro et al. (2004), Carvalho-Fernandes et al. (2016).

Plinia cauliflora Kausel

1. Gall on leaf, marginal roll, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Angra dos Reis: Ilha Grande). Reference: Maia & Oliveira (2010).
2. Gall on bud, cylindrical, green, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Angra dos Reis: Ilha Grande). Reference: Maia & Oliveira (2010).

Psidium cattleianum Sabine

1. Gall on leaf, biconical, green, glabrous, one-chambered. Galler: *Tectococcus ovatus* Hempel (Eriococcidae, Hemiptera). Locality: RJ (Angra dos Reis: Ilha Grande). Reference: Maia & Oliveira (2010).
2. Gall on leaf or bud, globoid, yellow, glabrous, one-chambered. Galler: Cecidomyiidae. Locality: RJ (Angra dos Reis: Ilha Grande). Reference: Maia & Oliveira (2010).
3. Gall on stem, fusiform, brown, glabrous, multichambered. Galler: undetermined. Locality: RJ (Angra dos Reis: Ilha da Marambaia). Reference: Rodrigues et al. (2014).
4. Gall on leaf, tubular, green, glabrous, one-chambered. Galler: Lasiopteridi (Cecidomyiidae). Locality: SP (Bertioga). Reference: Maia et al. (2008).
5. Gall on bud, green, rosette, glabrous. Galler: *Dasineura gigantea* Angelo & Maia, 1999 (Cecidomyiidae). Locality: SP (Bertioga). Reference: Maia et al. 2008.
6. Gall on leaf, conical, with small apical projections, green, glabrous. Galler: Cecidomyiidae. Locality: SP (Bertioga). Reference: Maia et al. (2008).
7. Gall on leaf, globoid, one-chambered. Galler: undetermined. Locality: SP (Bertioga). Other dwellers: Lepidoptera – inquiline. Reference: Maia et al. (2008).
8. Gall on leaf or leaf vein, lenticular. Galler: Cecidomyiidae. Locality: SP (Bertioga). Reference: Maia et al. 2008.

Discussion

In Brazilian restingas, Myrtaceae included from 18.18% to 11.38% of the total number of galled plant species (Table 1). In other physiognomies of the Atlantic Forest, the rates were lower, 0.00% in Semidecidual Seasonal Forest (Flor et al. 2018) and Altitude Fields (Coelho et al. 2013), from 3.86% to 11.11% in Ombrophilous Forest (Maia 2014, Maia et al. 2014 and Maia & Mascarenhas 2017), 8.16% in High Altitude Wetland Forest (Santos et al. 2011), and 9.75% in Tableland Forest (Maia & Carvalho-Fernandes 2015). Furthermore, Myrtaceae were not the plant family with the greatest number of galled species in these physiognomies. This position is occupied by Sapindaceae (in Semidecidual Seasonal Forest – Flor et al. 2018), Asteraceae (in Altitude Fields – Coelho et al. 2013 and Ombrophilous Forest – Maia et al. 2014 and Maia & Mascarenhas 2017), Melastomataceae (Ombrophilous Forest – Maia 2014), Nyctaginaceae (High Altitude Wetland Forest – Santos et al. 2011), and Fabaceae (in Tableland Forest – Maia & Carvalho-Fernandes 2015). The rates of gall morphotypes on Myrtaceae varied from 34.72% to 13.13% of the total number of gall morphotypes in Brazilian restingas (Table 1). In other physiognomies of the Atlantic Forest, these rates were lower, 0.00% in Semidecidual Seasonal Forest (Flor et al. 2018) and Altitude Fields (Coelho et al. 2013), from 6.40% to 10.89% in Ombrophilous Forest (Maia 2014, Maia et al. 2014 and Maia & Mascarenhas 2017), 8.86% in High Altitude Wetland Forest (Santos et al. 2011), and 9.09% in Tableland Forest (Maia & Carvalho-Fernandes 2015). These data revealed that Myrtaceae are an important host plant family mainly in restingas, while in other physiognomies of the Atlantic Forest this family can contribute to the gall richness as in the Ombrophilous Forest, High Altitude Wetland Forest and Tableland Forest or not as in Semidecidual Seasonal Forest and Altitude Fields. *Eugenia* and *Myrcia* highlighted as the Myrtaceae genera with the greatest number of galled species and gall morphotypes. Both are important components of the restinga flora, being the best represented genera of Myrtaceae in this physiognomy (Lourenço & Barbosa 2012, Souza & Morim 2008). The hypothesis of taxon size (Fernandes 1992) could explain the greater insect galls richness on Myrtaceae, *Eugenia* and *Myrcia*. It predicts that richer taxa have potentially more hosts and, consequently, a greater number of associated galling insects.

Leaves have been reported as the most galled plant organ in all regions of the world. This pattern was observed for the first time by Felt 1940 and has been confirmed in several studies (Maia & Fernandes 2004, Santos et al. 2011, Maia & Carvalho-Fernandes 2016, Maia & Mascarenhas 2017), probably because leaves represent an abundant, frequent and predictable resource.

Green galls predominated, probably because this is the color of the most frequent galled organ, leaf. Most galls were glabrous and one-chambered. These features have been observed as the most frequent in several Brazilian inventories, not only in restingas, but also in other physiognomies of the Atlantic Forest, as well as in other domains phytogeographic (Maia & Fernandes 2004, Santos et al. 2011, Maia & Carvalho-Fernandes 2016, Maia & Mascarenhas 2017).

Only 19% of the galling species have been identified in species. These results show how the taxonomical knowledge of the gallers is still incipient. Cecidomyiidae induced most galls as in all other inventories in the world. Among the gall midges genera, *Clinodiplosis* Kieffer, 1894 and *Dasineura* Rondani, 1840 have a wide world distribution, while *Neolasioptera* Felt, 1908 occurs in the Nearctic and Neotropical regions, and the others, *Bruggmannia* Tavares, 1906, *Eugeniamyia* Maia, Mendonça & Romanovski, 1997, *Jorgenseniella* Maia, 2005, *Myrciamyia* Maia, 1995, *Myrciariamyia* Maia, 1994, *Neomitranthella* Maia, 1996, and *Stephomyia* Rondani, 1840 are exclusively Neotropical. Among them, five have been recorded only in Brazil: *Eugeniamyia*, *Myrciariamyia*, *Jorgenseniella*, *Myrciamyia*, and *Neomitranthella*, being the last three restricted to restingas. *Clinodiplosis*, *Dasineura*, and *Neolasioptera* are speciose genera, with 476, 103 and 134 described species, respectively. *Bruggmannia*, *Eugeniamyia, Myrciariamyia,* and *Stephomyia* include 19, 2, 3 and 7 species, respectively. The others are monotypic (Gagné & Jaschhof 2017). *Clinodiplosis*, *Dasineura*, and *Neolasioptera* induce galls on several plant families, whereas most *Bruggmannia* species occurs on Nyctaginaceae, the other genera are exclusively associated with Myrtaceae (Gagné & Jaschhof 2017).

Rio de Janeiro is the most investigated Brazilian State, for this reason, it harbors most records. The geographical distribution of all galled plants is wider than that of the gallers, indicating that these insects can probably have a greater area of occurrence.

Galling species that occur exclusively on endemic plants were proposed as endemic too, based on their specificity of hosts. Concerning the conservational status, the gallers could not be evaluated as data on the host plants are deficient.

The presence of parasitoids, inquilines and predators has been frequently reported in gall inventories, not only in restingas and in Myrtaceae, but also in other ecosystems and plant families (Maia 2001a, Maia & Fernandes 2004, Maia et al. 2014, Maia & Carvalho-Fernandes 2016). Parasitoids have been considered the most frequent natural enemies of galling insects (Gagné 1994). Although all parasitoid families found in the present study are associated with several plant families in restinga, they are more diverse and frequent on Myrtaceae (Maia & Azevedo 2009). Concerning the parasitoids genera, *Donquickeia* and *Proacrias* are associated exclusively with Myrtaceae in restingas, while *Rileya* and *Chrysonotomia* have been recorded in other families, too (Maia & Azevedo 2009). Most records are in family categories, revealing that their taxonomical knowledge is still poor in Brazilian restingas. Multiparasitisme have been recorded not only in Myrtaceae, but also in other plant families (Maia & Monteiro 1999, Maia & Tavares 2000, Maia & Azevedo 2009). All predaceous and most inquilinous taxa recorded on galls in Myrtaceae of restingas have been reported in other plant families too (Maia 2001a, 2002), as well as in other phytogeographic domains (Maia 2001a, Maia & Fernandes 2004, Maia et al. 2014, Maia & Carvalho-Fernandes 2016). But some of them, as *Leptothorax* sp. (Hymenoptera), *Stenoma annosa* (Lepidoptera), and *Gynaikothrips uzeli* (Thysanoptera) are known only on Myrtaceae at restingas. These diverse associated fauna highlights the importance of the galling species as ecosystem engineers, as they provide a new niche, the gall, which can be used by several arthropods.

Although restingas are the best studied phytophysiognomy of Brazil, new records were added, as many areas have not yet been studied.

Conclusions

Myrtaceae harbor a great richness of insect galls in Brazilian restingas, 111 morphotypes, in 25 host plant species (15 endemic). Most morphotypes are induced by Cecidomyiidae. Twelve gall midge species (Diptera, Cecidomyiidae), one beetle species (Coleoptera, Curculionidae) and one scale-insect species (Hemiptera, Eriococcidae) are associated with endemic hosts. These gallers are proposed as endemic.

Eugenia L. is the plant genus with the greatest richness of host species and gall morphotypes. Leaves are the most frequent galled organ. Globoid shape, green color, absence of trichomes and a single internal chamber are the predominant gall traits. Many arthropods, mainly insects, have been reported as dwellers of galls, where they play the role of parasitoids, inquilines and predators, highlighting the importance of the gallers as ecosystem engineers.The taxonomical knowledge of the galling species and other dwellers is still deficient. Great efforts are necessary to refine it, involving field trips, rearing, and taxonomist participation.

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Author Contributions

Valéria Cid Maia: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of interest

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

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Erratum

In the article “*Non-invasive hair sampling of Neotropical otters*” with DOI code number http://dx.doi.org/10.1590/1676-0611-bn-2018-0579 published at Biota Neotropica 18(4): e20180579, the “Acknowledgements” section had not been included:

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Inventory

Ichthyofauna of streams of the Rio Sapucaí basin, upper Rio Paraná system, Minas Gerais, Brazil

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Abstract: The Rio Sapucaí basin, in Minas Gerais State, Brazil, is one of the many watersheds of the upper Rio Paraná system. Ichthyofauna surveys in this basin, in general, are scarce. In addition, small rivers and streams of the region have been targets of anthropogenic actions (e.g., pollution) – which suggest that more ichthyological studies must be performed within the watershed. In this study we provide a survey of species that occur within three streams of the lower Rio Sapucaí basin. Samples were collected in April, July, and November 2017 and in May 2018. Collections resulted in 349 individuals belonging to 28 species, five orders, and 12 families. Among our findings are three putatively undescribed species and the first record of *Oligosarcus argenteus* and *Pareiorhina hyptiorhachis* within the Rio Paraná system.

Keywords: *fishes; inventory; Oligosarcus argenteus; Pareiorhina hyptiorhachis; undescribed species.*

Ictiofauna de riachos da bacia do Rio Sapucaí, sistema do alto Rio Paraná, Minas Gerais, Brasil

Resumo: A bacia do Rio Sapucaí, no Estado de Minas Gerais, Brasil, é uma das muitas bacias hidrográficas do sistema do alto Rio Paraná. Levantamentos de ictiofauna nessa bacia, em geral, são escassos. Além disso, rios de pequeno porte e córregos da região têm sido alvos de ações antrópicas (e.g., poluição) – o que sugere que mais estudos ictiológicos devem ser realizados na bacia. Neste trabalho nós fornecemos um levantamento de espécies que ocorrem em três riachos da porção baixa da bacia do Rio Sapucaí. As amostras foram coletadas em Abril, Julho, e Novembro de 2017 e em Maio de 2018. As coletas resultaram em 349 indivíduos pertencentes a 28 espécies, cinco ordens e 12 famílias. Dentre os nossos achados estão três espécies possivelmente não descritas e o primeiro registro de *Oligosarcus argenteus* e *Pareiorhina hyptiorhachis* no sistema do Rio Paraná.

Palavras-chave: *espécies não descritas; inventário; Oligosarcus argenteus; Pareiorhina hyptiorhachis; peixes.*

Introduction

Ichthyofauna surveys are important for the conservation of freshwater fishes at both short and long-term scales. These studies provide additional information about species' distribution (e.g., Valdiviezo-Rivera et al. 2017, Bertora et al. 2018, Delariva et al. 2018, Honorio & Martins 2018, Oliveira-Silva et al. 2018) – which, in turn, may assist in new assessments about their "conservation status" (e.g., Melo et al. 2017). Additionally, surveys provide useful data for the establishment of freshwater protected areas (sensu Azevedo-Santos et al. 2018a). Therefore, ichthyological surveys should be carried out more frequently, especially in Brazilian freshwaters.

The Rio Sapucaí basin (~ 560,000 hectare; Magalhães Jr & Diniz 1997), Minas Gerais, Brazil, is part of the upper Rio Paraná system (Magalhães Jr & Diniz 1997). To our knowledge, only two ichthyological surveys have been published for this watershed. Ingenito & Buckup (2007) provided a list with the fishes of three localities in the upper portion of the basin near the Serra da Mantiqueira. Subsequently, Belei & Sampaio (2012) publishes a work with the fishes from the Rio Lourenço Velho, a direct tributary of the Rio Sapucaí. However, streams of the lower region of the watershed remain understudied.

Countless rivers and streams of the Rio Sapucaí basin have been targets of anthropogenic actions (e.g. small dams; see Belei & Sampaio 2012), which can significantly impact the overall biodiversity (Pelicice et al. 2017). These actions coupled with the lack of biodiversity knowledge suggest that more surveys must be conducted within the watershed. In this study we provide the results of a fish survey conducted in three different streams of the lower portion of the Rio Sapucaí basin, in Minas Gerais, Brazil.

Material and Methods

Fishes were collected in April, July, and November 2017, and in May 2018 (totaling four collections, one per month), across three different streams of the Rio Sapucaí basin (Table 1; Figure 1-2). Sampling occurred during daytime roughly 100 to 200 meters upstream of each stream. Collections were carried out with a small cast net (1.4 cm of mesh in opposite nodes), a hand net (~1.5 mm mesh), gill nets (1 and 2 cm in opposite nodes), and fishhooks of different sizes. Collections were performed with permission issued by Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, in Portuguese) – license numbers 46904-1 and 63177-1.

Vouchers specimens were euthanized with successive dosages of anesthetic, and transferred to a 10% formalin solution. Following fixation, individuals were transferred to a 70% alcohol solution and deposited at LBP (Laboratório de Biologia de Peixes, Departamento de Morfologia, Universidade Estadual Paulista "Júlio de Mesquita Filho", Botucatu, São Paulo, Brazil), DZSJRP (Departamento de Zoologia e Botânica, Universidade Estadual Paulista "Júlio de Mesquita Filho", São José do Rio Preto, São Paulo, Brazil), and LIRP (Laboratório de Ictiologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil).

Species reported in Table 2 were classified according to Fricke et al. (2018).

Results

A total of 349 individuals representing five orders, 12 families, and 28 fish species (Table 2) were collected from all reaches (i.e., R1, R2, R3). The order and family with highest species richness, considering all reaches, was Siluriformes and Characidae, respectively (Figure 3-4). We found the highest species richness at R3, with a total of 23 species, followed by R1 with nine and R2 with seven (Table 3).

Three putatively undescribed species were also collected: *Astyanax* sp. and '*Heptapterus'* sp., both from R1, and *Imparfinis* sp., from R3. Additionally, we found individuals of *Oligosarcus argenteus* and *Pareiorhina hyptiorhachis*, which represent the first record of these two species within the Rio Paraná system (Table 3). Individuals of *Trichomycterus septemradiatus* were collected at R1, which also

Table 1. Localities sampled from the lower Rio Sapucaí basin, Rio Paraná system, Minas Gerais, Brazil

Reaches	Acronym	Coordinates	Altitude (meters)	Municipality	Remarks
Reach 1	R1	20°54'57.59"S, 45°56'21.15"W	~828	Carmo do Rio Claro	Stream of unknown name. Affluent of Ribeirão Itací, Rio Sapucaí basin. Reach in the stream well-preserved. Riparian vegetation present. Reach with substrate, in general, composed by rocks juxtaposed.
Reach 2	R2	20°55'25.12"S, 45°58'21.63"W	~783	Carmo do Rio Claro	Stream of unknown name. Affluent of Ribeirão Itací, Rio Sapucaí basin. Reach in the stream with total (in some local partial) absence of riparian vegetation. Substrate composed by sandy, rarely with some rocks.
Reach 3	R3	21° 3'25.73"S, 46° 8'47.10"W	~787	Conceição da Aparecida	Stream known as Ribeirão Fortaleza, Rio Sapucaí basin. Reach in the stream with riparian vegetation completely removed. Substrate composed by sandy, with a local composed by rocks. Some locals impacted due the cattle breeding.

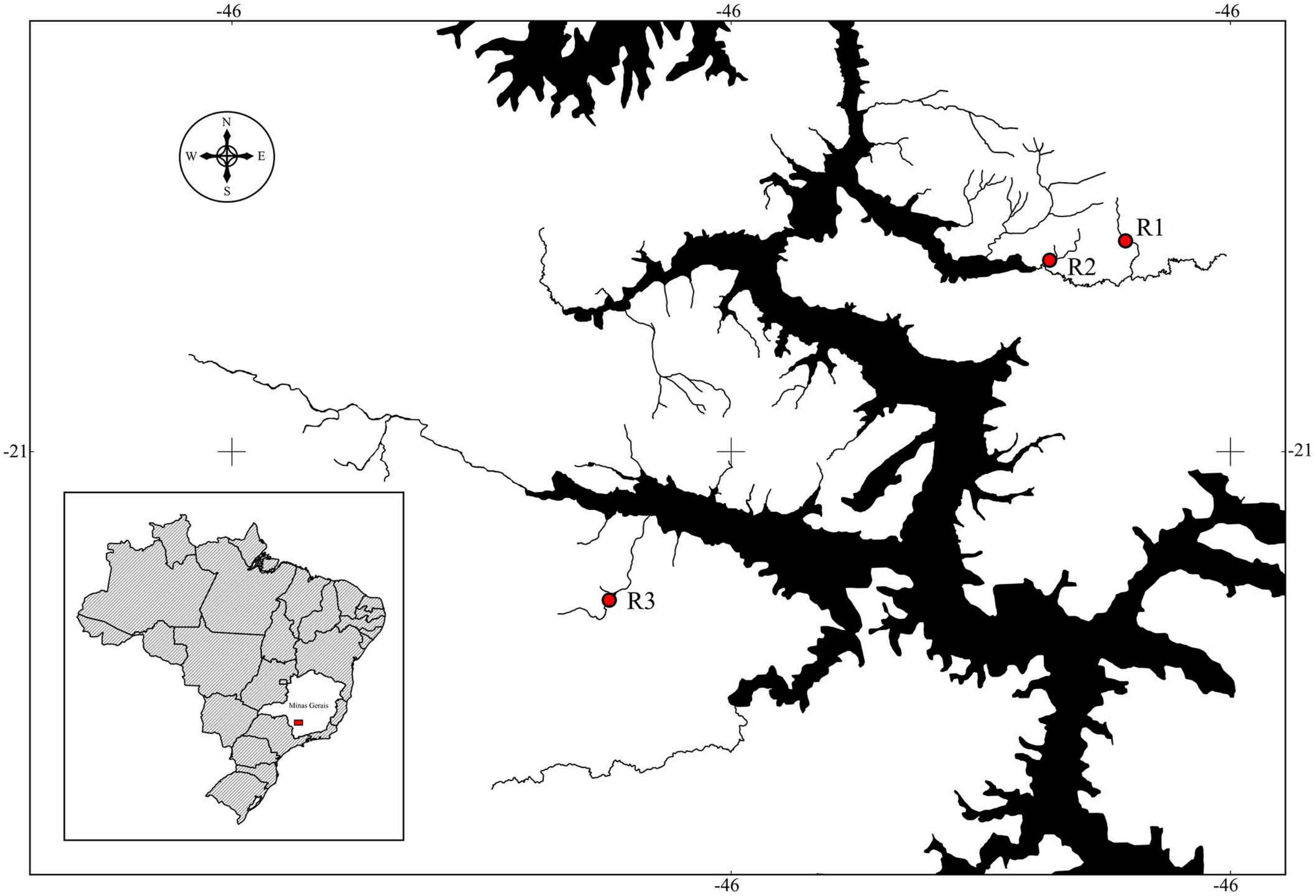

Figure 1. Partial view of Rio Sapucaí (under influence of the Furnas reservoir), in Minas Gerais, Brazil, with the location of each reach (R1, R2, and R3) sampled.

expands the distribution of this species into the Rio Paraná system. Lastly, we recorded *Knodus moenkhausii* and *Poecilia vivipara*, two non-native fish species within the Rio Sapucaí basin.

Discussion

Overall, members of the orders Siluriformes and Characiformes comprise the majority of species found in the three sampled streams of the Rio Sapucaí basin (see Figure 3). Dozens of investigators who have conducted fish surveys of rivers, reservoirs, or streams of the upper Paraná basin (e.g., Casatti et al. 2003, Smith et al. 2007, Smith & Petrere Jr 2007, Fagundes et al. 2015, Frota et al. 2016, Santos et al. 2017, Cavalli et al. 2018) have also found species richness to be highest in these orders. Therefore, the relatively high species counts in these two orders, as we found in this study, is an expected result for many regions of the upper Rio Paraná system.

The families with highest species richness in the lower Rio Sapucaí region are Characidae and Heptapteridae. However, in context of the Rio Paraná basin as a whole, Loricariidae has been reported to contribute higher species richness than Heptapteridae (Langeani et al. 2007). This suggests that loricariid species may have been undersampled in this survey. Specimens were collected only during the day (see Material and Methods section), which may have contributed to an undersampling of loricariids and possibly other groups (see below). Therefore, for future studies we recommend sampling at each stream during the night as well.

Odontostilbe weitzmani Chuctaya, Bührnheim, & Malabarba, 2018, a species recently described from the upper Paraná system (Chuctaya et al. 2018), has previously been collected at R1 (DZSJRP 20445, 2014 year). However, this species was not collected during this survey.

Three putatively undescribed species (i.e., *Astyanax* sp., '*Heptapterus*' sp., and *Imparfinis* sp.) were collected during this survey (Figure 5a, b, c). In addition to this study, Ingenito & Buckup (2007) discovered six undescribed species within the upper Rio Sapucaí basin. With these results we believe more ichthyological surveys in rivers and streams of the Rio Sapucaí basin are necessary, as additional undescribed species likely remain to be discovered.

Langeani et al. (2007) did not report *Oligosarcus argenteus* (Figure 5e) within the upper Rio Paraná system. Additionally, in a recent revision of the genus *Oligosarcus*, Ribeiro & Menezes (2015) reported this species as endemic to the Rio São Francisco and Rio Doce basins. In turn, *Pareiorhina hyptiorhachis* (Figure 5f) was recently described from the Rio Paraíba do Sul basin (Silva et al. 2013). Our study reports individuals of *O. argenteus* at R1 and individuals of *P. hyptiorhachis* at R1 and R2. Therefore, these findings represent the first records of these two species in the Rio Sapucaí basin, as well as in the Rio Paraná system in general.

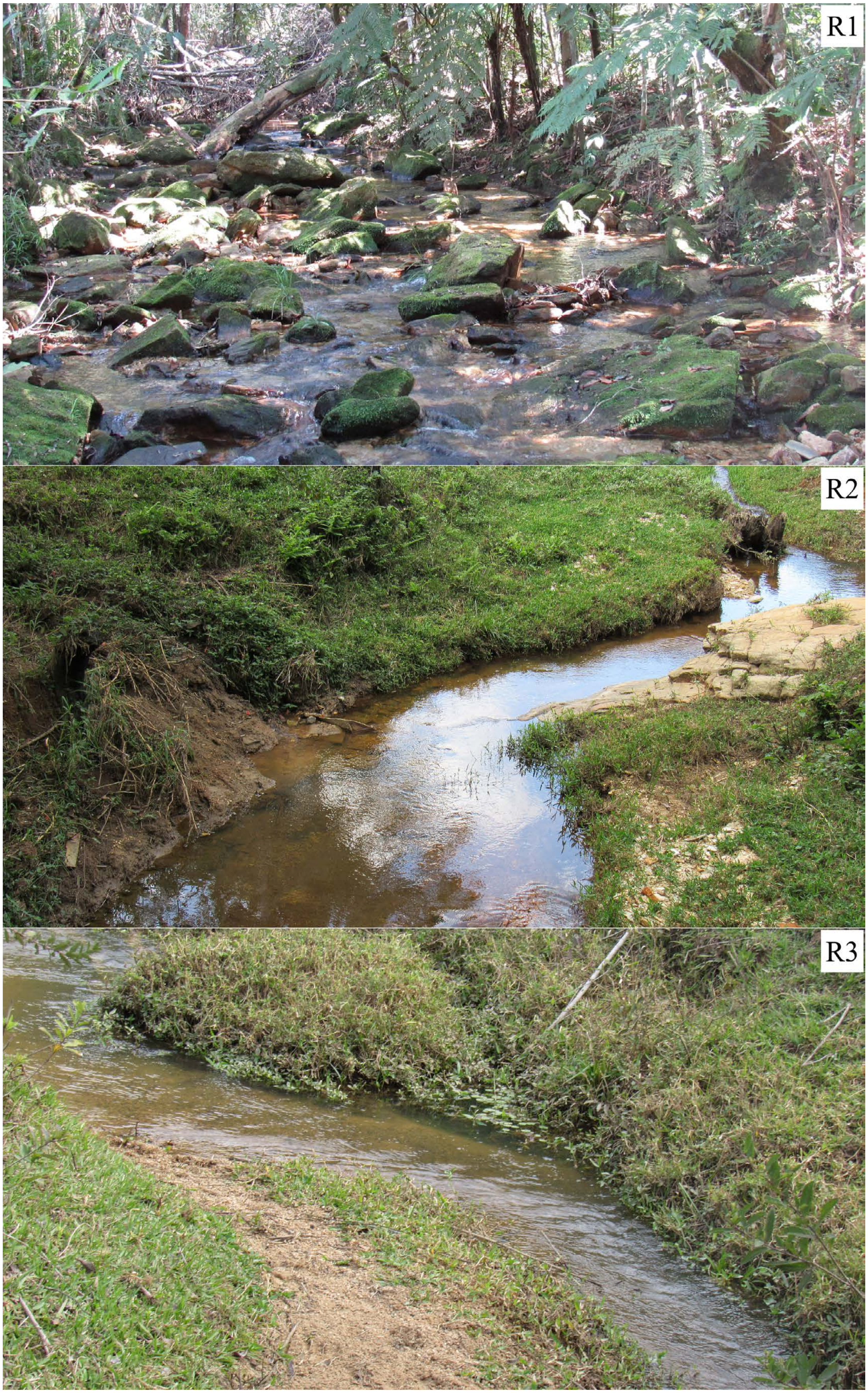

Figure 2. Partial view of each reach (R1, R2, and R3) sampled in streams of the Sapucaí basin, Minas Gerais, Brazil.

Table 2. Fish species captured in three reaches of streams of the Rio Sapucaí basin, upper Rio Paraná system, Minas Gerais, Brazil.

SPECIES	VOUCHER
CHARACIFORMES	
Crenuchidae	
Characidium zebra Eigenmann, 1909	DZSJRP 21138; LBP 26604
Characidium gomesi Travassos, 1956	DZSJRP 21136
Erythrinidae	
Hoplias malabaricus (Bloch, 1794)	LBP 23576; LBP 23580; LBP 23592; LBP 26591
Characidae	
Astyanax fasciatus (Cuvier, 1819)	LBP 23600; LBP 26585; LBP 26587; LBP 26608
Astyanax lacustris (Lütken, 1875)	LBP 26586; LBP 26589
Astyanax sp. **	LBP 23573; LBP 26571
Knodus moenkhausii (Eigenmann & Kennedy, 1903)***	DZSJRP 21131; LBP 26578; DZSJRP 21132; LBP 26583; LBP 26584; LBP 26605
Oligosarcus argenteus Günther, 1864*	LBP 23572
Oligosarcus paranensis Menezes & Géry, 1983	LBP 23591; LBP 26596
Piabarchus stramineus (Eigenmann, 1908)	LBP 26607
Piabina argentea Reinhardt, 1867	LBP 23601; LBP 26592; LBP 26611
GYMNOTIFORMES	
Gymnotidae	
Gymnotus carapo Linnaeus, 1758	LBP 23597; LBP 26602
Sternopygidae	
Eigenmannia cf. *trilineata* Lopez & Castello, 1966	LBP 23595; LBP 26599
SILURIFORMES	
Heptapteridae	
Cetopsorhamdia iheringi Schubart & Gomes, 1959	LBP 23574; LBP 26568; LBP 26574; LBP 26576; LBP 26582; LBP23599; LBP 26606
Rhamdia cf. *quelen* (Quoy & Gaimard, 1824)	LBP 26609
‘*Heptapterus*’ sp.**	LBP 23577; LBP 26570; LBP 26575
Imparfinis schubarti (Gomes, 1956)	LIRP 14326; LBP 26601
Pimelodella gracilis (Valenciennes, 1835)	LIRP 14327
Imparfinis sp.**	LIRP 14325; LBP 26600
Pimelodidae	
Pimelodus maculatus Lacepède, 1803	LBP 26594
Trichomycteridae	
Trichomycterus candidus (Miranda-Ribeiro, 1949)	LBP 23575; LBP 26567; LBP 26579; LBP 26581; LBP23596
Trichomycterus septemradiatus Katz, Barbosa & Costa 2013	LBP 23578; LBP 26569; LBP 26573; LBP 26577
Callichthyidae	
Callichthys callichthys (Linnaeus, 1758)	LBP 23598; LBP 26603
Loricariidae	
Hisonotus cf. *alberti* Roxo, Silva, Waltz, Melo 2016	LBP 23590; LBP 26593; LBP 26595
Hypostomus ancistroides (Ihering, 1911)	LBP 23594; LBP 26598
Pareiorhina hyptiorhachis Silva, Roxo, & Oliveira 2013 *	LBP 23571; LBP 26566; LBP 26572; LBP23579; LBP 26580
CICHLIFORMES	
Cichlidae	
Geophagus brasiliensis (Quoy & Gaimard, 1824)	LBP 23593; LBP 26588; LBP 26590; LBP 26597
CYPRINODONTIFORMES	
Poeciliidae	
Poecilia vivipara Bloch & Schneider, 1801***	LBP 26610

* First record for the upper Rio Paraná system.

** Putatively undescribed species.

*** Non-native species.

Table 3. Species captured (X) in each reach of the three different streams, Rio Sapucaí basin, Minas Gerais, Brazil.

SPECIES	REACH		
	R1	**R2**	**R3**
Astyanax fasciatus		X	X
Astyanax lacustris		X	X
Astyanax sp.	X		
Callichthys callichthys			X
Cetopsorhamdia iheringi	X	X	X
Characidium gomesi			X
Characidium zebra			X
Eigenmannia cf. *trilineata*			X
Geophagus brasiliensis			X
Gymnotus carapo			X
'*Heptapterus*' sp.	X		
Hisonotus cf. *alberti*			X
Hoplias malabaricus	X	X	X
Hypostomus ancistroides			X
Imparfinis schubarti			X
Imparfinis sp.			X
Knodus moenkhausii	X	X	X
Oligosarcus argenteus	X		
Oligosarcus paranensis			X
Pareiorhina hyptiorhachis	X	X	
Piabarchus stramineus			X
Piabina argentea			X
Pimelodella gracilis			X
Pimelodus maculatus			X
Poecilia vivipara			X
Rhamdia cf. *quelen*			X
Trichomycterus candidus	X	X	X
Trichomycterus septemradiatus	X		

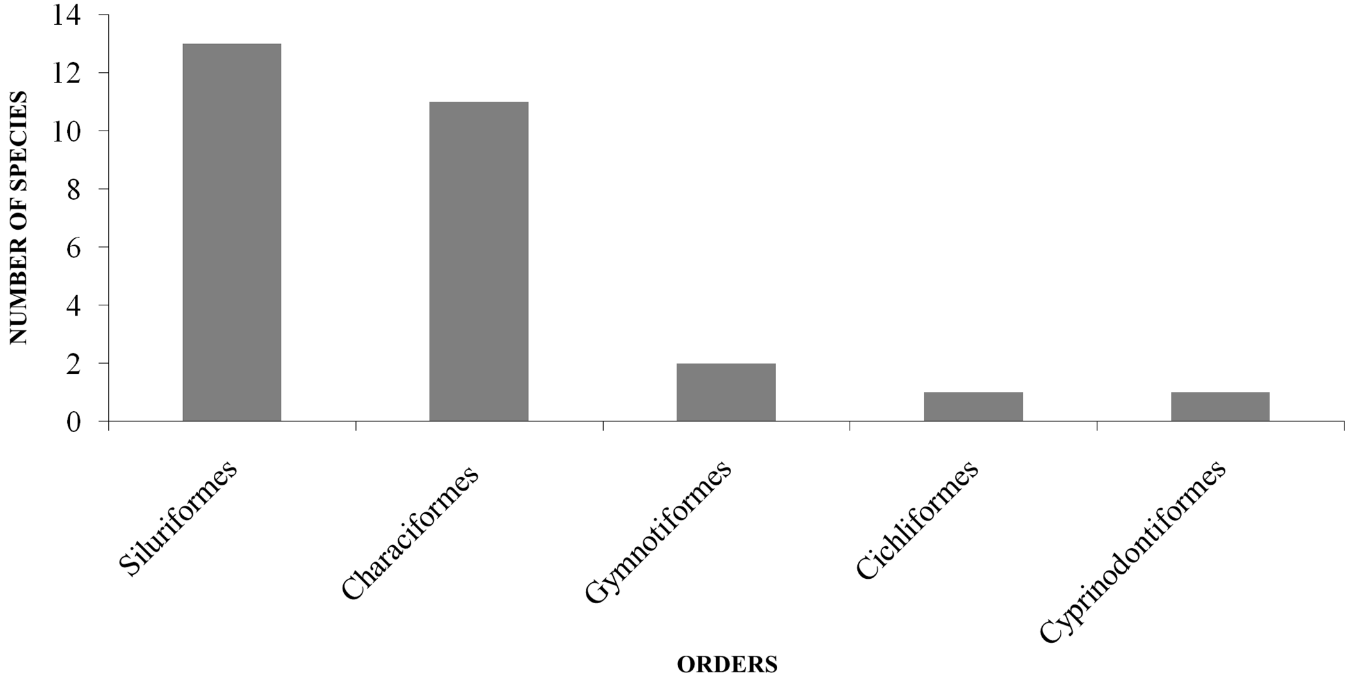

Figure 3. Species richness by orders collected in reaches of three different streams of the Rio Sapucaí basin, Minas Gerais, Brazil.

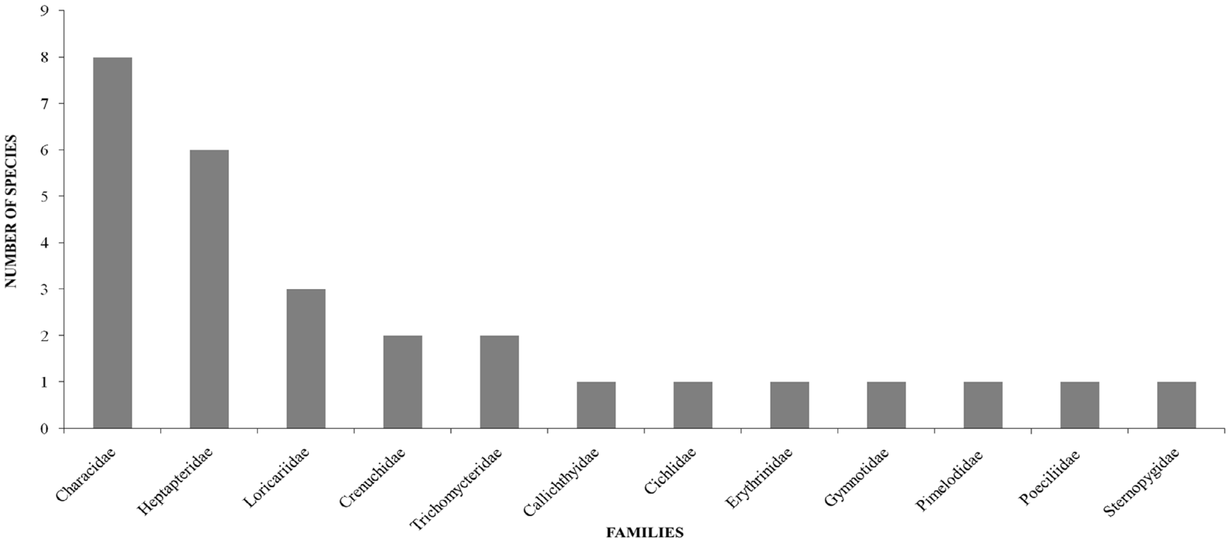

Figure 4. Species richness by families collected in reaches of three different streams of the Rio Sapucaí basin, Minas Gerais, Brazil.

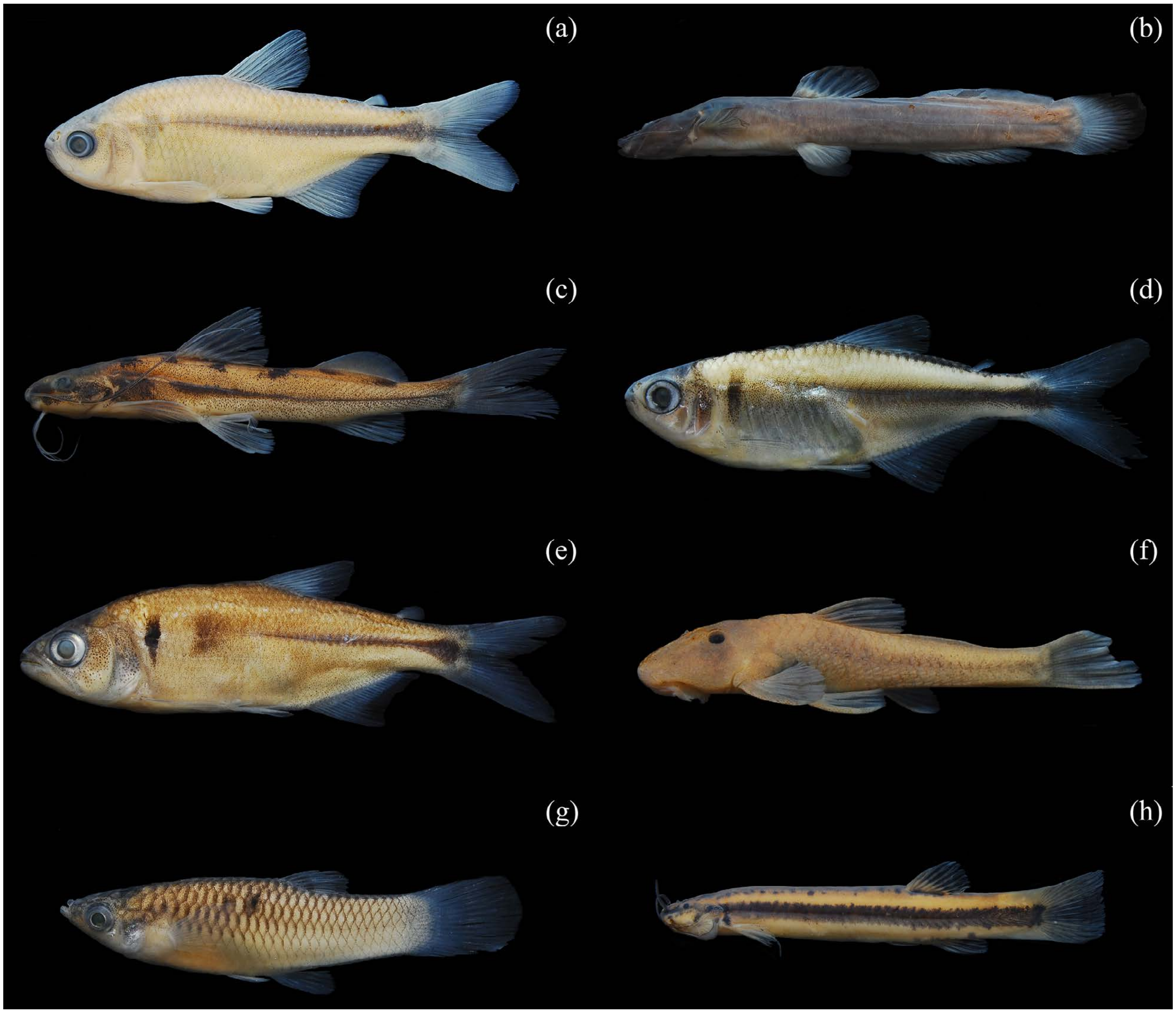

Figure 5. Representative individuals of eight species collected in this study: (a) *Astyanax* sp., 43.9 mm SL, LBP 26571; (b) '*Heptapterus*' sp., 104.8 mm SL, LBP 23577; (c) *Imparfinis* sp., 58.7 mm SL, LBP 26600; (d) *Knodus moenkhausii*, 34.5 mm SL, LBP 26584; (e) *Oligosarcus argenteus*, 61.8 mm SL, LBP 23572; (f) *Pareiorhina hyptiorhachis*, 28.5 mm SL, LBP 26572; (g) *Poecilia vivipara*, 29.6 mm SL, LBP 26610; (h) *Trichomycterus septemradiatus*, 39.6 mm SL, LBP 26573.

Trichomycterus septemradiatus (Figure 5h) was previously known only from its type locality, a single stream in the Rio Sapucaí basin (Katz et al. 2013). Our study reports individuals of *T. septemradiatus* at R1; therefore, we extend the distribution of this species within the basin.

Individuals of two non-native species, *Knodus moenkhausii* and *Poecilia vivipara*, were collected in this survey (see Table 3). *Knodus moenkhausii* (Figure 5d) has previously been assigned by different authors as non-native to the upper Rio Paraná system (e.g., Langeani et al. 2007, Souza et al. 2015, Azevedo-Santos et al. 2018b). *Poecilia vivipara* (Figure 5g) has also been reported by Langeani et al. (2007) as a non-native species introduced to the upper Rio Paraná system. Therefore, we consider *K. moenkhausii* and *P. vivipara* as non-native species within the Rio Sapucaí basin (sensu Langeani et al. 2007). However, sources of these introductions remain unknown.

Here we contribute to the knowledge of the fish fauna of the Rio Sapucaí basin, upper Paraná system. However, we recognize this study likely represents a small fraction of what remains to be sampled within this basin. The presence of putative undescribed species coupled with increasing anthropogenic effects highlights the need to conduct more surveys of the ichthyofauna of waterbodies of this region.

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Author Contributions

Valter M. Azevedo-Santos: idealized the survey and collected all individuals; identified fish species; wrote this manuscript.

Heraldo A. Britski: identified fish species; wrote this manuscript.

Claudio Oliveira: wrote this manuscript.

Ricardo C. Benine: wrote this manuscript.

Conflicts of interest

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

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Article

Spatial and temporal distribution and abundance of two species of *Persephona* (Decapoda: Brachyura: Leucosiidae) on the southern coast of the state of São Paulo, Brazil

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Abstract: Crabs of the genus *Persephona* are intensely captured in shrimp fisheries as bycatch in the Cananéia region off the coast of the state of São Paulo, Brazil. The analysis of the spatial and temporal distribution of *Persephona punctata* and *Persephona mediterranea* could provide information about variation in the abundance of these species, as well as the environmental variables affecting their distribution and the existence of a possible habitat partitioning. Using a shrimp fishery boat equipped with double-rig nets, crabs were monthly captured from July 2012 to June 2014 in seven sites: four in the coastal area adjacent to the Cananéia region and three in the Mar Pequeno estuarine area. The abundances of both species were compared according to spatial (among sites) and temporal (years and seasons) scale distribution. A total of 396 individuals of *P. punctata* and 64 of *P. mediterranea* were captured. The abundance of both species was higher in the second sampling year (July 2013–June 2014) and in coastal areas; only one individual of each species was captured in the estuarine area due to the low salinity at this location (approximately 27.7‰). The temperature was the environmental variable that most affected the distribution of both species, which was more abundant in warmer periods. The temporal variation in abundance was modulated by temperature, while salinity modulated the spatial distribution of *P. punctata* and *P. mediterranea*. The spatial-temporal distribution of both species differered in Cananéia, pointing to a similar use of the environment's resources.

Keywords: *Ecological distribution, abiotic factors, Brachyura, habitat partitioning, bycatch.*

Distribuição espacial e temporal e abundância de duas espécies do gênero *Persephona* (Decapoda: Brachyura: Leucosiidae) no litoral sul do estado de São Paulo, Brasil

Resumo: Os caranguejos *Persephona* são intensamente capturados na pesca do camarão como fauna acompanhante, inclusive na região de Cananéia no litoral do estado de São Paulo, no Brasil. A avaliação da distribuição espaço-temporal pode, consequentemente, fornecer informações sobre variações na abundância de *Persephona punctata* e *Persephona mediterranea*, bem como as variáveis ambientais que afetam suas distribuições e uma possível partilha de habitat desses congêneres. Com um barco de pesca de camarão equipado com redes de arrasto duplo, os indivíduos foram coletados mensalmente de julho de 2012 a junho de 2014 em sete locais: quatro na área costeira adjacente à região de Cananéia e três na área estuarina Mar Pequeno. A abundância dessas espécies foi comparada de acordo com a distribuição espacial (entre sítios) bem como entre anos e estações. Um total de 396 indivíduos de *P. punctata* e de 64 de *P. mediterranea* foram capturados. A abundância de ambas as espécies foi maior no segundo ano de coleta (julho de 2013 a junho de 2014). A abundância de ambas as espécies foi maior na área costeira, e apenas um indivíduo de cada espécie foi capturado na área estuarina devido à baixa salinidade neste local (27.7‰ aproximadamente). A temperatura foi o fator ambiental que afetou significativamente a distribuição das espécies, com maiores abundâncias em períodos mais quentes. A variação temporal da abundância foi modulada pela temperatura, enquanto a salinidade modulou a distribuição espacial de *P. punctata* e *P. mediterranea*. Em Cananéia as espécies não apresentaram diferenças na distribuição espaço-temporal, o que nos permite assumir elas habitam a área de uma forma muito similar.

Palavras-chave: *Distribuição ecológica, fatores abióticos, braquiúros, partilha de habitat, fauna acompanhante.*

Introduction

The family Leucosiidae Samouelle, 1819 is commonly found in different abundances along the Brazilian coast. *Persephona* Leach, 1852 is the most representative genus of the family (Rieger 1999, Bertini et al. 2001), and *Persephona mediterranea* (Herbst, 1794) and *P. punctata* (Linnaeus, 1758) are the most common species found in coastal areas of the state of São Paulo (Mantelatto et al. 2016). These crabs are widely distributed in the Atlantic Ocean, from intertidal to subtidal zones at depths of 60 m and 50 m, respectively, in substrates composed of mud, sand, shells, calcareous algae, and/or corals (Melo 1996). They are frequently captured in shrimp trawling fisheries on the southeast coast of Brazil and, although they are not economically important, their populations are subjected to the same impacts as the commercially exploited crabs and shrimps (Pereira et al. 2014).

Fisheries using non-selective trawls as fishing gear accidentally extract a highly diverse bycatch fauna composed of small or non-commercial individuals that are usually returned to the sea dead or weakened. This activity may lead to a decrease in fishery stocks (Lewison et al. 2004). Considering this scenario, knowing the distribution patterns of organisms captured in trawling fisheries is fundamental to assess possible impacts and contribute to the development of mitigation activities (Carvalho et al. 2010).

There are few studies addressing the biology of the genus *Persephona*, most of them performed in the Ubatuba region, along the northern coast of the state of São Paulo. Negreiros-Fransozo et al. (1989), described the larval development of *P. mediterranea*; Bertini et al. (2001) and Pereira et al. (2014) reported the spatial-temporal distribution of three species of *Persephona*; Bertini et al. (2010) estimated the reproductive period and sexual maturity of *P. mediterranea* in Ubatuba; and Almeida et al. (2013) compared the relative growth, sexual maturity, and reproductive period of three species of the genus. A taxonomic review of the genus was published by Magalhães (2016), and comparative analysis of the distribution and sexual maturity of *P. lichteinsteiini* and *P. punctata* was performed by Carvalho et al. (2010) on the coast of Ilhéus, state of Bahia.

The two species studied herein have a wide distribution. *Persephona mediterranea* is found in the eastern Gulf of Mexico, the Caribbean Sea, the Antilles, Venezuela, Suriname, French Guiana, Brazil, and Uruguay (Magalhães et al. 2016). *Persephona punctata* is distributed in the Carribean Sea, the Antilles, Colombia, Venezuela, Guianas, Suriname, and Brazil (Magalhães et al. 2016). Despite their wide distribution, there are gaps in the knowledge about their ecology and studies in areas with different environmental features are extremely important to assess how environment modulates their distribution and abundance.

The well-conserved Cananéia-Iguape system is considered one of the most important humid areas in the Brazilian coast due to its biodiversity and primary productivity (Mendonça 2007, Leme et al. 2014, Pinheiro et al. 2018). The Federal Environmental Protection Area of Cananéia, Iguape, and Peruíbe – APA-CIP – was established in 1984 aiming at protecting the biological diversity and ensuring the sustainable use of natural resources (Mendonça 2007). The area is located beyond the tropic line and is subjected to a higher climatic variation than systems located within the tropics (Schaefer-Novelli et al. 1990).

An evaluation of the spatial and temporal distribution of *P. mediterranea* and *P. punctata* in the Cananéia-Iguape system could clarify the variation in the abundance of these congeners and the environmental variables affecting their distribution. This study investigated the habitat partitioning by *P. mediterranea* and *P. punctata* considering (a) the temporal variation and (b) the spatial variation in their abundances between sampling sites, and (c) the environmental variables affecting their distribution.

Materials and Methods

1. *Study area*

The Cananéia lagoon estuarine system is located on the southern coast of the state of São Paulo, Brazil (25ºS, 48ºW). The system is bordered by the municipality of Iguape to the north, by the Ilha Comprida island to the east, by the Serra do Mar mountains to the west, and by Cananéia and Cardoso Islands to the south. The lagoon has two main connections with the ocean, by a single channel to the north (Mar Pequeno – Barra de Icapara) and by two segments to the south (Mar de Cananéia and Mar de Cubatão – Baía Trapandé), which surround the Cananéia Island (Mendonça & Katsuragawa 2001). This region is mainly influenced by the tropical water mass brought by the Brazilian Current, Coastal Water, and South Atlantic Central Water (Matsuura 1986). Circulation within the system is primarily driven by the action of tidal waves and fresh water from several rivers (Miyao & Harari 1989).

Intense artisanal fishing occurs in the Cananéia system and in the adjacent coastal area involving more than 3,000 fishermen from the municipalities of Iguape, Cananéia, and Ilha Comprida (Mendonça & Katsuragawa 2001).

2. *Sampling*

Crabs were monthly captured from July 2012 to June 2014. Due to adverse environmental conditions in March 2013 and February 2014, no animals were captured in these months. Crabs were captured using a shrimp fishing boat equipped with 10 m long double-rig nets (4 m mouth opening, 20 mm mesh size, and 18 mm cod-end).

Seven sampling sites were established and marked using global positioning system coordinates. Four sites were placed in the coastal area adjacent to the Cananéia region: sites 1, 2, and 3 located in 10–15 m isobaths and site 4 located in 5–10 m isobaths. The other three sampling sites were located in the Mar Pequeno estuarine area (sites 5, 6, and 7) at 5–10 m isobaths and are influenced by fresh water from the Cananéia-Iguape estuarine complex (Besnard 1950) (Figure 1).

Nets were dragged parallel to the shore for 30 min in each site, totalling an area of approximately 16,000 m^2. After each trawling, crabs were stored in labeled plastic bags and kept frozen until analysis. In the laboratory, individuals were identified to the species level according to Melo (1996).

Water temperature (measured with a mercury thermometer, ºC) and salinity (measured with a specific optical refractometer, ‰) were recorded at all sampling sites. Bottom water samples were obtained with a 5-L Van Dorn bottle. Pluviosity data were taken from the Portal Agrometeorológico e Hidrológico do Estado de São Paulo website (www.ciiagro.org.br).

Sediment samples were collected at each site using a Van Veen grab (0.06 m^2 area) to determine the mean sediment grain size (ϕ) and organic matter content (OM). In the laboratory, samples were dried in an oven at 70ºC for 72 h. Grain composition was estimated based on three

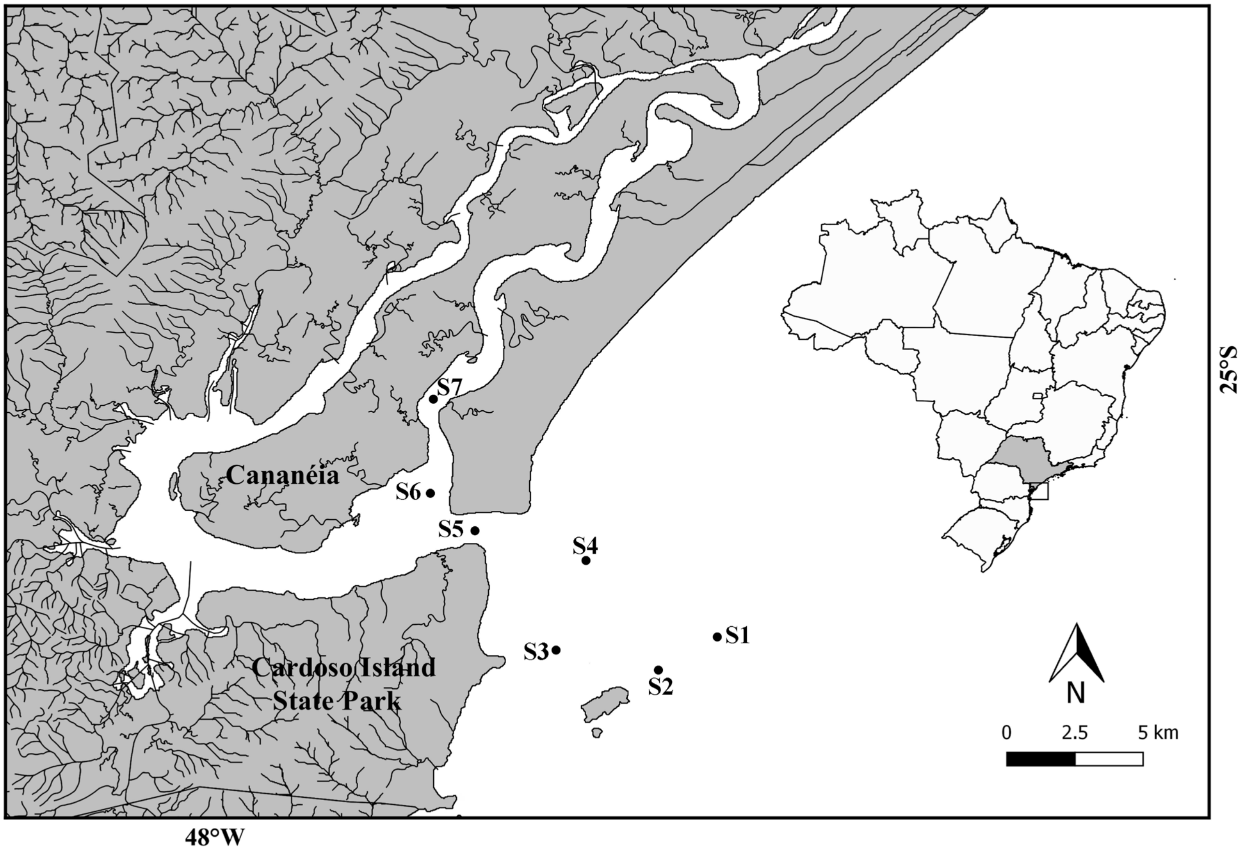

Figure 1. Map showing sampling sites in marine area (S1, S2, S3, S4) and in mar pequeno (S5, S6, S7) in Cananéia.

100-g subsamples treated with 0.2 N NaOH (250 mL), stirred for 5 min to separate silt and clay particles, and rinsed using a 0.063 mm mesh sieve. Sediments were sieved through different mesh sizes to determine the grain size: 2 mm sieve (gravel), 2.0–1.01 mm sieve (very coarse sand), 1.0–0.51 mm sieve (coarse sand), 0.50–0.26 mm sieve (medium sand), 0.25–0.126 sieve (fine sand), and 0.125–0.063 mm sieve (very fine sand). Smaller particles were classified as silt-clay (Tucker 1988).

Grain size categories followed the American standard (Wentworth 1922) and fractions were expressed on the phi (ϕ) scale to estimate the central tendency of sediment samples. For instance: $-1 = \phi < 0$ (gravel), $0 = \phi < 1$ (coarse sand), $1 = \phi < 2$ (intermediate sand), $2 = \phi < 3$ (fine sand), $3 = \phi < 4$ (very fine sand), and $\phi \geq 4$ (silt + clay) (Hakanson & Jansson 1983, Tucker 1988). Cumulative particle size curves were plotted using the ϕ scale with values corresponding to the 16th, 50th, and 84th percentiles used to determine the mean diameter of sediment via the formula:

$$Md = (\phi_{16} + \phi_{50} + \phi_{84}) / 3$$

Finally, ϕ was calculated using the formula $\phi = \log_2 d$, where d is the grain diameter (mm) (Tucker 1988).

To estimate the OM content, oven dried sediment samples (10 g) from each site were placed in porcelain crucibles, and heated in an oven at 500ºC for 3 h. The percentage of organic matter was estimated as the difference between the initial and final crucible weight (Mantelatto & Fransozo 1999).

3. *Statistical analysis*

Data were tested for homoscedasticity (Levene test) and normality (Shapiro-Wilk test) as a pre-requisite for the statistical analysis (Zar 1999). The abundance of *Persephona mediterranea* and *Persephona punctata* were analysed separately and compared considering their spatial and temporal distribution. The spatial distribution (between sites) was evaluated using an Analysis of Variance (one-way ANOVA) and a post-hoc Tukey test ($\alpha = 5\%$) and the temporal distribution (years and seasons) was evaluated using a nested ANOVA and a post-hoc Tukey test ($\alpha = 5\%$). For statistical purposes, seasons were grouped as follows: summer = January–March, autumn = April–June, winter = July–September, and spring = October–December. When necessary, the data were logarithmized to satisfy the analyses' premises (Zar 1999). Catch-per-unit-of-effort (CPUE) was calculated for each species and expressed on numbers of individuals per hour of trawling (ind/h).

A canonical redundancy analysis (RDA) was performed to assess the influence of variation in environmental variables (bottom water temperature and salinity, phi and organic matter content) on the monthly data of the species abundance using R (R Development Core Team 2013), considering $\alpha = 5\%$ (Zar 1999). The statistical package "vegan" was used (RDA, "envifit" Oksanen et al. 2013).

A time series analysis was performed (Cross Correlation Statistica 7.0, Statsoft, Inc) ($\alpha = 5\%$) (Statsoft 2004) to investigate the relationships between environmental variables (temperature, salinity, sediment texture, and percentage of organic matter in the sediment) and species abundance. In a cross-correlation analysis, two data series were compared as a function of a time lag (n), using the Pearson correlation coefficient to measure the relationship between values of the first and second data series n months earlier (in negative lags) or later (in positive lags), and at lag 0. The correlation coefficient values are equivalent to the standard Pearson correlation (Legentil et al. 2013).

An exploratory data analysis was also performed to establish classes of environmental variables and calculate the frequency of crabs in these classes.

Results

1. *Environmental variables*

The bottom water temperature (BT) ranged from 17.2–29.8ºC (mean: 23.1ºC ± 3.1). The highest mean BT was recorded in January 2014 (28.9ºC ± 0.86) and the lowest mean BT occurred in July 2013 (17.5ºC ± 0.31) (Figure 2A). The highest spatial mean BT variation was recorded in sites 5, 6, and 7 (Figure 2B).

Bottom water salinity (BS) ranged from 16.9–38‰ (mean: 31.4‰ ± 5). The highest mean BS was recorded in October 2012 (36.1‰ ± 2.38) and the lowest in November 2013 (25.7‰ ± 4.07) (Figure 2C). Spatially, the lowest and the highest mean salinity were recorded in sites 7 and 1, respectively (Figure 2D).

Sediment samples were mainly composed of very fine sand ($3\ \phi < 4$). Exceptions were recorded in site 4 (silt and clay; $\phi \geq 4$) and site 6 (fine sand; $2\ \phi < 3$) (Figure 3). The highest percentage of organic matter was found in site 4 (6.56%) and the lowest in site 6 (2.07%) (Figure 3).

The highest mean pluviosity in the study area was recorded in March 2013 (48.9 mm ± 30.8) and the lowest in August 2012 (2.2 mm ± 4) (Figure 4).

2. *Temporal and spatial distribution*

A total of 460 crabs were captured (64 *P. mediterranea* individuals and 396 *P. punctata* individuals). During the first year of sampling, 2.29 ind/h of *P. mediterranea* and 15.43 ind/h of *P. punctata* were captured (Figure 5). In the second year, these numbers increased to 16 ind/h of *P. mediterranea* and 97.71 ind/h of *P. punctata* (Figure 5). The highest abundances of *P. mediterranea* (3.57 ind/h; 39%) and *P. punctata* (63.14 ind/h; 56%) occurred in January 2014 (Figure 5). In both years, the abundance of *P. punctata* was not significantly different considering the temporal scales ($p > 0.05$). As for *P. mediterranea*, the abundance was different when the year was considered (1 df, F = 5.17, p = 0.03).

Spatially, more crabs were sampled in site 1, 3.18 ind/h of *P. mediterranea* and 18.55 ind/h of *P. punctata* (Figure 6). There was a significant difference in the abundance of both species depending on the site. *Persephona punctata* was more abundant in sites 1 and 2 than in sites 5, 6, and 7 (one-way ANOVA, 6 df, F= 6.02, p = 0.000013; Tukey Test $p < 0.05$). *Persephona mediterranea* was more abundant in site 1 than in sites 2, 5, 6 and 7 (one-way ANOVA, 6 df, F= 4.38, p = 0.000435; Tukey Test $p < 0.05$). No crabs were captured in sites 6 and 7 (Figure 6).

3. *Abundance vs. environmental variables*

According to the RDA analysis, only bottom temperature was significantly correlated with the abundance of both species (Table 1); the first axis of the biplot chart explained 99% of the total variance (RDA, r^2 0.0393, p = 0.044).

Persephona punctata and *P. mediterranea* were more abundant at higher temperatures (27–29°C), higher salinities (>34‰), in sites with sediment composed of very fine sand rich in silt and clay fractions ($\phi > 4$), and with organic matter concentrations lower than 9% (Figure 7, Figure 8). The highest abundance of both species was found in site 1, which is predominantly composed of very fine sand; silt and clay were also present in this site and varied according to the sampling month. As a result, the cross-correlation showed no significant correlation between environmental variables and abundance (Time series, $p > 0.05$).

Discussion

The water temperature along the coast of Cananéia varied as expected for subtropical regions, with higher temperatures in summer and lower temperatures in winter. This variation determined the fluctuations in *P. punctata* and *P. mediterranea* abundance throughout the year; e.g. in January 2014, BT and abundance were the highest for both species.

Bertini et al. (2001) and Pereira et al. (2014), also found a higher abundance of these species in periods of high temperatures in surveys along the northern coast of the Ubatuba region (São Paulo). Both studies indicated the highest crab abundances in autumn and winter when temperatures were higher than in spring and summer. This temperature difference is linked to the influence of the South Atlantic Central Water (SACW) in the Ubatuba region during spring and summer, which decreases the water temperature. However, in Cananéia, differences in abundance were seen mainly between summer and autumn. The lowest temperatures were recorded during winter and autumn, limiting the capture of both species.

Higher temperatures act on energy demanding events, such as moulting and spawning, play an important role in triggering reproductive events, and favour high abundances of individuals (Stelle & Bert 1994). The seasonal nature of moulting can be influenced by temporal variation in temperature and adequate food availability (Aiken 1969). This relationship was confirmed in the Cananéia region by Garcia et al. (2018) that found a positive correlation between the monthly temperature variation and chlorophyll-*a* concentration.

Coastal waters are subjected to a higher variation in salinity due to freshwater inflow and this may influence the species distribution (Carvalho et al. 2010). Miyao et al. (1986), classified the salinity in the Cananéia estuary as partially mixed, increasing according to depth. This explains the higher abundance of crabs recorded in site 1 (10–15 m) in comparison with site 5 (5–10 m), which is located in the estuarine region. Sites 6 and 7 are located in a region called Mar Pequeno, which is shallower and influenced by continental waters, resulting in a lower salinity when compared with marine coastal areas. Garcia et al. (2018), characterized this area as a mesohaline estuary, with salinity near to 15‰. No crabs were captured in sites 6 and 7, demonstrating that *P. punctata* and *P. mediterranea* do not inhabit sites with low salinity, corroborating features of their larval development which

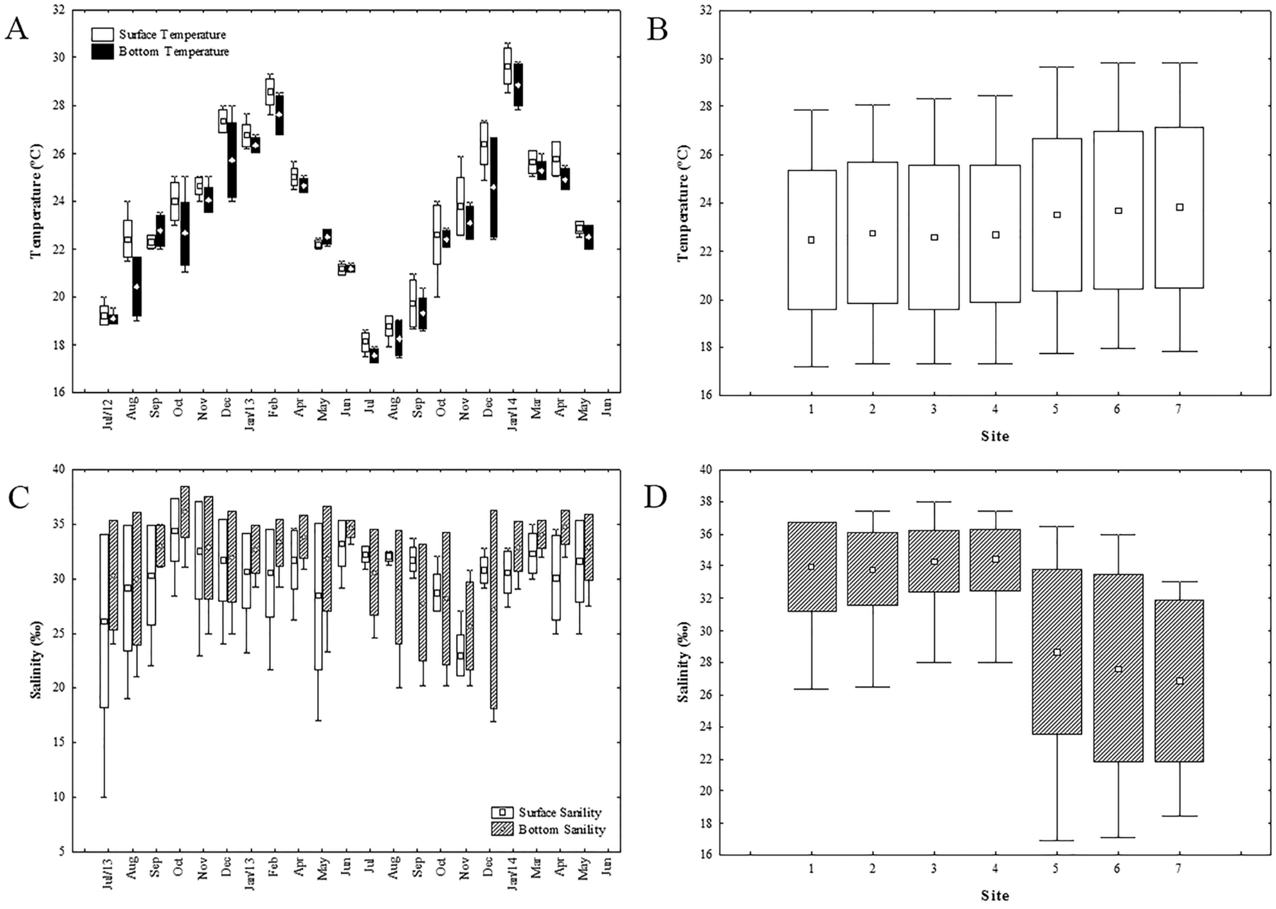

Figure 2. [A] monthly variation of mean values of surface (S) and bottom (F) water temperature; [B] Mean values and minimum and maximum amplitudes of bottom water temperature; [C] Monthly variation of mean values of surface (S) and bottom (F) water salinity; [D] Mean values and minimum and maximum amplitudes of bottom water salinity. Abiotic factors were sampled from July/12 to May/14 in Cananéia region. (Mean; SD = standard deviation; Min = minimum; Max = maximum).

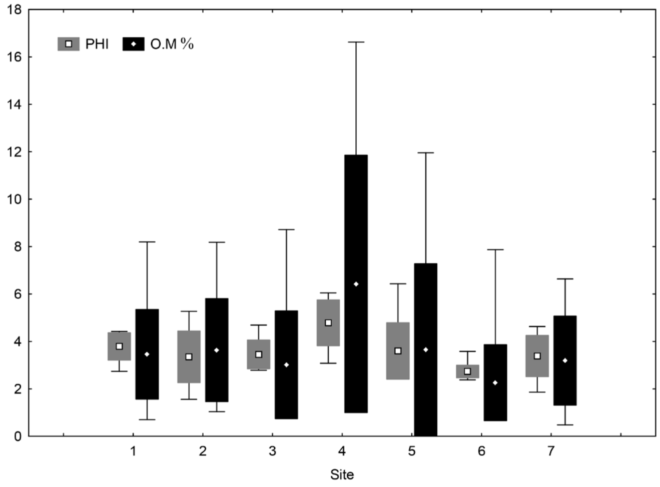

Figure 3. Mean values and minimum and maximum amplitudes of grain size (PHI) and organic matter content (O.M %), sampled from July/12 to May/14 in Cananéia region. (Mean; SD = standard deviation; Min = minimum; Max = maximum).

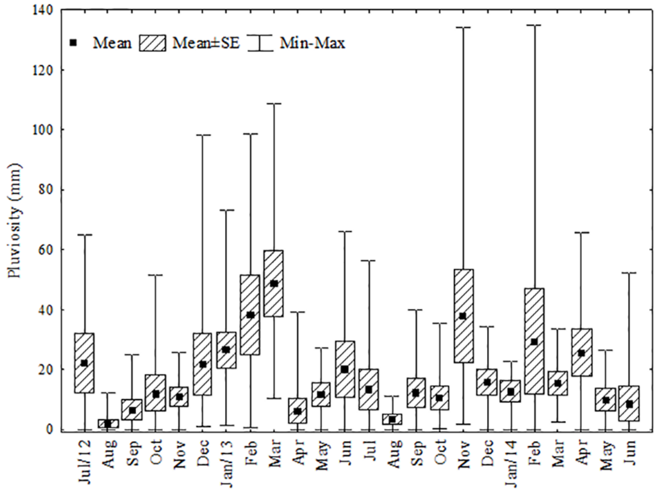

Figure 4. Mean values and minimum and maximum amplitudes of pluviosity (mm) sampled from July/12 to May/14 in Cananéia region. (Mean; SD = standard deviation; Min = minimum; Max = maximum).

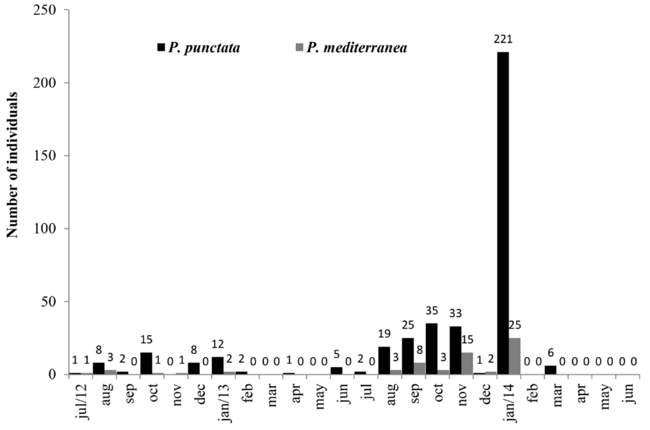

Figure 5. Number of individuals of *Persephona punctata* (Linnaeus, 1758) and *Persephona mediterranea* (Herbst, 1794) per month in Cananéia/SP, from July/12 to June/14.

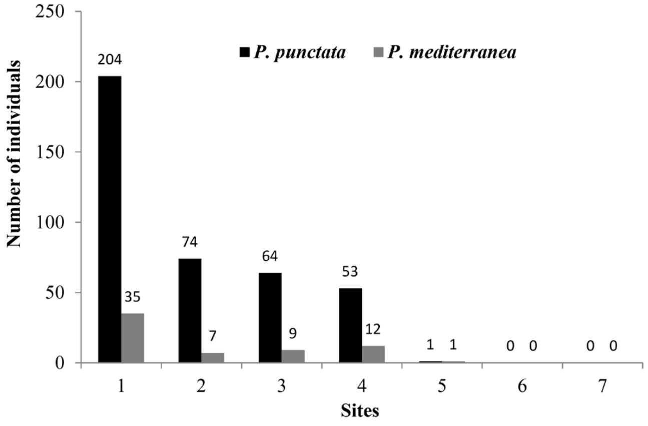

Figure 6. Number of individuals of *Persephona punctata* (Linnaeus, 1758) and *Persephona mediterranea* (Herbst, 1794) per site in Cananéia/SP, from July/12 to June/14.

Table 1. Results from Redundancy Analysis (RDA) among abundances of *Persephona mediterranea* (Herbst, 1794) and *Persephona punctata* (Linnaeus, 1758) and environmental factors, sampled in Cananéia region, São Paulo State, from July 2012 to June 2014. Species Score (RDA1) *P. mediterranea* = 0.1958 and *P. punctata* = 2.2951.

Explained proportion		Environmental factors	RDA1	RDA2	r^2	P
RDA1	0.9985	BT	0.95987	0.28043	0.0393	0.044*
RDA2	0.00152	BS	0.98540	0.17024	0.0078	0.568
		Phi	-0.04175	-0.99913	0.0089	0.126
		OM	0.99959	0.02850	0.0100	0.414

* = P<0,05. BT – bottom temperature, BS – bottom salinity, Phi – granulometric fraction, OM – organic matter content.

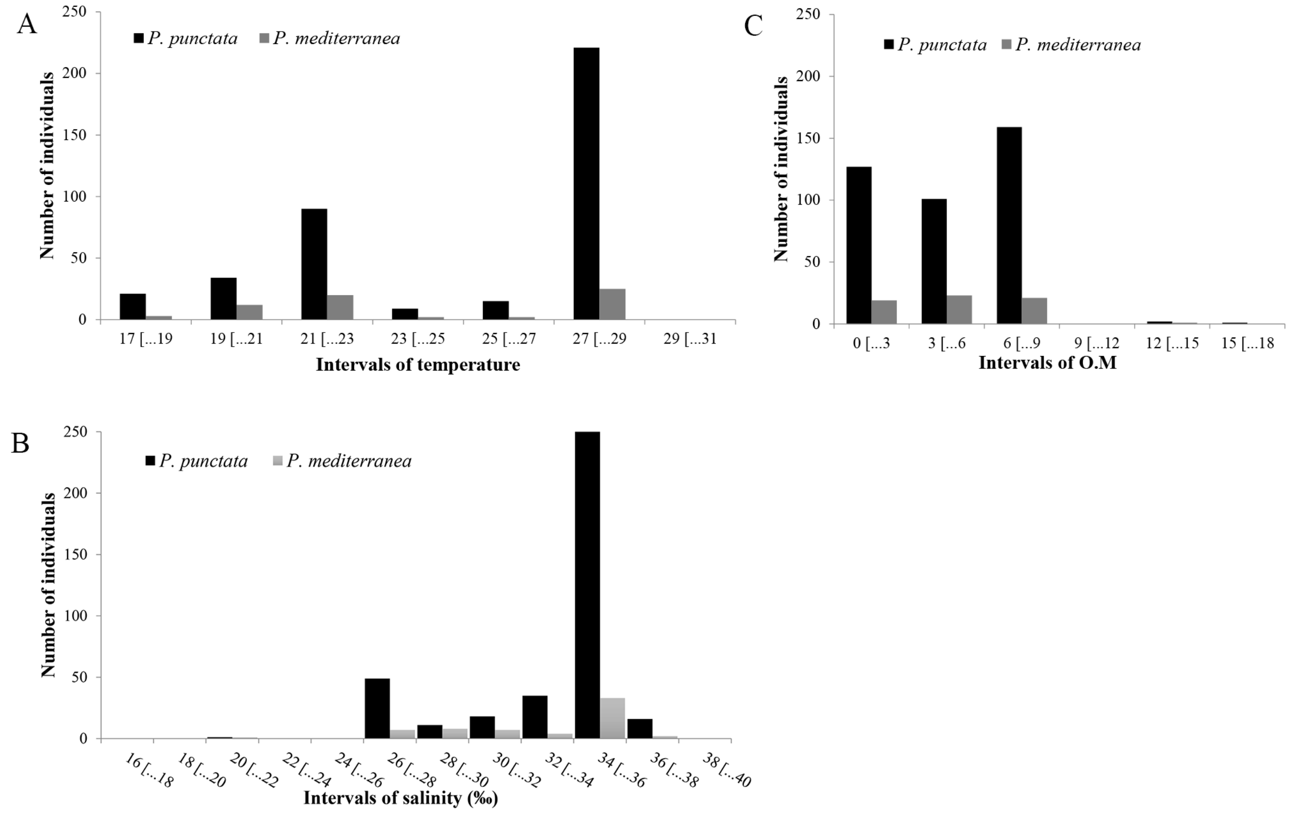

Figure 7. Number of individuals of *Persephona punctata* (Linnaeus, 1758) and *Persephona mediterranea* (Herbst, 1794) by intervals of abiotic factors: [A] temperature; [B] salinity; [C] organic matter content (O.M). All data was sampled from July/12 to June/14 in Cananéia region.

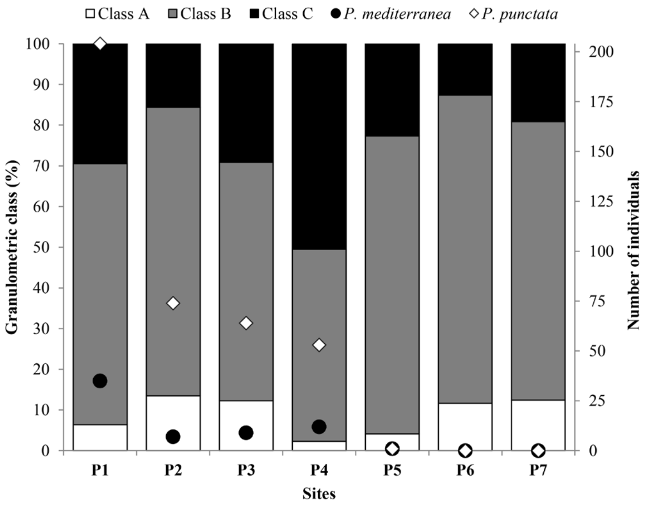

Figure 8. Grain size categories and number of individuals of *Persephona punctata* (Linnaeus, 1758) and *Persephona mediterranea* (Herbst, 1794) by site. [Class A] gravel, coarse sand and intermediate sand; [Class B] fine sand and very fine sand; [Class C] silt and clay. All data was sampled from July/12 to June/14 in Cananéia region.

occurs in inshore and offshore areas with increased salinity (34/35 ‰) (Negreiros-Fransozo et al. 1989, Hirose 2009).

Carvalho et al. (2010), evaluating populations of *P. punctata* and *P. lichteinsteinii* in Ilhéus, Bahia, found that individuals were more abundant in autumn when salinity was reduced to an average of 35‰. In our survey, we found that both species were more abundant in areas with a salinity range of 32–34‰, which is considered high for the region since the study area is strongly influenced by fresh waters. During the first year, higher crab abundances were recorded in spring; abundance might have decreased in summer due to the higher pluviosity during this period, which consequently lowered the salinity. In the second year, pluviosity was higher in spring and salinity was correspondingly lower during this season. In the following summer, pluviosity decreased and salinity increased, and the abundance of both species increased. Nevertheless, Garcia et al (2018), did not found a correlation between salinity and pluviosity in Cananéia, probably because after the construction of the Valo Grande channel in 1852 (GEOBRÁS 1966) salinity is controlled by the water input from the Ribeira Iguape River. Tidal influx might also have influenced these results since the Cananéia strait is located in the southern portion of the Cananéia system and do not have significant barriers between the region and the ocean.

Sediment granulometry did not have a significant influence on the species distribution, both species often inhabit sites composed mainly of very fine sand, as seen in site 1. However, the seasonal variation in abundance demonstrated that when more silt and clay were present, species abundance also increased. Bertini et al. (2001) and Pereira et al. (2014), reported that both *P. punctata* and *P. mediterranea* inhabit sites with sediment composed of silt and clay in the Ubatuba region; these substrate types allow crabs to bury themselves for protection and food foraging, results that were corroborated by our observations.

Considering that the substrate in site 4 is mainly composed of silt and clay, which retain more organic particles, it is clear why the percentage of organic matter content in site was the highest (Castilho et al. 2008). The organic matter was not directly related to the abundance of *P. punctata* and *P. mediterranea*, but it influences the abundance of Polychaeta which is used as a food resource by both species (Petti et al. 1996).

Marine crustaceans often vigorously defend shelter and food resources, and similar-sized crabs fight more intensely for these resources (Dingle 1983, Huntinford et al. 1995). Historically, biologists have postulated that competition should be more intense between species more closely related phylogenetically (Elton & Miller 1954). Other authors, however, have reported that the competition pressure may be reduced when organisms use strategies that allow them to exploit the same niche in different ways (Peterson & Andre 1980, Evans 1983, Abrams 1986, 1990). This is common in coastal lagoons, estuaries, and shallow marine environments due to the abundance of resources and the high diversity of species colonising these habitats (González-Gurriaran et al. 1989).

In Cananéia, *P. punctata* and *P. mediterranea* inhabit the same area and lack differences in spatial and temporal distribution, suggesting that they have the same requirements regarding environmental variables. This probably occurs because shallow areas (sites 6 and 7) have very low salinity, which hinder the occurrence of *P. punctata* and *P. mediterranea*. Furthermore, habitat partitioning does not necessarily mean a diet overlap. Rosas et al. (1994), found that the feeding habits of three coexisting species of *Callinectes* varied seasonally, suggesting that the variation is more related to the differences in the energy requirements of each species than to the abundance of prey. This is probably the reason why, although *P. punctata* is more abundant than *P. mediterranea*, both species are coexisting in the same habitat. Dominance among crabs could have a proximal cause based on behavioural interactions, and behavioural hierarchies should be considered when considering explanations for co-occurrence and distribution of crabs (Brown et al. 2005).

Overall, the present study showed that increased temperatures determined the temporal variation in abundance, and salinity modulated the spatial distribution of *P. punctata* and *P. mediterranea*, limiting these crabs to the marine area. The Cananéia-Iguape System has very distinctive oceanographic features and our study contributed with information about ecological aspects of both species, which have been rarely studied despite their wide distribution.

Future studies about population structure, reproductive period, and sexual maturity are essential to better understand the factors driving the biology of these species. Studies considering the species distribution in different latitudes would be crucial to understand the life history patterns of *P. punctata* and *P. mediterranea*.

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Author Contributions

Júlia Fernandes Perroca: The manuscript was an undergraduate research project of the author.

Daphine Ramiro Herrera: contributed to the definition of statistical analyzes and the interpretation of results and discussion.

Rogerio Caetano da Costa: contributed to the methodological design and assisted in the interpretation of the results and the discussion.

Conflicts of interest

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

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Article

Leaf Morphoanatomy of *Diploon* Cronquist (Sapotaceae Juss.)

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Abstract: *Diploon* is a monospecific genus represented by *Diploon cuspidatum*, an arboreal species that has morphological characteristics distinct from those of other Sapotaceae species. In this study, *Diploon cuspidatum* leaves were characterized morphoanatomically in order to reveal additional diagnostic characters of their external morphology of the genus. The *Diploon* petiole presents shape and arrangement of the vascular system flat-convex, occasionally with one or two accessory bundles, many laticifers, and many prismatic crystals. The midrib is biconvex with a U-shaped cuticle on the abaxial side, and laticifers are associated with the vascular tissues. Mesophyll is dorsiventral, palisade parenchyma has two cell layers, T- and Y-shaped malpighiaceous trichomes are on the abaxial epidermis with a small stalk cell and long arm. The venation pattern is brochidodromous. Intersecondary veins run parallel to the secondary veins, and quaternary veins branch freely. Higher order veins are not present. Morphoanatomical analysis revealed important characteristics that reveal a set of structures common to Sapotaceae, in addition to characters that are important for the recognition and identification of *D. cuspidatum*.

Keywords: *Leaf anatomy, leaf architecture, micromorphology, taxonomy, Chrysophylloideae.*

Morfoanatomia Foliar de *Diploon* Cronquist (Sapotaceae Juss.)

Resumo: *Diploon* é um gênero monoespecífico representado por *Diploon cuspidatum*, espécie arbórea com características morfológicas peculiares em relação a outros gêneros de Sapotaceae. A espécie teve suas folhas caracterizadas morfoanatomicamente, a fim de fornecer caracteres diagnósticos adicionais à morfologia externa, subsidiar pesquisas no âmbito da anatomia vegetal, dendrologia e filogenia. *D. cuspidatum* evidenciou pecíolo plano-convexo, com feixe vascular plano-convexo, presença ocasional de até dois feixes acessórios, presença de muitos laticíferos e cristais prismáticos. A nervura central é biconvexa, com cutícula em forma de U no lado abaxial, laticíferos associados aos elementos vasculares. Mesofilo dorsiventral, parênquima paliçádico com duas camadas descontínuas, tricomas malpighiáceos do tipo T e Y na epiderme abaxial com pedúnculo pequeno e braço longo. O padrão de venação é do tipo broquidódroma. Veias intersecundárias paralelas as veias secundárias, veias quaternárias em ramos livres. Ausência de veias de ordem superior. A análise morfoanatômica realizada evidenciou caracteres importantes que retratam um conjunto de estruturas comuns a Sapotaceae e também importantes para reconhecimento e identificação *D. cuspidatum*.

Palavras-chave: *Anatomia foliar, Arquitetura foliar, micromorfologia, taxonomia, Chrysophylloideae.*

Introduction

Sapotaceae is a family with a pantropical distribution that includes about 60 genera and 1300 species (Pennington 1991; Govaerts *et al*. 2001, The PlantList 2013). It is one of the largest families of eudicots. It is known for having species of great morphological diversity that are especially important in lowland wet tropical forests because they provide essential resources to native fauna and humans (Lawrence 1951, Barroso 1978, Pennington 1991, Felippi *et al.* 2008, Gomes *et al.* 2008, Reis *et al.* 2013). In Brazil, there are approximately 234 species distributed in 12 genera (Carneiro *et al.* 2015). Among these is *Diploon* Cronquist, which is of particular importance because of its peculiar reproductive morphology.

Diploon is a monospecific genus represented by *Diploon cuspidatum* (Hoehne) Cronquist, which has as its basionym *Chrysophyllum cuspidatum* Hoehne. This arboreal species has alternating leaves, axillary flowers, basally connate petals, and it lacks staminodes. Its ovary is glabrous and unilocular with two ovules, and it has basal placentation, in addition to succulent fruits containing a single seed that has a small, basilateral scar and no endosperm (Pennington 1990, 1991). Together, these characters contribute to a good generic delimitation because some features of the genus *Diploon* are not common in other Sapotaceae, which facilitates its identification. Despite having a distinct reproductive morphology (unilocular ovary and biovular with basal placentation), *Diploon* shares morphological, anatomical, chemical, and phylogenetic characteristics with other Sapotaceae genera (Cronquist 1946, Kukachka 1979, Pennington 1990, Swenson & Andeberg 2005).

Diploon cuspidatum, popularly known as "bapeba-preta" or "bapeba-roxa," is native to Brazil, but it can also be found in Bolivia, Guyana, and Peru (Pennington 1990). In Brazil, it is distributed throughout two phytogeographic domains: the Amazon and the Atlantic Forest, occurring in the North, Northeast, Southeast, and South regions of the country (Carneiro *et al.* 2015). Although it is a relatively well-distributed species, *D. cuspidatum* is present in biomes regarded as biodiversity hotspots, which face serious environmental threats caused by the most diverse anthropic disturbances. Because of the richness in flora and fauna, these areas require more studies to expand our knowledge of the biology of their species. Concerning *D. cuspidatum*, the literature has been largely restricted to references to local flora and floristic surveys (Kurtz & Araújo 2000, Alves-Araújo & Alves 2010, Palazzo *et al*. 2010, Barreto & Catharino 2015) that gathered data on geographic distributions, identification keys, or morphological descriptions. Anatomical studies are even scarcer, even though they are relevant to our understanding of a species' biology and they provide data to support taxonomic, dendrological, micromorphological, and phylogenetic studies.

In the last proposals regarding the taxonomic classification of Sapotaceae (Pennington 1991, Swenson & Andeberg 2005), the positioning of the genus *Diploon* in the family was mainly based on its floral morphology and the presence of chemical components (flavonoids). Kukachka (1979) showed common anatomical characters between *Diploon* and the other genera of the family after analyzing woods of neotropical Sapotaceae. Regarding leaf architecture, Pennington (1990) used different terminology to describe the foliar venation pattern in *Diploon* and other genera, revealing useful features to identify *D. cuspidatum*, which, in the vegetative state, may be similar to some *Micropholis* (Griseb.) Pierre species. These data, along with current research on other Sapotaceae genera (Monteiro *et al*. 2007a, Almeida-Jr *et al*. 2012), show that morphoanatomical studies can contribute significantly to plant systematics at different hierarchical levels. Therefore, the present study aimed to characterize and discuss the leaf morphoanatomy of *D. cuspidatum* from a taxonomic perspective in order to identify diagnostic characters that contribute to our knowledge of this genus.

Material and Methods

This study was based on the collection of *Diploon* specimen branches found in the Atlantic and Amazon Forest (in Brazil's North and Northeast areas), in addition to the analysis of material deposited in the national herbarium (BHCB, ESA, HEPH, HRBC, IAC, IAN, INPA, MBML, PEUF, SPSF, UB, and VIC).

The material used to perform the anatomical analysis was obtained during field expeditions and from herbarium samples (voucher: IAC43362, IAN123562, PEUFR53995, PEUFR53996 and SPSF3507). Samples were selected according to the following criteria: 1) herbal material in good preservation state, 2) reliable taxonomic identification, and 3) presence of fully expanded leaves.

The botanical material was submitted to a process of reverse herborization according to Smith & Smith (1942). The process consisted of treating the samples with 2% sodium hydroxide for 2 h, washing them in distilled water, and dehydrating them in graded ethanol solutions. Subsequently, samples were stored in 70% ethanol until the anatomical slides were prepared.

Free-hand transverse sections were cut from the petiole (apex, middle, and base regions) and the middle region of the leaf blade. Sections were clarified in 50% sodium hypochlorite and stained with Astra blue and basic fuchsin. Samples were mounted on slides using glycerinated gelatin and sealed with colorless nail polish (Kraus & Arduin 1997).

For the analysis of leaf architecture, diaphanization was carried out according to the protocol developed by Shobe & Lersten (1967 *apud* Kraus & Arduin 1997). The clarified samples were then washed in distilled water, dehydrated in an ethanol series, and stained with basic fuchsin in 50% ethanol. Samples were mounted with glycerinated gelatin (Kaiser 1880), and slides were sealed with colorless nail polish (Kraus & Arduin 1997).

Morphoanatomical analysis was performed using an optical microscope (L2000i, Bioval), and images were obtained using a digital camera (Digital Microscope Imager, 44421, Celestron) attached to the microscope.

Leaf blades, petioles, and trichome characters were described in accordance with the terminology of Metcalfe & Chalk (1983), Howard (1979), and Theobald *et al.* (1979), respectively. The description of leaf venation patterns was based on the terminology of Ellis *et al.* (2009).

Results

D. cuspidatum (Figure 1a) leaves are simple, alternate distichous or weakly spiralled. The leaf blade varies from elliptic to oblanceolate, the apex is acuminate or rarely attenuate and the base is attenuate-cuneate, chartaceous. The adaxial side is glabrous, while the abaxial side is glabrous to glabrescent (Figure 1b, 1c). The leaf blade has a striated appearance created by the course of secondary and intersecondary veins (Figure 1c). The petiole is canaliculate and subglabrous.

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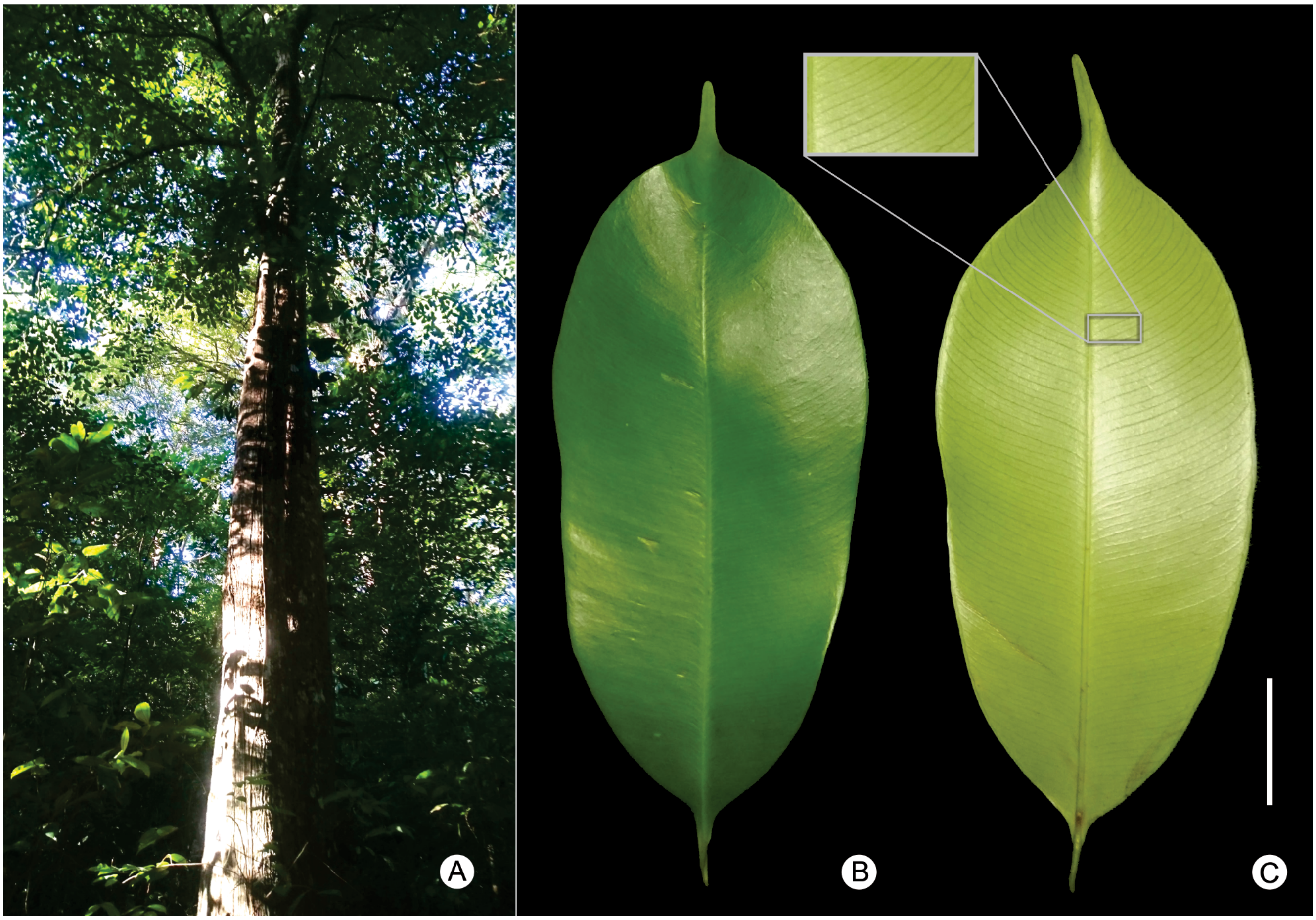

Figure 1. *Diploon cuspidatum* (Hoehne) Cronquist. **A.** Individual located at the Parque Estadual Dois Irmãos, PE, Brazil; **B.** Leaf blade showing the adaxial side; **C.** Leaf blade showing the abaxial side and detailing the venation pattern. * Scale bar: 2.00 cm.

The analysis of leaf architecture allowed the identification of a brochidodromous venation pattern with a pinnate midrib (Figure 2a). Secondary veins are excurrent from the primary vein, and they branch out without reaching the margin, which is formed by submarginal veins (Figure 2a). The spaces between adjacent secondary veins are regular, and the angle between the primary vein and secondary veins increases smoothly as these approach the leaf base. Intersecondary veins are 50% greater in length than lower secondary veins, and they run parallel to secondary veins. It is possible to observe one or more per intercostal area (Figure 2a). Intercostal tertiary veins display an alternate percurrent course, crossing the midrib in a reticulate pattern (Figure 2b). External tertiary veins are also observed, ending at the leaf margin parallel to the secondary veins. Quaternary veins end freely in a single branch (Figure 2b). Higher order veins were not observed.

In cross-section, the petiole has a flat-convex shape (Figure 2c), and when it is close to the leaf base, it presents two lateral projections in the adaxial portion, resulting in the canaliculate morphology of the petiole observed as part of the external morphology. The epidermis is unstratified with cells that are usually quadrangular and covered by a thin layer of cuticle. It is possible to observe scars left by trichomes. Below the epidermis, there is a continuous layer of collenchyma tissue followed by parenchyma. This region has a higher concentration of solitary prismatic crystals and laticifers, but few druses. Prismatic crystals and laticifers, however, can be found over the entire petiole at a lower frequency. The vascular bundle, which is present in the middle and the petiole, is flat-convex (Figure 2c). One or two accessory bundles surrounded by a sclerenchymatous sheath are observed. Laticifers and prismatic crystals are present in the medullar region.

The leaf blade has a thicker cuticle on the adaxial side compared to the abaxial side. On both sides, the epidermis is unstratified and formed mainly by quadrangular cells. The abaxial side, however, displays simple, unicellular, T- or Y-shaped malpighiaceous trichomes with a short peduncle and long arms, which are concentrated close to the midrib (Figure 2g). Leaves are hypostomatic and have anisocytic stomata. In cross-section, guard cells are on the same level as other epidermal cells. The mesophyll is dorsiventral and consists of two discontinuous layers of palisade parenchyma followed by 10 layers of spongy parenchyma (Figure 2d). The palisade parenchyma of the mesophyll sometimes extends into the region of the midrib that is interrupted by annular collenchyma. Laticifers and collateral bundles occur throughout the mesophyll, and sclereids are present in the abaxial side of the leaf blade (Figure 2d).

In cross-section, the midrib is biconvex (Figure 2e). The epidermis displays quadrangular cells, and is covered by a U-shaped cuticle that is thicker on the abaxial side. There are four to five layers of annular collenchyma just below the epidermis. It should be noted that laticifers and prismatic crystals are more frequent in the midrib than in the mesophyll (Figure 2f, 2h). The vascular system is collateral, and it has

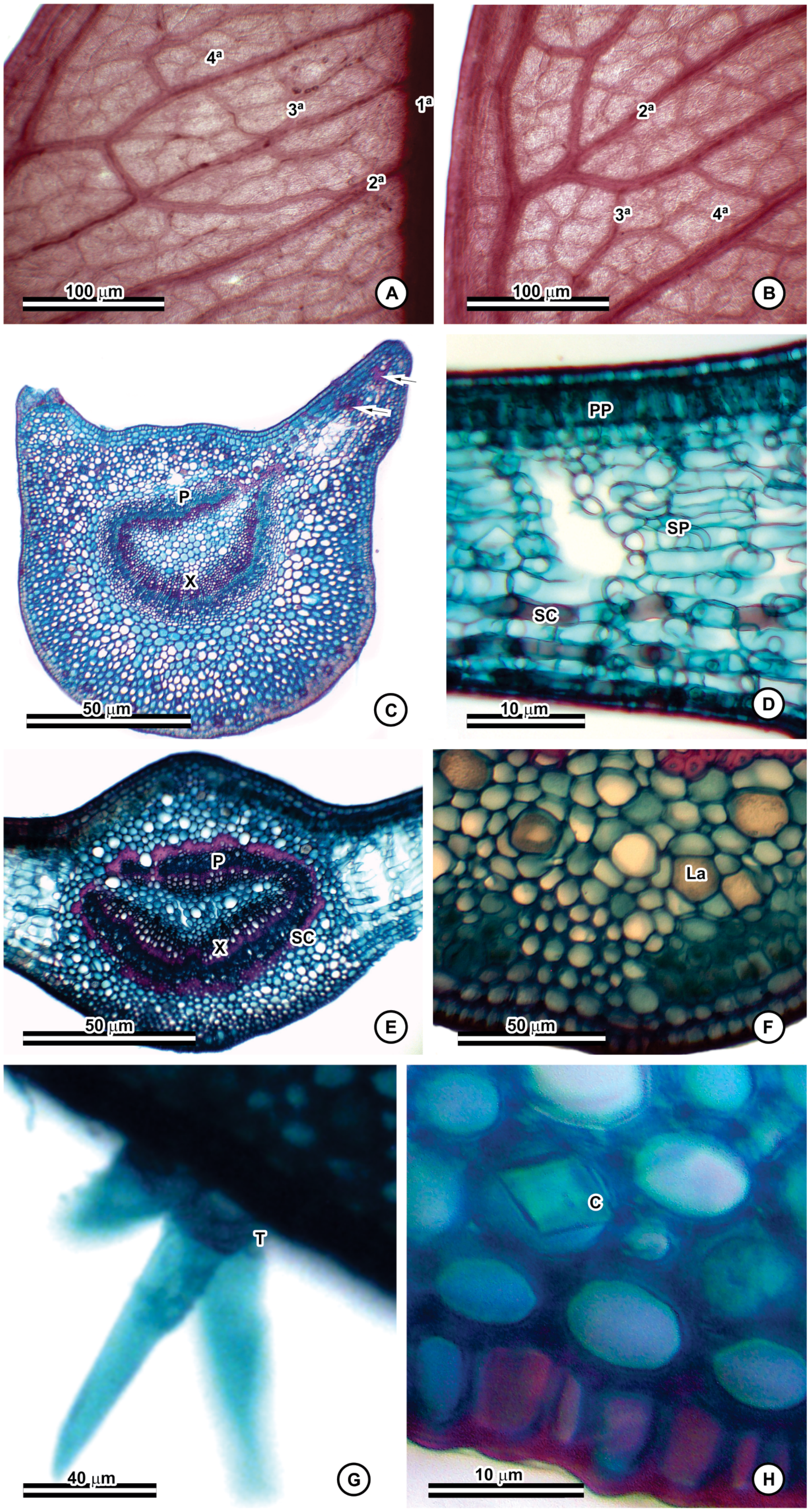

Figure 2. Morphoanatomic characters of *D. cuspidatum*. **A–B.** Venation pattern showing primary, secondary, intersecondary, tertiary, and quaternary veins; **C.** Cross-section of the petiole with accessory bundles (arrows); **D.** Cross-section of the leaf blade; **E.** Cross-section of the mesophyll; **F.** Laticifers in the midrib; **G.** Malpighiaceous trichomes on the abaxial face; **H.** Detail of prismatic crystals in the midrib. **Abbreviations.** C: prismatic crystals; La: laticifers; P: phloem; Pp: palisade parenchyma; Sc: sclereids; Sp: spongy parenchyma; T: malpighiaceous trichomes; X: xylem. * Scale bar: 650 µm.

a plano-convex shape. It is also surrounded by a sclerenchymatous sheath. Laticifers are present around every vascular bundle. The rest of the structure is filled with parenchyma.

Discussion

Some morphoanatomical characters described by previous authors (Solereder 1908, Metcalfe & Chalk 1950, Pennington 1990, Monteiro *et al.* 2007a, Almeida-Jr *et al.* 2012) as characteristic of the Sapotaceae family were corroborated by the taxonomic analysis in the present study. The literature shows that leaf venation patterns can provide a set of characters that are variable among different Sapotaceae genera, sections, and species. Pennington (1990) described leaf venation using characters of primary to quaternary veins and by analyzing shape, organization, course, branching, and areolation. In this family, secondary veins can be craspedodromous, eucamptodromous, or brochidodromous, and they can have intersecondary veins. The course of tertiary and quaternary veins is variable with or without areolation.

The leaf vein system may vary in response to the environmental conditions of the different biomes. (Boeger *et al.* 2009, Sack & Scoffoni 2013). Despite this, the leaf venation pattern is an important character used for taxonomic distinction (Ellis *et al.* 2009, Borrero *et al.* 2016, Coutinho 2016). We highlight some conservative characters that can help in the identification of *D. cuspidatum* in the vegetative stage: both in the Atlantic forest biome and in the Amazon forest. These characters include the course of the midrib, the types of spaces between secondary veins, the angle between primary and secondary veins, and the pattern of intersecondary veins.

Laticifers and malpighiaceous trichomes are notable anatomical characters, which together allow any member of the Sapotaceae family to be easily recognized, even in sterile stages (Solereder 1908, Metcalfe & Chalk 1950, Monteiro 2007b, Almeida-Jr *et al.* 2012). Additionally, the U-shaped cuticle on the abaxial side (which resembles smooth ondulations), hypostomatic leaves, anisocytic stomata, the predominance of a dorsiventral mesophyll, and the occurrence of sclerenchymatous elements and solitary or clustered crystals are also potentially diagnostic characters of Sapotaceae (Solereder 1908, Metcalfe & Chalk 1950, Monteiro 2007a, Almeida-Jr *et al.* 2012).

The shape and arrangement of the vascular system of the petiole and midrib have been deemed useful in plant systematics (Araújo *et al.* 2010, Leandro *et al.* 2015, Coutinho *et al.* 2016, Feio *et al.* 2018, Guesdon *et al.* 2018, Pereira *et al.* 2018). Metcalfe & Chalk (1950) described eight shapes for the petiole of Sapotaceae. Of these, *Diploon* is classified as "adaxially flattened closed cylinder" or flat-convex. The vascular bundle of the petiole is also flat-convex, occasionally displaying one or two accessory bundles. In the midrib, the contour and conformation of the vascular bundle is biconvex. For *Diploon*, as a monospecific genus, these characters are also taxonomically relevant. In other genera of Sapotaceae, the aforementioned characteristics were also present; however, the shape of the petiole, the midrib, and the conformation of the vascular bundles may vary among species, which was observed for *Manilkara* Adans. and *Pouteria* Aubl. (Monteiro *et al.* 2007a, Almeida-Jr *et al.* 2012).

The thickness and number of layers of the palisade parenchyma are usually related to the habitat of the individual species, and it is mainly affected by the humidity and the extent of luminous intensity (Hlwatika & Bhat 2002, Rossatto & Kolb 2010; Schmidt *et al.* 2017; Muniz *et al.* 2018). However, the number of layers is fixed for some species (Solereder 1908). Generally, for Sapotaceae, the palisade tissue consists of one or more cell layers, and it is present only on the adaxial side (dorsiventral mesophyll). In most Sapotaceae species, the spongy parenchyma has large intercellular spaces (Solereder 1908, Metcalfe & Chalk 1950, Monteiro 2007a, Almeida-Jr *et al.* 2012). Variations in the number of layers, a dorsiventral mesophyll, two discontinuous layers of palisade parenchyma, and a spongy parenchyma with large intercellular spaces were observed in *D. cuspidatum*.

In *Diploon*, laticifers are present between the parenchyma cells of the midrib and the petiole, and they are easily distinguished from other epidermal and parenchymal elements. In Sapotaceae, laticifers are arranged in longitudinal rows and they have resinous substances that are sometimes accompanied by calcium oxalate crystal sand and starch (Solereder 1908). In the leaf blade, they are distributed predominantly in the midrib region, but are also found along the mesophyll and petiole (Solereder 1908, Metcalfe & Chalk 1950, Metcalfe & Chalk 1983, Monteiro *et al.* 2007a). In different genera of this family, the location of laticifers throughout these structures (leaf blade, mesophyll, and petiole) is variable, and they may occur along the vascular bundles of the veins or they can be immersed in the mesophyll (in *Chrysophyllum* L. and *Pouteria* Aubl.) or distributed in the cortical and medullary regions of the leaf blade (in *Manilkara* Adans.) (Solereder 1908, Metcalfe & Chalk 1950, Monteiro *et al.* 2007a, Almeida-Jr *et al.* 2012). This study emphasizes the marked presence of laticifers in the family, which can be found in different parts of the leaf blade depending on the genera and species.

According to Pennington (1990), *D. cuspidatum* displays glabrous leaves, but the anatomical analysis only allowed the visualization of trichomes along the midrib. Trichomes are unicellular malpighiaceous with long arms and a short peduncle. In Sapotaceae, most species are covered by an indumentum formed by persistent or deciduous adpressed trichomes with arms of different sizes and of variable density on the abaxial and adaxial side (Pennington 1990). The present study demonstrated the occurrence of trichomes in *D. cuspidatum*, which may be deciduous. In many cases, it was possible to observe only the scars of the trichomes.

For Solereder (1908) and Metcalfe & Chalk (1950), the occurrence of large numbers of cells rich in solitary or clustered prismatic crystals is a good generic diagnostic characteristic for Sapotaceae, especially when combined with other characters. We observed the presence of large prismatic crystals dispersed in the midrib and petiole regions in *D. cuspidatum*, which were more frequent in the first subepidermal layers of the petiole. Crystal idioblasts present in the palisade parenchyma were important for the taxonomic delimitation of *Pouteria* Aubl. and *Pradosia* Liais (Solereder 1908, Monteiro *et al.* 2007a).

The description of *D. cuspidatum* leaf architecture and anatomy has revealed a set of characters that have been reported for the Sapotaceae family and characters that aid in the identification of the species. In addition, the findings of the present study contribute to the morphoanatomic characterization of the *Diploon* and provide important information that can be used in taxonomic and phylogenetic studies.

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Author Contributions

Renata Gabriela Vila Nova de Lima: 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.

Liliane Ferreira Lima: 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.

Angélica Cândida Ferreira: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Josiane Silva Araújo: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Carmen Silvia Zickel: 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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Mammals recorded in isolated remnants of Atlantic Forest in southern Goiás, Brazil

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Abstract: Habitat fragmentation is one of the principal causes of the decline of species worldwide, and the Brazilian Atlantic Forest and Cerrado savanna biomes are among the most severely affected by this process. While highly fragmented, remnants of Atlantic Forest can still be found within the Cerrado domain of southern Goiás, where previous studies have revealed high levels of biodiversity. To inventory the mammalian species that occur in the region, two fragments of semideciduous Atlantic Forest were sampled between 2011 and 2016, using line transect surveys and camera trapping. A total of 1016 records were obtained of 30 mammal species, of which eleven are under some threat of extinction. The species richness recorded on this study was similar to or higher than the values reported from other areas of Goiás, which reinforces the importance of the maintenance of these remnants, located in private properties, for the conservation of the region's mammals.
Keywords: *survey, cameras traps, linear transect, medium- and large-sized mammals, spatial distribution, species richness.*

Mamíferos registrados em áreas isoladas de Mata Atlântica no sudoeste de Goiás

Resumo: A fragmentação de hábitat é uma das principais causas do declínio das espécies e tanto a Mata Atlântica quanto o Cerrado são considerados um dos biomas mais afetados do mundo. As regiões sul e sudoeste de Goiás são intensamente fragmentadas, mas ainda abrigam alguns dos últimos remanescentes relictuais de Mata Atlântica dentro do domínio do Cerrado e estudos prévios revelaram uma alta biodiversidade. Com o objetivo de elaborar a lista de espécies de mamíferos que ocorrem para a região, dois fragmentos de florestas semideciduais da Mata Atlântica foram amostrados entre 2011 a 2016, através de transecções lineares e armadilhas fotográficas. Foram obtidos um total de 1.016 registros de 30 espécies de mamíferos, das quais onze estão ameaçadas de extinção. A alta riqueza registrada foi similar ou maior ao de outras áreas no Estado e reforça a importância da manutenção desses remanescentes localizados em propriedades particulares para a conservação das espécies.
Palavras-chave: *amostragem, armadilhas fotográficas, transecto linear, médios e grandes mamíferos, distribuição espacial, riqueza.*

Introduction

Brazil is home to 10% of the world's terrestrial biota, and is considered to be one of the planet's most biodiverse countries (Mittermeier et al. 1997). The country's fauna includes more than 700 recognized mammal species, and additional taxa are expected to be discovered in the near future (Paglia et al. 2012). The Atlantic Forest is not only one of Brazil's most diverse biomes, but also has a high rate of endemism (Feio & Caramaschi 2002), which led to its inclusion, together with the Cerrado savanna, in the world's 34 conservation hotspots defined by Mittermeier et al. (2005). The reduction and isolation of the remaining forests in these hotspots represent one of the principal and most pressing threats to global biodiversity (Cullen Jr et al. 2005, Schnell et al. 2013).

The Brazilian Atlantic Forest originally covered a total area of 1,315,460 km², including part of 17 different states, corresponding to approximately 15% of the total area of Brazil (Ribeiro et al. 2009). Most of the state of Goiás is covered by Cerrado savanna formations (Morellato & Haddad 2000), although 3% of the southern and southwestern extreme of the state were originally covered with Atlantic Forest, with a total area of 1,190,184 ha (Galindo-Leal & Câmara 2003). However, only 30,386 ha of this forest has survived to the present day (Fundação SOS Mata Atlântica 2017). In Goiás, only two types of Atlantic Forest formation exist, seasonal deciduous forest and seasonal semi-deciduous forest, which are both characterized by leaf-fall during the dry season, in addition to the impacts of habitat fragmentation and the loss of local biodiversity (Campanili & Prochnow 2006, Fundação SOS Mata Atlântica 2017).

Given the importance of the Atlantic Forest and its critical situation in the state, the government of Goiás created the Atlantic Forest State Park (Parque Estadual da Mata Atlântica: PEMA) with the support of the MAB/UNESCO program. As this conservation unit was created less than 13 years ago, its biodiversity is still relatively poorly-known, despite the small area of Atlantic Forest it protects. These remnant areas of Atlantic Forest require basic surveys to provide a reliable assessment of their conservation status, and in particular of the principal groups of organisms that provide the best indices of environmental quality. Vertebrates are among the most useful of these groups, especially as they represent the fauna for which the most scientific data are available. In the specific case of the Atlantic Forest, the birds and mammals are especially important, due to the fact that the include the majority of the endangered species (Chiarello et al. 2008).

Despite the importance of the Atlantic Forest, and its reduced area in Goiás, which as already been decimated by anthropogenic impacts, there are virtually no studies of the biota of this local ecosystem. Given this, the present study was conducted with the aim of expanding the inventory of medium and large mammals species available for the study area and contributing to the evaluation of the conservation status of the fragments, given that mammals are relatively reliable indicators of habitat quality.

Material and Methods

1. *Study Area*

The study area is composed of two fragments of Atlantic Forest, one of 2100 hectares (18º55'41'' S, 51º42'41'' W) and the other of 2000 hectares (18º55'37'' S, 51º39'12'' W), set within a matrix of pasture, which are part of the PELD/Jataí Long-Term Ecological Monitoring Project, located in the municipalities of Aporé and Itajá, in Goiás, Brazil (Figure 1). This region encompasses the last remnants of the Atlantic Forest left in Goiás, which are complex and heterogeneous environments that have a high species richness (Melo et al. 2013). The region's climate is humid tropical, *Aw* in the Köppen classification system, with dry winters (April to September) and rainy summers (October to March), a mean temperature of 30ºC, and mean annual precipitation of 1486 mm (Melo et al. 2013).

2. *Data Collection*

The inventory of medium and large mammals was based on the data collected in two studies. The first study was conducted between April and July 2011, and consisted of visual surveys along three linear transects of 500 m in length established within the study fragments and on the roads that run along its edges. These surveys were conducted in the early morning (06:00–12:00 h) and late afternoon/early night (16:00–00:00 h). Additional data were collected using four Tigrinus camera traps, installed within the fragments, but outside the transects, during the first field trip, which were only removed during the last trip, in July 2011. The cameras were in operation 24 hours a day, and were visited monthly to change film and batteries, and to replace the bait, which was composed of sardine, pineapple, and salt. During three field trips, a total of 19 days of line transect surveys were conducted, and photographic records were collected during 192 trap-days.

During the second study, between December 2015 and March 2016, data were collected only by camera-trapping, with 10 Bushnell HD Trophy Cam camera traps being used in the same fragments surveyed during the first study. On this occasion, the camera traps were set in a linear arrangement, following the principal axis of the fragment, at a mean distance of 1 km from one another, 30 cm above the ground. No bait was used. A threshold of 1 hour was used to consider different records of the same species captured by the same trap to be independent sampling events. A total of 3337 trap-days were collected over a 15-month study period during this second sampling period.

The conservation status of each mammalian species recorded during the present study was obtained from the red list of the International Union for Conservation of Nature and Natural Resources (IUCN 2017), and the Brazilian list of threatened fauna (ICMBio-MMA 2016). Species taxonomy followed Paglia et al. (2012), with the updates of Delsuc et al. (2016) for the Cingulata, and the proposed order Cetartiodactyla (see Montgelard et al. 1997).

2.1. Data Analysis

For the statistical analysis, a species accumulation curve was obtained from the whole data set from the two study periods combined, using the Jackknife I estimator. As the detection probability of most mammals is highly heterogeneous, due to their mobility and variable behavior, the Jackknife estimator is considered to be the most appropriate procedure for the assessment of species richness (Brose et al. 2003, Brose & Martinez 2004, Tobler et al. 2008). The Jackknife I procedure takes the frequency of the rarest species in the sample into account (Heltshe & Forrester 1983) and was calculated in the EstimateS program (Colwell 2009).

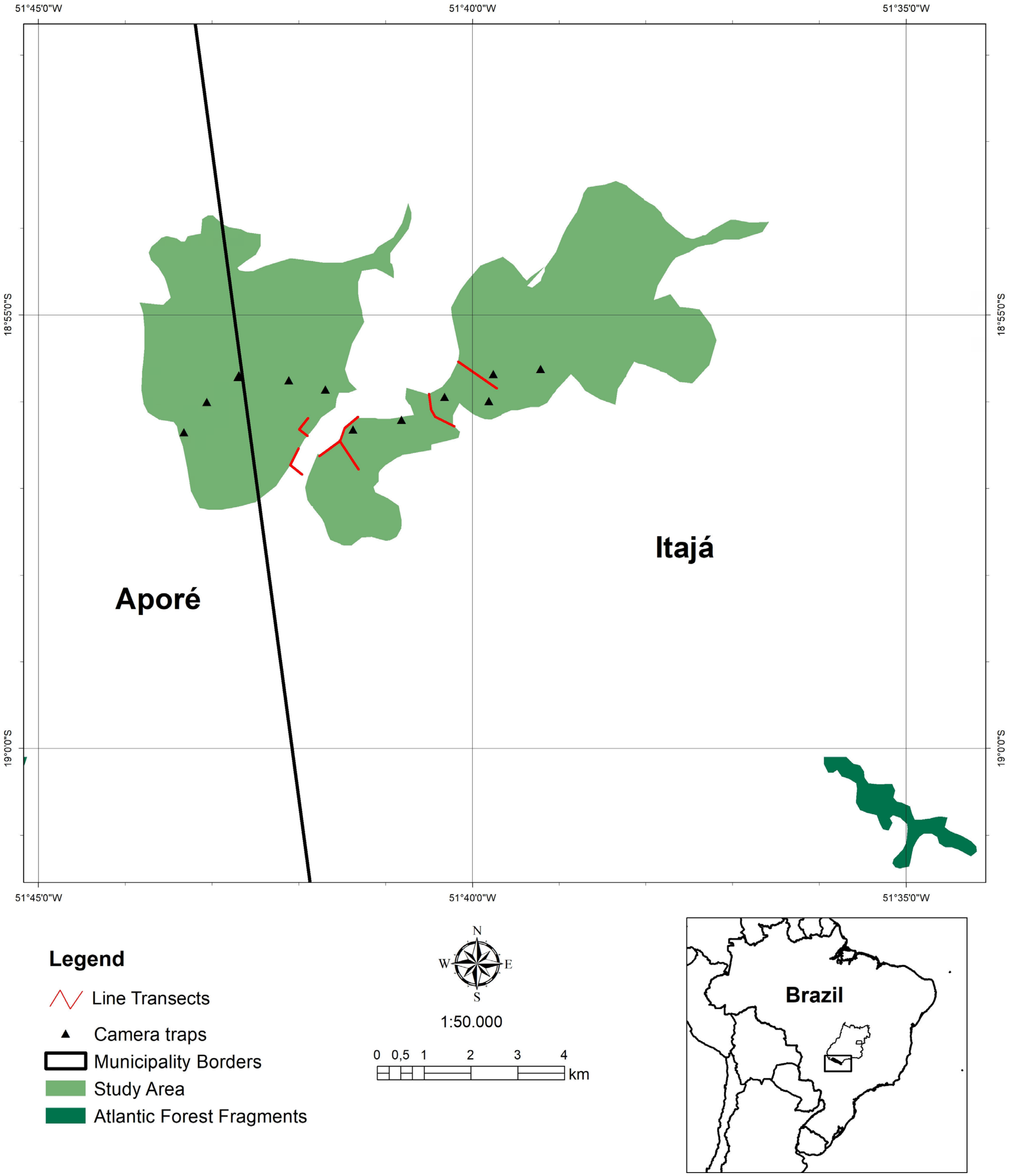

Figure 1. Location of the study area in the municipalities of Itajá and Aporé, in Goiás state, Brazil. The black dots indicate the locations at which the camera traps were installed during the second period and red lines are the linear transects.

Results

A total of 1016 records were collected in the present study, which confirmed the presence of 27 mammal species of medium to large size. Three other species, the maned wolf (*Chrysocyon brachyurus*), the Neotropical otter (*Lontra longicaudis*), and the Brazilian porcupine (*Coendou prehensilis*), were also observed within the study area, but were not recorded during the fieldwork, so they were not included in the quantitative analyses. The final inventory thus contained 30 species of medium/large mammals, representing seven families and nine orders (Table 1, Figure 2).

Overall, 22 of the 27 species recorded in the fieldwork were registered in the line transect surveys, and five of these species were only recorded by this method. These species include the black howler monkey (*Alouatta caraya*), which rarely comes to the ground, the six-banded armadillo (*Euphractus sexcinctus*), the tapeti (*Sylvilagus brasiliensis*), and two carnivores, the hoary fox (*Lycalopex vetulus*) and the Pantanal cat (*Leopardus braccatus*). The camera traps also recorded 22 species and five exclusive taxa, all of which were terrestrial forms, including two armadillos, the southern naked-tailed armadillo (*Cabassous unicinctus*) and the nine-banded armadillo (*Dasypus novemcinctus*), the margay

Table 1. Mammals recorded in fragments of Atlantic Forest, southern Goiás state, Brazil. Type of record: VO = visual observation, V = vocalization, T= track, F = feces, B = burrow, CT = camera trap. Status of Conservation: VU= Vulnerable; NT= Near Threatened; LC= Least Concern; NC= Not Cited.

TAXON	COMMON NAME	TYPE OF RECORD	CONSERVATION STATUS	
			IUCN	MMA
Order Didelphimorphia				
Family Didelphidae				
Didelphis albiventris Lund, 1840	White-eared Opossum	VO, CT	LC	NC
Order Cingulata				
Family Dasypodidae				
Dasypus novemcinctus (Linnaeus, 1758)	Nine-banded Armadillo	CT	LC	NC
Family Chlamyphoridae				
Cabassous unicinctus (Linnaeus, 1758)	Southern Naked-tailed Armadillo	CT	LC	NC
Euphractus sexcinctus (Linnaeus, 1758)	Yellow Armadillo	VO, T, B	LC	NC
Priodontes maximus (Kerr,1792)	Giant Armadillo	B, T, CT	VU	VU
Order Pilosa				
Family Myrmecophagidae				
Myrmecophaga tridactyla Linnaeus, 1758	Giant Anteater	VO, T, CT	VU	VU
Tamandua tetradactyla (Linnaeus, 1758)	Southern Tamandua	VO, CT	LC	NC
Order Primates				
Family Cebidae				
Sapajus cay (Illiger, 1815)	Azara's Capuchin	VO, V, CT	LC	VU
Family Atelidae				
Alouatta caraya (Humboldt, 1812)	Black-and-gold Howler Monkey	VO	LC	NC
Order Carnivora				
Family Canidae				
Cerdocyon thous (Linnaeus, 1766)	Crab-eating Fox	VO, V, T, F, CT	LC	NC
Chrysosyon brachyurus (Illiger, 1815)	Lobo-guará	Vi, Vo	NT	VU
Lycalopex vetulus Lund, 1842	Hoary Fox	VO, T	LC	VU
Family Felidae				
Leopardus braccatus (Molina, 1782)	Pampas Cat	VO, T	NT	VU
Leopardus pardalis (Linnaeus, 1758)	Ocelot	T, CT	LC	NC
Leopardus wieddi (Schinz, 1821)	Margay	CT	NT	VU
Puma concolor (Linnaeus, 1771)	Puma	T, CT	LC	VU
Puma yagouaroundi (É. Geoffroy, 1803)	Jaguarundi	CT	LC	VU
Family Mustelidae				
Eira barbara (Linnaeus, 1758)	Tayra	VO, V, CT	LC	NC

Continuation Table 1.

TAXON	COMMON NAME	TYPE OF RECORD	CONSERVATION STATUS	
Family Procyonidae				
Nasua nasua (Linnaeus, 1766)	South American Coati	T, CT	LC	NC
Procyon cancrivorus (G. [Baron] Cuvier, 1798)	Crab-eating Raccoon	VO, T, CT	LC	NC
Order Perissodactyla				
Family Tapiridae				
Tapirus terrestris (Linnaeus, 1758)	Lowland Tapir	VO, T, F, CT	VU	VU
Order Artiodactyla				
Family Tayassuidae				
Pecari tajacu (Linnaeus, 1758)	Collared Peccary	VO, V, T, CT	LC	
Tayassu pecari (Link, 1795)	White-lipped Peccary	VO, V, T, CT	VU	VU
Family Cervidae				
Mazama americana (Erxleben, 1777)	Red Brocket Deer	CT	DD	NC
Mazama gouazoubira (G. Fisher, 1814)	Gray Brocket Deer	T, CT	LC	NC
Order Rodentia				
Family Cuniculidae				
Cuniculus paca (Linnaeus, 1766)	Spotted Paca	T, CT	LC	NC
Family Dasyproctidae				
Dasyprocta azarae (Lichtenstein, 1823)	Azara's Agouti	VO, V, T, CT	DD	NC
Erithizontidae				
Coendou prehensilis (Linnaeus, 1758)	Brazilian Porcupine	Vi	LC	
Order Lagomorpha				
Family Leporidae				
Sylvilagus brasiliensis (Linnaeus, 1751)	Tapeti	VO, T	LC	NC

(*Leopardus wiedii*) and the jaguarundi (*Puma yagouaroundi*), and the red brocket deer (*Mazama americana*).

The most diverse order was the Carnivora, with 10 species (37.0% of the total number of species recorded), followed by the Cingulata and Cetartiodactyla, each with four species (14.8% each). The number of species recorded per order varied negligibly between sampling procedures (Figure 3). The species accumulation curve reached the asymptote (Figure 4), which indicates that the sampling was sufficient to detect all the mammals in the study area, and while the estimate indicated the presence of an additional four species, three of these species would have been those recorded outside the fieldwork period.

Discussion

Local species inventories are essential for the assessment of the conservation status of a region's biodiversity, providing guidelines for the selection of priority areas for conservation (Diniz-Filho et al. 2008, Jenkins et al. 2015). In the present study, the use of different approaches for the collection of data maximized the chances of recording species, in particular the more cryptic forms that are difficult to observe, such as carnivores, providing the most representative possible sample of the local mammalian fauna, given the available time and resources (Silveira et al. 2003).

While comparisons with other studies may not be totally reliable, given differences in sampling effort and habitat characteristics, they do provide an important baseline for the quantification of a region's biodiversity (Gomes et al. 2015). The number of species recorded in the present study (30) was similar to or higher than those recorded in previous studies in southern Goiás. In the Atlantic Forest State Park, in the municipality de Água Limpa, for example, Rocha et al. (2015) recorded 23 species of medium to large-bodied mammals within a landscape that has few remnants of natural habitat, due to intense fragmentation, but is important for the maintenance of the region's fauna. Hannibal et al. (2015) recorded 25 species of medium/large mammals, including a number of endangered forms, in fragments of seasonal forest located on private properties in southern Goiás. In southwestern Goiás, within an area dominated by Cerrado savanna formations, Estrela et al. (2015) recorded 25 species in a fragment in Urutaí and Gomes et al. (2015) recorded 33 species in different phytophysionomies in the Serra do Facão range, in the municipalities of Catalão and Campo Alegre de Goiás.

On an ampler, and more directly comparable scale, the mammalian faunas recorded in fragments of Atlantic Forest in other regions of Brazil, such as São Paulo and Minas Gerais, were between 52% and 67% similar to that recorded in the present study (Magioli et al., 2014; Machado et al., 2016; Laurindo et al., 2017; Bovo et al., 2018).

Figure 2. Some of the mammals recorded in fragments of Atlantic forest in southern Goiás state, Brazil: A) *Myrmecophaga tridactyla*; B) *Leopardus braccatus*; C) *Mazama americana*; D) *Tayassu pecari*; E) *Tapirus terrestris*; F) *Puma concolor*.

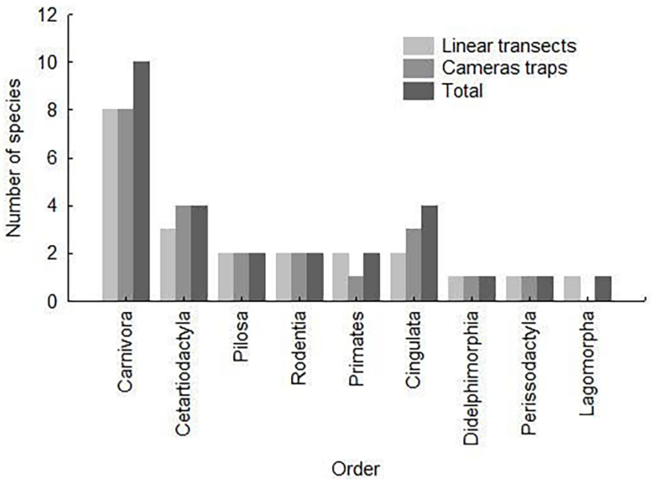

Figure 3. Records of the different mammalian orders obtained by linear transect surveys and cameras trapping in fragments of Atlantic Forest in southern Goiás state, Brazil.

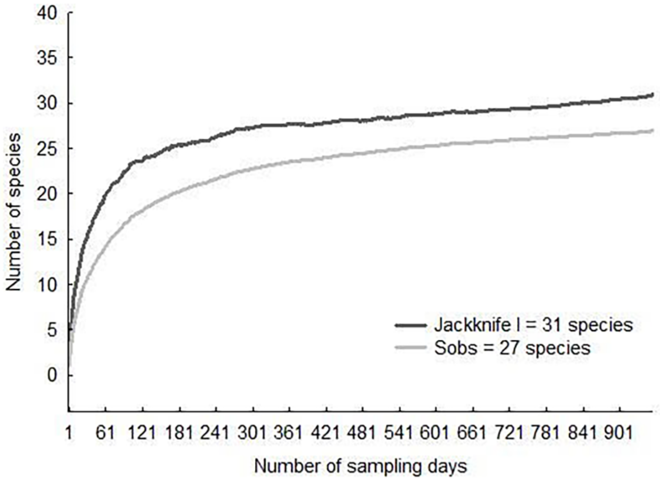

Figure 4. Accumulation curve of mammal species recorded in fragments of Atlantic Forest in southern Goiás state, Brazil.

The similarities were even greater in comparison with fragments of Cerrado savanna, protected or otherwise, with an average of 83% of species in common (Rodrigues et al., 2002; Bocchiglieri et al., 2010; Campos et al., 2013; Leite et al., 2016; Cabral et al., 2017), reflecting the considerable influence of this biome on the mammalian fauna of the study area.

The species richness recorded in the present study is probably also a reflection of the vegetation structure of the fragments surveyed, which are formed predominantly by seasonal semi-deciduous Atlantic Forest in transition with areas of Cerrado savanna (Melo et al. 2013). This vegetation mosaic is occupied by species that are common to both biomes, as well as species characteristic of the Cerrado, such as the maned wolf (*Chrysocyon brachyurus*), hoary fox (*Lycalopex vetulus*), and Pantanal cat (*Leopardus braccatus*), in addition to Azaras' capuchin monkey (*Sapajus cay*), whose range within the Cerrado domain is extended here (Gusmão et al. 2018). No exclusive Atlantic Forest species were recorded in this study.

These findings reinforce the importance of the study fragments for the conservation of the mammals, in particular the species under some threat of extinction. Just over a third (36.6%) of the species recorded in the present study are classified in some category of threat in the two listings consulted (ICMBio-MMA, 2016; IUCN, 2017). A number of the species appear in both lists, and of these, the giant armadillo (*Priodontes maximus*), giant anteater (*Myrmecophaga tridactyla*), lowland tapir (*Tapirus terrestris*), and white-lipped peccary (*Tayassu pecari*) are all listed as vulnerable in both cases. Three other species, the Pantanal cat (*L. braccatus*), the margay (*L. wiedii*), and the maned wolf (*C. brachyurus*), are also listed as vulnerable by ICMBio-MMA (2016), but as "Near Threatened" by the IUCN (2017). Azaras' capuchin (*S. cay*), the hoary fox (*Lycalopex vetulus*), puma (*Puma concolor*), and jaguarundi (*P. yagouaroundi*) are all listed as vulnerable by the Brazilian environment ministry (ICMBio-MMA 2016).

As medium/large mammals, in particular carnivores, require relatively large areas to support a viable population (Costa et al. 2005), the environmental diversity of the fragments surveyed in the present study may be vital to the maintenance of the local populations of many of the species recorded in this study (Calaça et al. 2010, Rocha et al. 2015, Laurindo et al. 2017). Southern Goiás has suffered intense habitat fragmentation to make way for plantations of cash crops and cattle ranching, and these habitat remnants may represent vital refuges for many mammals. The protection of a region's biodiversity is essential to guarantee ecosystem function, in particular in fragmented landscapes, where interactions tend to break down as key species are lost (Camargo-Sanabria et al. 2015). Overall, then, the findings of the present study reemphasize the importance of the fragments surveyed for the conservation of the region's mammals and reinforce the urgent need for the creation of a conservation unit in this region, as proposed by Melo et al. (2013).

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Author Contributions

Analice Calaça: Substancial 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.

Marluci Fachi: Contribution to data collection; Contribution to manuscript preparation.

Diego Afonso Silva: Contribution to data collection.

Seixas Rezende Oliveira: Contribution to data collection; Contribution to manuscript preparation.

Fabiano Rodrigues de Melo: 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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Inventory

Butterflies (Lepidoptera: Papilionoidea) of an urban park in northeastern Brazil

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Abstract: Despite being one of the groups most well studied in Brazil, the butterfly fauna of northeastern Brazil, especially north of the mouth of São Francisco River, is poorly known. The aim of this study was to inventory the butterfly fauna in a remnant of Atlantic Forest in the state of Pernambuco as a contribution to understanding the distribution of these insects. The study was carried out in a 384.7 hectares fragment of Parque Estadual Dois Irmãos, in the metropolitan region of Recife. The butterflies were sampled monthly with insect nets from August 2011 to July 2012 and from January to August 2016. Additional records were obtained from two entomological collections and from two previous visits to the area in July 2003 and August 2006. A total of 273 species was sampled in 464 hours, with an additional 15 species recorded from the Entomological Collections, totaling 288 butterfly species recorded. The richest family was Hesperiidae (108 species), followed by Nymphalidae (80), Lycaenidae (43) Riodinidae (37), Pieridae (16) and Papilionidae (4). The fauna was dominated by generalist species with a broad geographical distribution, many of them commonly found on open areas, forest edges and secondary vegetation. *Roeberella lencates* (Hewitson, 1875) and *Pheles atricolor atricolor* (Butler, 1871) (Riodinidae) represent new records for northeastern Brazil.
Keywords: *Atlantic Forest, Conservation, Inventory, northeastern Brazil.*

As borboletas (Lepidoptera: Papilionoidea) de um parque urbano do nordeste do Brasil

Resumo: Apesar de ser um dos grupos mais bem estudados no Brasil, as borboletas da região Nordeste, especialmente ao norte da foz do Rio São Francisco, são ainda pouco conhecidas. O objetivo deste estudo foi realizar um inventário da fauna de borboletas em um fragmento de Floresta Atlântica no estado de Pernambuco, como uma contribuição para compreensão sobre a distribuição desses insetos. O estudo foi realizado em um fragmento de Floresta Atlântica de 384.7 hectares do Parque Estadual Dois Irmãos, localizado na região metropolitana de Recife. As borboletas foram amostradas mensalmente, de agosto de 2011 a julho de 2012 e de janeiro a agosto de 2016, com auxílio de redes entomológicas. Registros adicionais foram obtidos de duas Coleções Entomológicas e de duas coletas prévias em julho de 2003 e agosto de 2006. Um total de 273 espécies foi registrado em 464 horas e 15 espécies adicionais registradas a partir de Coleções Entomológicas, totalizando 288 espécies de borboletas registradas. A família mais rica foi Hesperiidae (108 espécies), seguida por Nymphalidae (80), Lycaenidae (43), Riodinidae (37), Pieridae (16) e Papilionidae (4). A fauna de borboletas foi dominada por espécies generalistas com uma ampla distribuição geográfica, muitas delas encontradas comumente em áreas abertas, bordas de mata e em vegetação secundária. *Roeberella lencates* (Hewitson, 1875) e *Pheles atricolor atricolor* (Butler, 1871) (Riodinidae) representam um novo registro para o Nordeste do Brasil.
Palavras-chave: *Conservação, Floresta Atlântica, Inventário, Nordeste Brasileiro.*

Introduction

Although the butterfly fauna of Brazil is relatively well sampled, there is still a "knowledge gap" in northeastern Brazil (Santos et al. 2008). The entire region is relatively under-sampled and poorly known, especially in the highly fragmented forests north of the mouth of São Francisco River (Freitas & Marini-Filho 2011, Iserhard et al. 2017). This region, which is known as the "Pernambuco Center of Endemism" (Brown Jr. 1977, Santos et al. 2007), is the most endangered sector of the Atlantic Forest, with less than 12% of the original vegetation remaining (Ribeiro et al. 2009), almost half as fragments smaller than 10 ha (Ranta et al. 1998).

Research on the butterfly fauna in northeastern Brazil started in the 19th century with Bates (1867) and continued in the next century (e.g. D'Almeida 1935, Cardoso 1949, Silva 1967, Kesselring & Ebert 1982). Research activity on butterflies in the region has recently blossomed, with papers on threatened species (Kerpel et al. 2014, Melo et al. 2014) and the natural history of poorly-known species (Cardoso 2005, Freitas & Brown Jr. 2005, Nobre & Schlindwein 2011, 2012, Callaghan & Nobre 2014, Dolibaina et al. 2014, Nobre & Robbins 2014). Although several partial lists are now available (e.g. Duarte Jr. et al. 2001, Vasconcelos et al. 2009, Zacca et al. 2011, Zacca & Bravo 2012, Kerpel et al. 2014), very few comprehensive inventories have been published for northeastern Brazil. Examples of recently published well-sampled lists for the region includes those of Nobre et al. (2008, 2012) and Lima & Zacca (2014) for areas of semi-arid "Caatinga" and the inventories for an altitudinal and coastal Atlantic Forest (Paluch et al. 2011 and 2016, respectively).

The purpose of the present study was to inventory the butterfly species found in "Parque Estadual Dois Irmãos", a forest fragment in the metropolitan region of Recife, Pernambuco, northeastern Brazil, and to compare the results with those of previous inventories in the same region. These baseline results will also serve as a point of comparison for future inventories as ecological succession proceeds in this park.

Material and Methods

1. *Study area*

Parque Estadual Dois Irmãos (PEDI) (08º 00' 48" S, 34º 56' 42.9" W) is a state park in Recife, Pernambuco, northeastern Brazil (Figure 1). PEDI was created by State Law nº 11.622 of December 29, 1998 with ca. 384.7 ha, and has recently expanded (state decree nº 40.547 of March 28, 2014) with the inclusion of adjacent forest (the "Fazenda Brejo dos Macacos"), now totaling 1,157.72 ha. This coastal Atlantic Forest consists of a mix of successional regenerating stages. The present study was carried out in the 384.7 ha area now called "Mata de Dois Irmãos". This area is the original part of the Park, including a 14 ha zoo. The climate is tropical monsoon (Am of Köppen), hot and humid, with a monthly average temperature of 25.8ºC. Rainfall, with an annual precipitation up to 2,400 mm, is seasonal, with a wet season from March to August and a dry season from September to February (Coutinho et al. 1998, Alvares et al. 2014). The predominant soils are dystrophic red-yellow latosols (Coutinho et al. 1998), and the vegetation is classified as ombrophilic dense lowland forest (Veloso et al. 1991).

2. *Sampling and identification*

Sampling was carried out monthly from August 2011 to July 2012, following the protocol described in Brown Jr. (1972), Brown Jr. & Freitas (2000) and Iserhard et al. (2013), with four sampling days each month, totaling 48 field trips. In addition, occasional visits were conducted between January and August 2016, totaling 17 additional field trips. The daily sampling period was from 9:00 to 16:00 hours, with a total sampling effort of 455 hours. Sampling was conducted along pre-existing trails (up to 2 linear km) covering a variety of habitats by one collector using an insect net (with a handle 1 m in length). Special attention was devoted to forest edges, water bodies or their banks, rich soil and flower patches, small clearings, and other heterogeneous environments. Areas with notable concentrations of butterflies were observed until no additional species had been recorded for a period of five to ten minutes. Additional data were obtained from two previous visits by AVLF (July 29, 2003 and August 10, 2006, totaling 9 samplings hours) and from material deposited in the Entomological Collection, Biology Department, Universidade Federal Rural de Pernambuco (UFRPE), and the Entomological Collection, Universidade Federal de Pernambuco (UFPE). Specimens were mounted and deposited in the following Brazilian institutions: 1) Universidade Federal de Pernambuco, Recife, PE; 2) Museu de Zoologia, Universidade Estadual de Campinas, Campinas, SP; and 3) Coleção Entomológica Padre Jesus Santiago Moure, UFPR, Curitiba, PR.

The authors identified species using the literature, the museum collections for which they are responsible, and their taxonomic expertise, which is partly based on years of fieldwork in Brazil and other Neotropical countries. The Discussion is based on these same information sources. The nomenclature in Table 1 follows Lamas (2004), but higher taxonomic categories for Nymphalidae were updated from Wahlberg et al. (2009), for Riodinidae from Seraphim et al. (2018) and Hall (2018), for Hesperiidae from Warren et al. (2009). The higher classification of butterflies was updated from Heikkilä et al. (2012). All families were sampled, except for the Hedylidae.

Results

In total, 288 butterfly species in six families were recorded at PEDI (Table 1). The fieldwork yielded 273 species in 464 sampling hours. An additional 15 species had been previously sampled (vouchers in UFPE and UFRPE, as noted in Table 1). The most species rich family was Hesperiidae with 108 species (37,5%), followed by Nymphalidae with 80 (28%), Lycaenidae with 43 (15%), Riodinidae with 37 (13%), Pieridae with 16 (5,5%) and Papilionidae with 4 (1%).

A number of unusual riodinid species were found. The species *Roeberella lencates* (Hewitson, 1875) and *Pheles atricolor atricolor* (Butler, 1871) were recorded for northeastern Brazil for the first time (Table 1). A single individual of *R. lencates* was captured when resting in a "pitangueira" tree (*Eugenia uniflora*, Myrtaceae) in an open sunny area known as "Chapéu do Sol". A single individual of *P. atricolor atricolor* was found on a trail along a forest edge. It made short flights followed by landing on a leaf. A third riodinid *Eurybia gonzaga* Dolibaina, Dias, Mielke & Casagrande, 2014 is a recently described endemic for Pernambuco. It was observed frequently in PEDI. This species flies near the ground at dusk. It lands on the underside of leaves.

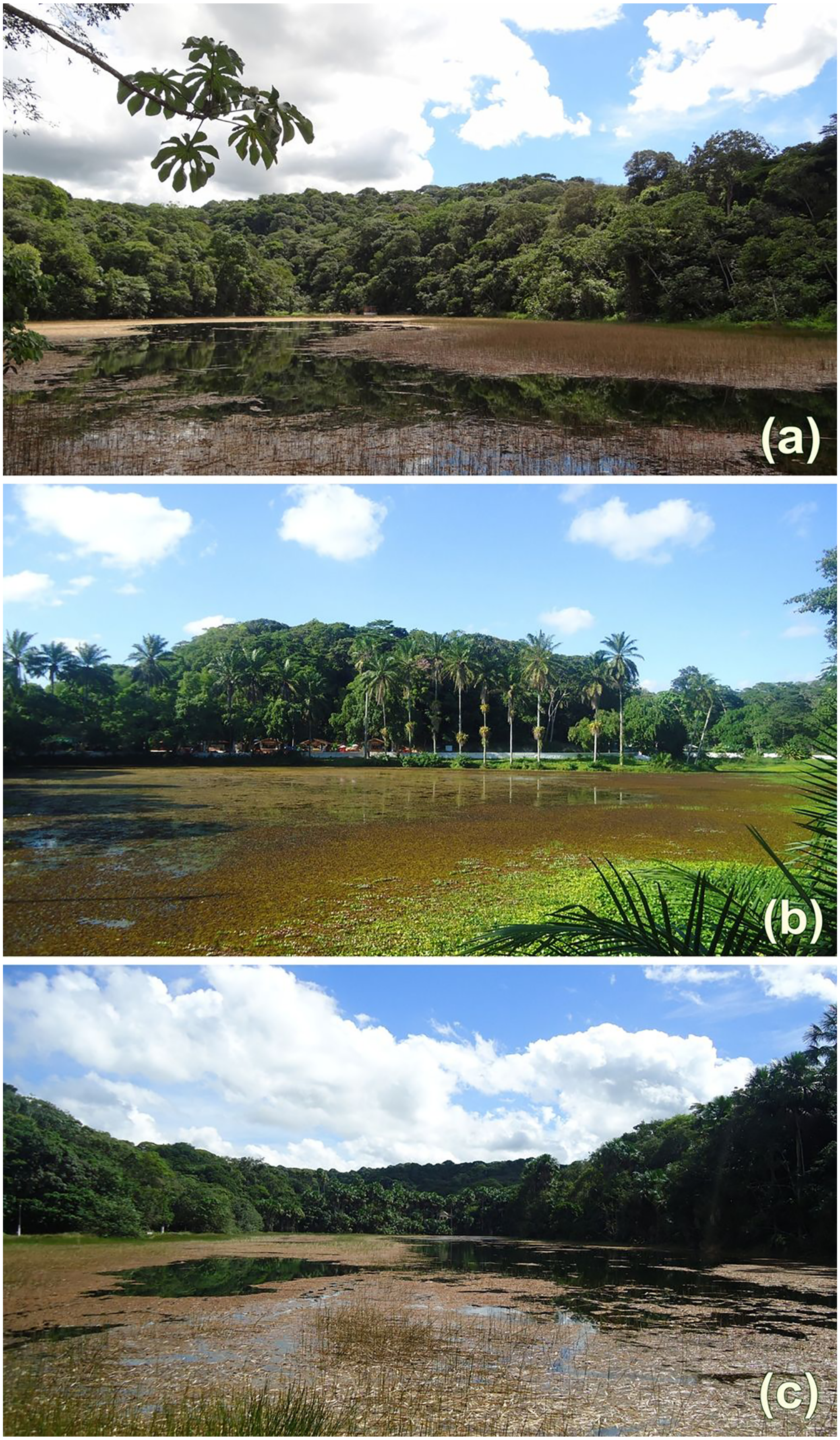

Figure 1. View of the "Parque Estadual Dois Irmãos", Recife, Pernambuco, Brazil, showing the vegetal formation next to the three following weirs: **(a)** Açude do Prata; **(b)** Açude do Parque; **(c)** Açude do Meio.

Table 1. Butterflies (Lepidoptera) from the "Parque Estadual Dois Irmãos", Recife, Pernambuco, Brazil. Number of species are noted within parenthesis for each major taxon. [1]Data from the UFPE Entomological Collection; [2]Data from the UFRPE Entomological Collection; [3]new record for northeastern Brazil.

- **PAPILIONOIDEA (288)**
  - **Papilionidae (4)**
    - Papilioninae (4)
    - Troidini (2)
      - *Battus polydamas polydamas* (Linnaeus, 1758)
      - *Parides zacynthus polymetus* (Godart, 1819)
    - Papilionini (2)
      - *Heraclides anchisiades capys* (Hübner, [1809])
      - *Heraclides thoas brasiliensis* (Rothschild & Jordan, 1906)
  - **Pieridae (16)**
    - Coliadinae (12)
      - *Anteos clorinde* (Godart, [1824])
      - *Anteos menippe* (Hübner, [1818])
      - *Aphrissa statira statira* (Cramer, 1777)
      - *Eurema agave pallida* (Chavannes, 1850)
      - *Eurema albula albula* (Cramer, 1775)
      - *Eurema deva deva* (Doubleday, 1847)[2]
      - *Eurema elathea flavescens* (Chavannes, 1850)
      - *Leucidia elvina* (Godart, 1819)
      - *Phoebis argante argante* (Fabricius, 1775)
      - *Phoebis philea philea* (Linnaeus, 1763)
      - *Phoebis sennae marcellina* (Cramer, 1777)
      - *Pyrisitia nise tenella* (Boisduval, 1836)
    - Pierinae (4)
      - *Ascia monuste orseis* (Godart, 1819)
      - *Glutophrissa drusilla drusilla* (Cramer, 1777)
      - *Hesperocharis anguitia anguitia* (Godart, 1819)[1]
      - *Itaballia demophile nimietes* (Fruhstorfer, 1907)[1]
  - **Lycaenidae (43)**
    - Polyommatinae (3)
      - *Hemiargus hanno hanno* (Stoll, 1790)
      - *Leptotes cassius cassius* (Cramer, 1775)
      - *Zizula cyna* (Edwards, 1881)
    - Theclinae (40)
      - *Allosmaitia strophius* (Godart, [1824])
      - *Aubergina hesychia* (Godman & Salvin, 1887)
      - *Brangas torfrida* (Hewitson, 1867)
      - *Calycopis caulonia* (Hewitson, 1877)
      - *Calycopis demonassa* (Hewitson, 1868)
      - *Calycopis* sp. 1
      - *Calycopis* sp. 2
      - *Celmia celmus* (Cramer, 1775)
      - *Chalybs janias* (Cramer, 1779)
      - *Cyanophrys amyntor* (Cramer, 1775)

Continuation Table 1.

- *Gargina emessa* (Hewitson, 1867)
- *Gargina* sp.
- *Kisutam syllis* (Godman & Salvin, 1887)
- *Magnastigma hirsuta* (Prittwitz, 1865)
- *Michaelus jebus* (Godart, [1824])
- *Michaelus phoenissa* (Hewitson, 1867)
- *Ministrymon cleon* (Frabricius, 1775)
- *Ministrymon una* (Hewitson, 1873)
- *Ministrymon zilda* (Hewitson, 1873)
- *Nesiostrymon hyccara* (Hewitson, 1868)
- *Nicolaea ophia* (Hewitson, 1868)
- *Oenomaus* sp.
- *Ostrinotes tarena* (Hewitson, 1874)
- *Panthiades hebraeus* (Hewitson, 1867)
- *Pseudolycaena marsyas* (Linnaeus, 1758)
- *Rekoa palegon* (Cramer, 1780)
- *Strephonota ambrax* (Westwood, 1852)
- *Strymon astiocha* (Prittwitz, 1865)
- *Strymon bubastus* (Stoll, 1780)
- *Strymon mulucha* (Hewitson, 1867)
- *Strymon ziba* (Hewitson, 1868)
- *Symbiopsis* sp.
- *Theclopsis gargara* (Hewitson, 1868)
- *Thereus cithonius* (Godart, [1824])
- *Thereus enenia* (Hewitson, 1867)
- *Theritas hemon* (Cramer, 1775)
- *Theritas triquetra* (Hewitson, 1865)
- *Thestius lycabas* (Cramer, 1777)
- *Tmolus echion* (Linnaeus, 1767)
- *Ziegleria hesperitis* (Butler & H. Druce, 1872)
- **Riodinidae (37)**
  - Nemeobiinae (1)
  - Euselasiini (1)
    - *Euselasia cafusa* (H. W. Bates, 1868)
  - Riodininae (36)
  - Eurybini (6)
    - *Ionotus alector* (Geyer, 1837)
    - *Mesosemia nyctea lato* Stichel, 1910
    - *Perophthalma tullius* (Fabricius, 1787)
    - *Voltinia phryxe* (C. Felder & R. Felder, 1865)
    - *Eurybia gonzaga* Dolibaina, Dias, Mielke & Casagrande, 2014
    - *Eurybia halimede halimede* (Hübner, [1807])
  - Riodinini (7)
    - *Calephelis braziliensis* McAlpine, 1971
    - *Isapis agyrtus* (Cramer, 1777)
    - *Melanis smithiae* (Westwood, 1851)

Continuation Table 1.

- *Panara soana* Hewitson, 1875
- *Pheles atricolor atricolor* (Butler, 1871)[3]
- *Rhetus arcius* (Linnaeus, 1763)
- *Rhetus periander* (Cramer, 1777)
- Helicopini (3)
  - *Anteros formosus formosus* (Cramer, 1777)
  - *Anteros renaldus* (Stoll, 1790)
  - *Sarota acanthoides* (Herrich-Schäffer, [1853])
- Nymphidiini (11)
  - *Ariconias glaphyra* (Westwood, 1851)
  - *Parvospila lucianus* (Fabricius, 1793)
  - *Periplacis pretus* (Cramer, 1777)
  - *Nymphidium mantus* (Cramer, 1775)
  - *Nymphidium olinda* H. W. Bates, 1865
  - *Synargis calyce pernambuco* Callaghan, 1999
  - *Synargis gela* (Hewitson, [1853])
  - *Synargis paulistina* (Stichel, 1910)
  - *Roeberella lencates* (Hewitson, 1875)[3]
  - *Theope pieridoides* C. Felder & R. Felder, 1865
  - *Theope* sp.
- Symmachiini (6)
  - *Mesene florus* (Fabricius, 1793)
  - *Mesene monostigma monostigma* (Erichson, [1849])
  - *Mesene phareus* (Cramer, 1777)
  - *Mesene* sp.
  - *Panaropsis inaria* (Westwood, 1851)
  - *Pirascca sagaris satnius* (Dalman, 1823)
- Calydnini (3)
  - *Calydna cea* Hewitson, 1859
  - *Calydna sturnula* (Geyer, 1837)
  - *Calydna* sp.

- **Nymphalidae (80)**
  - Nymphalinae (9)
  - Coeini (2)
    - *Colobura dirce dirce* (Linnaeus, 1758)
    - *Historis odius dious* Lamas, 1995
  - Nymphalini (1)
    - *Vanessa myrinna* (Doubleday, 1849)[2]
  - Kallimini (4)
    - *Anartia amathea* ssp.
    - *Anartia jatrophae jatrophae* (Linnaeus, 1763)
    - *Junonia evarete evarete* (Cramer, 1779)
    - *Siproeta stelenes meridionalis* (Fruhstorfer, 1909)
  - Melitaeini (2)
    - *Anthanassa hermas hermas* (Hewitson, 1864)
    - *Tegosa* sp.

Continuation Table 1.

- Danainae (11)
- Danaini (4)
  - *Danaus eresimus plexaure* (Godart, 1819)[2]
  - *Danaus erippus* (Cramer, 1775)
  - *Danaus gilippus gilippus* (Cramer, 1775)
  - *Lycorea halia discreta* Haensch, 1909
- Ithomiini (7)
  - *Dircenna dero celtina* Burmeister, 1878
  - *Episcada striposis* Haensch, 1909
  - *Hypothyris euclea laphria* (Doubleday, 1847)
  - *Hypothyris ninonia daetina* (Weymer, 1899)
  - *Mechanitis lysimnia nesaea* Hübner, [1820]
  - *Napeogenes inachia* ssp.
  - *Scada reckia reckia* (Hübner, [1808])
- Biblidinae (11)
  - *Biblis hyperia nectanabis* (Fruhstorfer, 1909)
  - *Callicore astarte codomannus* (Frabicius, 1781)
  - *Callicore sorana sorana* (Godart, [1824])[2]
  - *Dynamine athemon athemaena* (Hübner, [1824])
  - *Dynamine postverta postverta* (Cramer, 1779)
  - *Ectima thecla thecla* (Fabricius, 1796)
  - *Hamadryas amphinome amphinome* (Linnaeus, 1767)
  - *Hamadryas chloe rhea* (Fruhstorfer, 1907)[2]
  - *Hamadryas februa februa* (Hübner, [1823])
  - *Hamadryas feronia feronia* (Linnaeus, 1758)
  - *Pyrrhogyra neaerea susarion* Fruhstorfer, 1912[2]
- Cyrestinae (2)
  - *Marpesia chiron marius* (Cramer, 1779)
  - *Marpesia petreus petreus* (Cramer, 1776)
- Satyrinae (29)
- Morphini (1)
  - *Morpho helenor anakreon* Fruhstorfer, 1910
- Brassolini (9)
  - *Brassolis sophorae dinizi* D'Almeida, 1956
  - *Caligo brasiliensis brasiliensis* (C. Felder, 1862)
  - *Caligo illioneus illioneus* (Cramer, 1775)
  - *Caligo teucer* ssp.
  - *Dynastor darius darius* (Fabricius, 1775)
  - *Eryphanis automedon amphimedon* (C. Felder & R. Felder, 1867)
  - *Opsiphanes cassiae crameri* C. Felder & R. Felder, 1862
  - *Opsiphanes invirae remoliatus* Fruhstorfer, 1907
  - *Opsiphanes quiteria meridionalis* (Staudinger, 1887)
- Haeterini (2)
  - *Pierella hyalinus* ssp.
  - *Pierella kesselringi* Zacca, Siewert & Paluch, 2016

Continuation Table 1.

  - Satyrini (17)
    - *Chloreuptychia chlorimene* (Hübner, [1819])[2]
    - *Cissia myncea* (Cramer, 1780)
    - *Cissia terrestris* (Butler, 1867)[1]
    - *Hermeuptychia atalanta* (Butler, 1867)
    - *Hermeuptychia gisella* (Hayward, 1957)
    - *Hermeuptychia hermes* (Fabricius, 1775)
    - *Magneuptychia libye* (Linnaeus, 1767)
    - *Pareuptychia* sp.
    - *Paryphthimoides sylvina* (C. Felder & R. Felder, 1867)
    - *Taygetis echo echo* (Cramer, 1775)
    - *Taygetis laches laches* (Fabricius, 1793)
    - *Taygetis rufomarginata* Staudinger, 1888
    - *Taygetis sosis* Hopffer, 1874
    - *Taygetis virgilia* (Cramer, 1776)
    - *Yphthimoides affinis* (Butler, 1867)
    - *Yphthimoides renata* (Stoll, 1780)
    - *Zischkaia saundersii* (Butler, 1867)
  - Limenitidinae (2)
    - *Adelpha cytherea aea* (C. Felder & R. Felder, 1867)
    - *Adelpha iphicleola leucates* Fruhstorfer, 1915[2]
  - Charaxinae (6)
  - Anaeini (3)
    - *Fountainea ryphea phidile* (Geyer, 1837)
    - *Hypna clytemnestra forbesi* Godman & Salvin, [1884]
    - *Zaretis strigosus* (Gmelin, 1790)
  - Preponini (3)
    - *Archaeoprepona demophon thalpius* (Hübner, [1814])
    - *Archaeoprepona demophoon antimache* (Hübner, [1819])
    - *Prepona laertes laertes* (Hübner, [1811])
  - Heliconiinae (10)
  - Argynnini (1)
    - *Euptoieta hegesia meridiana* Stichel, 1938
  - Heliconnini (9)
    - *Agraulis vanillae maculosa* (Stichel, 1908)
    - *Dione juno juno* (Cramer, 1779)
    - *Dryadula phaetusa* (Linnaeus, 1758)
    - *Dryas iulia alcionea* (Cramer, 1779)
    - *Eueides isabella dianasa* (Hübner, 1806)
    - *Heliconius erato phyllis* (Fabricius, 1775)
    - *Heliconius ethilla flavomaculatus* Weymer, 1894
    - *Heliconius melpomene nanna* Stichel, 1899
    - *Heliconius sara apseudes* (Hübner, 1806)
- **Hesperiidae (108)**
  - Eudaminae (29)
    - *Aguna megaeles megaeles* (Mabille, 1988)
    - *Astraptes alardus alardus* (Stoll, 1790)

Continuation Table 1.

    - *Astraptes anaphus anaphus* (Cramer, 1777)
    - *Astraptes fulgerator fulgerator* (Walch, 1775)
    - *Astraptes talus* (Cramer, 1777)
    - *Augiades crinisus* (Cramer, 1780)
    - *Autochton itylus* Hübner, [1823]
    - *Autochton neis* (Geyer, 1832)
    - *Autochton zarex* (Hübner, 1818)
    - *Bungalotis astylos* (Cramer, 1780)
    - *Bungalotis erythus* (Cramer, 1775)
    - *Bungalotis quadratum barba* Evans, 1952
    - *Chioides catillus catillus* (Cramer, 1779)
    - *Entheus priassus pralina* Evans, 1952
    - *Epargyreus exadeus exadeus* (Cramer, 1779)
    - *Narcosius colossus granadensis* (Möschler, 1879)
    - *Nascus phocus* (Cramer, 1777)
    - *Phanus australis* L. D. Miller, 1965
    - *Phanus marshalli* (W. F. Kirby, 1880)[2]
    - *Phocides polybius phanias* (Burmeister, 1880)[2]
    - *Pseudonascus paulliniae* (Sepp, [1849])
    - *Salatis salatis* (Stoll, 1782)
    - *Typhedanus undulatus* (Hewitson, 1867)[2]
    - *Urbanus dorantes* (Stoll, 1790)
    - *Urbanus procne* (Plötz, 1880)
    - *Urbanus proteus proteus* (Linnaeus, 1758)
    - *Urbanus teleus* (Hübner, 1821)
    - *Urbanus simplicius* (Stoll, 1790)
    - *Urbanus velinus* (Plötz, 1880)
  - Pyrginae (22)
    - *Anastrus neaeris narva* Evans, 1953
    - *Anastrus sempiternus simplicior* (Möschler, 1877)
    - *Camptopleura auxo* (Möschler, 1879)
    - *Gesta gesta* (Herrich-Schäffer, 1863)
    - *Gorgythion begga begga* (Prittwitz, 1868)
    - *Gorgythion* sp.
    - *Helias phalaenoides palpalis* (Latreille, [1824])
    - *Heliopetes alana* (Reakirt, 1868)
    - *Heliopetes arsalte* (Linnaeus, 1758)
    - *Morvina morvus morvus* (Plötz, 1884)
    - *Mylon cristata* Austin, 2000
    - *Nisoniades macarius* (Herrich-Schäffer, 1870)
    - *Pachyneuria* sp.
    - *Pyrgus orcus* (Stoll, 1780)
    - *Pyrgus veturius* Plötz, 1884
    - *Pyrrhopyge amythaon amythaon* Bell, 1931
    - *Quadrus cerialis* (Stoll, 1782)
    - *Staphylus perna* Evans, 1953
    - *Telemiades antiope tosca* Evans, 1953

Continuation Table 1.

Telemiades sp.
Timochares trifasciata trifasciata (Hewitson, 1868)
Xenophanes tryxus (Stoll, 1780)
Hesperiinae (57)
Anthoptus epictetus (Fabricius, 1793)
Argon lota (Hewitson, 1877)
Arita arita (Schaus, 1902)
Callimormus alsimo (Möschler, 1883)
Callimormus corades (C. Felder, 1862)
Callimormus corus Bell, 1941
Callimormus radiola radiola (Mabille, 1878)
Calpodes ethlius (Stoll, 1782)
Cobalopsis miaba (Schaus, 1902)
Cobalopsis nero (Herrich-Schäffer, 1869)
Cobalus virbius hanta Evans, 1955
Corticea corticea (Plötz, 1882)
Corticea graziellae Bell, 1959
Cymaenes alumna (Butler, 1877)
Cymaenes tripunctus theogenis (Capronnier, 1874)
Cynea diluta (Herrich-Schäffer, 1869)
Cynea irma (Möschler, 1879)
Decinea lucifer (Hübner, [1831])
Flaccilla aecas (Stoll, 1781)
Hesperiinae sp. 1
Hesperiinae sp. 2
Hylephila phyleus phyleus (Drury, 1773)
Justinia justinianus justinianus (Latreille, [1824])
Methionsopis ina (Plötz, 1882)
Morys compta compta (Butler, 1877)
Morys sinta Evans, 1955
Mucia zygia (Plötz, 1886)
Naevolus orius orius (Mabille, 1883)
Niconiades nikko Hayward, 1948
Nyctelius nyctelius nyctelius (Latreille, [1824])
Onophas columbaria distigma Bell, 1930
Panoquina hecebolus (Scudder, 1872)
Panoquina lucas lucas (Fabricius, 1793)
Paracarystus hypargyra (Herrich-Schäffer, 1869)
Perichares philetes adela (Hewitson, 1867)
Phanes almoda (Hewitson, 1866)
Polites vibex catilina (Plötz, 1886)
Pompeius amblyspila (Mabille, 1898)
Pompeius pompeius (Latreille, [1824])
Quinta cannae (Herrich-Schäffer, 1869)
Remella remus (Fabricius, 1798)
Saliana esperi Evans, 1955
Saliana sp. 1

Continuation Table 1.

Saliana sp. 2
Saturnus metonidia (Schaus, 1902)
Synapte malitiosa equa Evans, 1955
Talides sergestus (Cramer, 1775)[2]
Talides alternata Bell, 1941
Thracides phidon (Cramer, 1779)
Vacerra bonfilius bonfilius (Latreille, [1824])
Vacerra sp.
Virga paraiba Nicolay, 1973
Vehilius celeus vetus Mielke, 1969
Vehilius stictomenes stictomenes (Butler, 1877)
Vettius artona (Hewitson, 1868)
Vettius marcus marcus (Fabricius, 1787)
Wallengrenia premnas (Wallengren, 1860)

Discussion

1. *Species richness*

The 288 butterfly species recorded at PEDI is considerably lower than at sites in SE Brazil (800+ species, Francini et al. 2011) and the Amazon Basin (up to 1700 species in Robbins et al. 1996 and Brown 2005), but is similar in richness to other inventories for the Atlantic Forest of northeastern Brazil. Kesselring & Ebert (1982) found 291 butterfly species in the urban park "Mata do Buraquinho" in João Pessoa, Paraíba after five years of sampling. Paluch et al. (2011, 2016) recorded 197 species in a forest area in Caruaru, Pernambuco (216 sampling hours) and 260 species in Itanagra, Bahia (288 sampling hours), respectively. Cardoso (1949) recorded 218 butterfly species in the forest of Maceió, Alagoas after many years of sampling. Even though PEDI is located in an urban area of Recife that is close to a large highway, its butterfly fauna is comparable in richness to these other sites in northeastern Brazil.

The richness of Nymphalidae at PEDI was similar to that recorded in Kesselring & Ebert (1982) and in other studies in the region (Cardoso 1949, Paluch et al. 2011, 2016). With seven species recorded, the number of Ithomiini is similar to that recorded in João Pessoa, Paraíba, with eight species (Kesselring & Ebert 1982) and in Itanagra, Bahia, with seven species (Paluch et al. 2016). In contrast, 11 species of Ithomiini were recorded in Maceió, Alagoas (Cardoso 1949), 11 in Areia, Paraíba (see Kerpel et al. 2014), 14 in Caruaru, Pernambuco (Paluch et al. 2011), 17 in Usina Serra Grande, São José da Lage, Alagoas and 18 in the RPPN Frei Caneca, Jaqueira, Pernambuco (DHAM, Carlos E. B. Nobre and AVLF unpublished data), all sites in northeastern Brazil. PEDI lacks typical forest species such as *Aeria olena* ssp., *Hypoleria alema oreas* Weymer, 1899 and *Heterosais edessa* (Hewitson, [1855]), species recorded in some sites in the region (DHAM and AVLF pers, obs.). Further expeditions to the large area of "Fazenda Brejo dos Macacos" could add more species of Ithomiini associated with shaded humid forests.

The richness of Hesperiidae and Lycaenidae in PEDI was similar to that recorded by Kesselring & Ebert (1982), but higher than other localities in the region (Cardoso 1949, Paluch et al. 2011, 2016). For

Pieridae, the number of recorded species is similar to most inventories conducted in the region (Cardoso 1949, Kesselring & Ebert 1982, Paluch et al. 2011, 2016). The richness of Papilionidae is low compared to that in southern Atlantic Forest sites (see Brown Jr. & Freitas 2000), though similar to other well-sampled sites on the Atlantic Forest of northeastern Brazil (Kesselring & Ebert 1982, Paluch et al. 2011, 2016). Finally, the number of Riodinidae species was similar to those in Kesselring & Ebert (1982) and Paluch et al. (2016), but was greater than the numbers reported in Cardoso (1949) and Paluch et al. (2011).

2. *Taxonomic composition*

The relative contribution of each butterfly family in PEDI mirrors the pattern reported in the list of Brazilian butterflies and in all well sampled sites in Brazil. Hesperiidae is the richest family, followed by Nymphalidae (Brown Jr. & Freitas 1999, 2000, Francini et al. 2011). Lycaenidae and Riodinidae are equivalent in richness in PEDI, a pattern similar to that observed in most southern Atlantic Forest sites and in montane forests. However, it diverges from that observed in the lowland forests of Rio de Janeiro and Espírito Santo (Brown Jr. & Freitas 2000, Francini et al. 2011), where Riodinidae surpasses Lycaenidae. Clearly, additional surveys in other sites in the Pernambuco Center of Endemism and in the forests of Bahia are needed to better document these patterns.

In the Atlantic Forest, Nymphalidae is considered a reliable surrogate of the total butterfly fauna, generally making up 25-29% of the total butterfly fauna (Brown Jr. & Freitas 2000). Based on this indicator, the fauna of PEDI would be expected to vary between 276 and 320 species, which is consistent with the 288 recorded species.

3. *Habitat*

The fauna of PEDI is dominated by species commonly found along forest edges, in open habitats and disturbed forests. As an example, all species of Pieridae recorded in the study site are typical of secondary forests and disturbed areas with the possible exception of *Leucidia elvina* (Godart, 1819). The same is true for Hesperiidae and Papilionidae, except for *Parides zacynthus polymetus* (Godart, 1819), and the major groups of Nymphalidae. The tribe Ithomiini (Nymphalidae: Danainae) is poorly represented, as noted, with most of those in PEDI associated with open disturbed habitats and forest edges (with the exception of *Napeogenes inachia* ssp.).

4. *Biogeography*

The biogeographic composition of the PEDI fauna shows a large contribution of widely distributed species, as illustrated with the Lycaenidae, Papilionidae and Pieridae. For Lycaenidae for example, many of the species have wide geographic distributions; with 24 of 43 species (56%) occurring from Central America to Brazil. Alternately, some species are primarily Amazonian and penetrate the Atlantic Forest only in the north. Examples are *Nesiostrymon hyccara* (Hewitson, 1868), *Ostrinotes tarena* (Hewitson, 1874), and *Thereus enenia* (Hewitson, 1867). Others are endemic to the Atlantic Forest, such as *Panthiades hebraeus* (Hewitson, 1867) and *Theritas triquetra* (Hewitson, 1865). Although none of the Lycaenidae, Pieridae and Papilionidae at PEDI are endemic to the Pernambuco Center of Endemism, this is not true for Riodinidae. For example, the recently described *Eurybia gonzaga* is known only from Pernambuco (Dolibaina et al. 2014). It was also recorded in "RPPN Frei Caneca", Jaqueira (as type material, see Dolibaina et al. 2014), in "Parque Natural Municipal Professor João Vasconcelos Sobrinho", Caruaru (cited as *Eurybia pergaea* by Paluch et al. 2011), and in "Estação Ecológica do Tapacurá", São Lourenço da Mata (DHAM pers, obs.). All sites are in Pernambuco, indicating that this species is a common faunistic component of this region. The Nymphalidae also present several taxa endemic or predominantly occurring in the Pernambuco Center of Endemism, such as *Napeogenes inachia* ssp. and *Hypothyris ninonia daetina* (Danainae: Ithomiini), *Pierella hyalinus* ssp. and *Pierella kesselringi* Zacca, Siewert & Paluch, 2016 (Satyrinae: Haeterini), *Morpho helenor anakreon* Fruhstorfer, 1910 (Satyrinae: Morphini) and *Heliconius ethilla flavomaculatus* Weymer, 1894 (Heliconinae).

5. *Conservation*

No threatened butterflies are recorded in PEDI, but there is a historical record (about 50 years ago) of *Morpho menelaus eberti* Fischer, 1962 (Nymphalidae: Satyrinae: Morphini) in the "Chapéu do Sol", deposited in the National Museum of Natural History in Paris (Blandin 2007). Although habitat loss, years of logging and other urban related disturbances could explain the local extinction of *M. melenaus eberti*, this butterfly could still persist in the area in low densities. Another threatened morphine that could be present in the area is *Morpho epistrophus nikolajewna* Weber, 1951. This butterfly has been recorded to the south in Maceió (Cardoso 1949), to the north in Igarassu (Carlos E. B. Nobre pers. comm.) and in João Pessoa (Kesselring & Ebert 1982), which are Atlantic Forest fragments near urban areas. Further sampling is needed before stating that both species of *Morpho* Fabricius, 1807 are absent in forested areas around Recife.

The reported results showed that PEDI possesses a relatively rich butterfly community. Considering the degree of threat to the fauna of the Atlantic Forest of northeastern Brazil, PEDI is of enormous importance for conserving butterflies, as it is for vertebrates such as birds and mammals (Machado et al. 1998). Conservation strategies should be developed in conjunction with public entities, such as the Environment Secretariat of Recife, and non-governmental organizations such as the "Centro de Pesquisas Ambientais do Nordeste" (CEPAN), and with the local communities surrounding the whole area. Priority actions for PEDI should include the proposition of a sustainable management plan focused on reintroduction of locally extinct species of plants and animals, an effective plan for restoring heavily disturbed areas, and establishment of programs of ecological monitoring and management of the surrounding environments so that the area can persist as an important Atlantic Forest remnant.

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Author Contribution

Douglas H. A. Melo: contributed in the concept and design of the study, data collection, data analysis and interpretation, and in the manuscript preparation.

Marcelo Duarte: contributed in the data analysis and interpretation, and in the critical revision, adding intelectual content.

Olaf H. H. Mielke: contributed in the data analysis and interpretation, and in the critical revision, adding intelectual content.

Robert K. Robbins: contributed in the data analysis and interpretation, and in the critical revision, adding intelectual content.

André V. L. Freitas: contributed in the concept and design of the study, data collection, data analysis and interpretation, manuscript preparation, and in the 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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Article

Tadpole of *Pithecopus rusticus* (Bruschi, Lucas, Garcia & Recco-Pimentel, 2014) (Anura, Phyllomedusidae): description of external morphology and natural history notes of a microendemic species

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Abstract: *Pithecopus rusticus* is a newly described species, of which information on its natural history, vocalization and tadpole morphology are still lacking. Here, we describe the larval external morphology of *P. rusticus* from the type locality, in the municipality of Água Doce, state of Santa Catarina, southern Brazil, comparing it with that of other species of the genus *Pithecopus* and providing information on its natural history. Eggs from two spawns were collected and kept in the laboratory until hatching. The tadpoles of *P. rusticus* belong to the suspension-rasper guild. At Gosner stage 37, the tadpoles showed: body shape oval in dorsal view and triangular in lateral view; a tooth row formula of 2(2)/3(1); the third lower row six times shorter than others; marginal papillae uniserial, interrupted by a wide dorsal gap and with rounded tips; and a single row of alternate marginal papillae on lower lip. External morphological features were compared with those of other tadpoles of *Pithecopus*. Observations on the natural history of *P. rusticus* are also reported.

Keywords: *Taxonomy, Grasslands, Atlantic Forest.*

Girino de *Pithecopus rusticus* (Bruschi, Lucas, Garcia & Recco-Pimentel, 2014) (Anura, Phyllomedusidae): descrição da morfologia externa e notas sobre a história natural de uma espécie microendêmica

Resumo: *Pithecopus rusticus* é uma espécie recém-descrita, cujas informações sobre a história natural, vocalização e morfologia larval permanecem ausentes. Aqui, descrevemos a morfologia externa larval de *P. rusticus* da localidade-tipo, município de Água Doce, estado de Santa Catarina, sul do Brasil, comparando-a com outras espécies do gênero *Pithecopus* e fornecendo informações sobre sua história natural. Ovos de duas desovas foram coletados e mantidos no laboratório até a eclosão. Os girinos de *P. rusticus* pertencem à guilda de raspador em suspensão. No estágio 37 de Gosner, os girinos apresentam: corpo oval em vista dorsal e triangular em vista lateral; fórmula de fileira de dente de 2(2)/3(1); terceira fileira inferior seis vezes mais curta que as demais; papilas marginais unisseriadas, interrompidas em amplo espaço dorsal e com pontas arredondadas; uma única fileira de papilas marginais alternadas no lábio inferior. Características morfológicas externas foram comparadas com as de outros girinos de *Pithecopus*. Observações sobre a história natural de *P. rusticus* também são relatadas.

Palavras-chave: *Taxonomia, Campos, Mata Atlântica.*

Introduction

The neotropical amphibian genus *Pithecopus* Cope, 1866 was recently resurrected (Duellman et al. 2016) to include the species that were formerly assigned to the *Phyllomedusa hypochondrialis* group (*sensu* Faivovich et al. 2005, 2010). *Pithecopus* is distributed from tropical South America east of the Andes and southern Venezuela to northern Argentina and southern Brazil (Frost 2018). The genus *Pithecopus* is currently diagnosed by the following combination of characteristics (Duellman et al. 2016): medium-size (snout-vent length ± 45 mm); toe I much longer than and opposable to toe II; vomerine teeth absent; and tadpoles with a somewhat small anteroventral oral disc. In general, the species are associated with a wide variety of habitats, such as temporary and permanent ponds and streams in forests or open formations (Brandão 2002, Brandão et al. 2009, Pezzuti et al. 2009). The species currently recognized in the genus *Pithecopus* are *P. araguaius* Haga, Andrade, Bruschi, Recco-Pimentel & Giaretta, 2017, *P. ayeaye* Lutz, 1966, *P. azureus* (Cope, 1862), *P. centralis* (Bokermann, 1965), *P. hypochondrialis* (Daudin, 1800), *P. megacephalus* (Miranda-Ribeiro, 1926), *P. nordestinus* (Caramaschi, 2006), *P. oreades* (Brandão, 2002), *P. palliatus* (Peters, 1873), *P. rohdei* (Mertens, 1926) and *P. rusticus* (Bruschi, Lucas, Garcia & Recco-Pimentel, 2014). On the basis of molecular, chromosomal, and morphological approaches, Bruschi et al. (2014) described *P. rusticus* as a new species, and it was later included in the genus *Pithecopus* (Segalla et al. 2016), but not considered in the review by Duellman et al. (2016).

Currently, nine species of this genus have a formal description of tadpoles: *P. ayeaye* (Lutz 1966, Cruz 1982, Pezzuti et al. 2009 – as *P. itacolomi*), *P. azureus* (Cei 1980, Schulze et al. 2015), *P. centralis* (Brandão et al. 2009), *P. hypochondrialis* (Pyburn & Glidewell1971, Martínez 1990), *P. megacephalus* (Cruz 1982 as *P. centralis* according to Brandão 2002 and Eterovick & Sazima 2004), *P. nordestinus* (Cruz 1982 – as *P. hypochondrialis*, according to Caramaschi 2006), *P. oreades* (Brandão 2002), *P. palliatus* (Duellman 1978), and *P. rohdei* (Cruz 1982). Herein, we describe the external morphology of *P. rusticus* tadpoles from natural grasslands (*sensu* Klein 1978) in the highlands of the Atlantic Forest biome in southern Brazil, comparing them with those of other species of the genus *Pithecopus* and providing information on natural history.

Material and Methods

We collected two leaf nests with spawns of *P. rusticus* from a pond of the type locality, in the municipality of Água Doce, state of Santa Catarina, southern Brazil (26°35’59.9”S, 51°34’39.4”W; 1,330 m a.s.l.), on December 6, 2016. We kept the tadpoles in an aquarium with water under the spawns until the tadpoles hatched. All tadpoles hatched in two days after collection of spawns. We kept tadpoles in the aquarium with pond vegetation. Half of the aquarium water was changed every seven days. The tadpoles were fed with pond vegetation, and every seven days were provided fish and turtle feed. Some tadpoles were reared until the end of metamorphosis to confirm species identity. Tadpoles were anesthetized with 0.1% lidocaine and then fixed in 10% formalin. Voucher specimens were submitted to the Scientific Collection of Amphibians of the Universidade Comunitária da Região de Chapecó, municipality of Chapecó, state of Santa Catarina, Brazil: tadpoles (lots CAUC 001, 003-007), froglet (CAUC 2396) and metamorphosed frog (CAUC 2243-2244). The pond where we collected the tadpoles has an area of approximately 2750 m² and depth ranging from 10 cm (near the margin) to 3 m (in the center of the pond) and is located next to an unpaved road (SC-452 Highway) of a private farmland. The central and marginal portions of the pond were covered with herbaceous vegetation. The marginal vegetation is often removed to facilitate cattle access to water. The ponds were surrounded by crops, which were less than 2 m from the margins.

External morphology descriptions were based on 15 tadpoles at stage 37 (Gosner 1960) and 21 morphological measurements were taken according to Lavilla & Scrocchi (1986): maximum body width (BW), maximum body height (BH), maximum eye diameter (ED), maximum nostril diameter (ND), nostril-snout distance (NSD), eye-snout distance (ESD) and oral disc diameter (ODD); McDiarmid & Altig (1999): maximum tail height (MTH); Grosjean (2005): maximum ventral fin height (VFH), maximum dorsal fin height (DFH); Altig (2007): total length (TL), body length (BL), tail length (TAL), tail musculature width (TMW), tail musculature height (TMH), interorbital distance (IOD), and internarial distance (IND). Additional measurements were recorded for jaw sheaths, following the dimensions proposed by McDiarmid & Altig (1999): lower jaw sheath length (LJSL), lower jaw sheath height (LJSH), upper jaw sheath length (UJSL), and upper jaw sheath height (UJSH). Tadpole measurements were made using a stereomicroscope with an ocular micrometer (0.01 mm precision), except for total length and body length, which were measured with a digital caliper (0.01 mm precision) (Table 1). Comparisons with tadpoles of other species of *Pithecopus*, as well as the description of possible intrapopulation morphological variation of *P. rusticus* were provided for an additional 25 individuals (stages 27, 33, 35-36) (Gosner 1960) using the same set of measurements described above (Table 2). All tadpoles were inspected for the presence of the lateral line system (Lannoo 1999).

Results

Tadpole description. Measurements for all available development stages are given in Table 1. The tadpoles of *P. rusticus* have an elongated body (BH/BW: 1.02); body length approximately one-third of the total length (BL/TL: 0.36); body oval in dorsal view and triangular in lateral view (Figure 1); snout slightly truncated in dorsal and lateral views; nostrils oval and anterolateral, closer to tip of snout than to eyes; IND larger than the NSD (IND/NSD: 3.06); eyes lateral; IOD greater than ESD (IOD/ESD: 1.94); spiracle ventral, sinistral, short and wide, posteriorly directed, opening on middle third of body; inner wall absent; vent tube dextral, short and narrow, posteriorly directed, fused to ventral fin, with free opening; tail longer and taller than the body (TAL/TL: 0.63; MTH/BH: 1.01) curved toward ventral surface, with flagellum; tail musculature higher than wide (TMH/TMW: 1.31), well developed throughout the tail length; dorsal fin lower than ventral fin (DFH/VFH: 0.47); dorsal fin beginning on the posterior third of the body, originating anteriorly to the body-tail junction, at a median slope and extending to the tail tip; ventral fin origin anterior to vent tube extending to tail tip; the lateral line system is not visible; oral disc anteroventral, anteriorly directed, not emarginated; marginal papillae uniserial with rounded tips, wide dorsal gap present (Figure 2); single row of alternate marginal papillae on lower lip and lateral portion; submarginal papillae laterally aggregate, not forming rows; jaw sheaths darkly pigmented, edge finely

Table 1. Morphological measurements (in mm) of 40 tadpoles of *Pithecopus rusticus* from the municipality of Água Doce, state of Santa Catarina, southern Brazil. Data presented as mean + standard deviation (range). The sample size is in parentheses, below the larval stage. TL – total length, BL – body length, BW – maximum body width, BH – maximum body height, TAL – tail length, MTH – maximum tail height, TMW – tail musculature width, TMH – tail musculature height, DFH – maximum dorsal fin height, VFH – maximum ventral fin height, IOD – interorbital distance, IND – internarial distance, ESD – eye-snout distance, NSD – nostril-snout distance, ED – maximum eye diameter, ND – maximum nostril diameter, ODD – oral disc diameter, LJSL – lower jaw sheath length, LJSH – lower jaw sheath height, UJSL – upper jaw sheath length, UJSH – upper jaw sheath height.

	Stage 27 (n = 8)	**Stage 33 (n = 3)**	**Stage 35 (n = 4)**	**Stage 36 (n = 10)**	**Stage 37 (n = 15)**
TL	17.43 ± 1.00 (16.39-19.36)	29.21 ± 1.42 (27.65-31.10)	33.44 ± 0.77 (33.65-34.55)	33.50 ± 0.63 (32.27-34.35)	34.15 ± 1.49 (32.43-37.54)
BL	6.09 ± 0.56 (5.15-7.26)	10.39 ± 0.31 (9.97-10.70)	11.78 ± 0.59 (11.06-12.71)	12.24 ± 0.40 (11.53-12.94)	12.43 ± 0.53 (11.53-13.52)
BW	3.14 ± 0.28 (2.71-3.71)	5.76 ± 0.06 (5.71-5.85)	6.17 ± 0.44 (5.42-6.57)	6.77 ± 0.36 (6.28-7.28)	6.66 ± 0.52 (5.85-8.00)
BH	3.26 ± 0.28 (2.85-3.85)	6.04 ± 0.06 (6.00-6.14)	6.46 ± 0.40 (5.85-7.00)	6.82 ± 0.31 (6.28-7.28)	6.81 ±0.41 (6.00-7.71)
TAL	11.33 ± 0.75 (10.27-12.75)	18.82 ± 1.15 (17.68-20.40)	21.65 ± 0.28 (21.37-22.03)	21.26 ± 0.73 (19.59-22.12)	21.71 ± 1.11 (20.41-24.27)
MTH	3.31 ± 0.27 (3.00-3.90)	6.13 ± 0.36 (5.70-6.60)	6.80 ± 0.29 (6.30-7.00)	6.97 ± 0.41 (6.10-7.50)	6.88 ± 0.44 (6.20-7.80)
TMW	0.91 ±0.12 (0.71-1.14)	2.09 ± 0.06 (2.00-2.14)	2.53 ± 0.11 (2.42-2.71)	2.57 ± 0.12 (2.42-2.71)	2.60 ± 0.10 (2.42-2.85)
TMH	1.42 ± 0.18 (1.28-1.71)	2.71 ± 0.11 (2.57-2.85)	3.60 ± 0.11 (3.42-3.71)	3.44 ± 0.20 (3.00-3.71)	3.43 ± 0.17 (3.00-3.57)
DFH	0.83 ± 0.08 (0.71-1.00)	1.52 ± 0.06 (1.42-1.57)	1.57 ± 0.10 (1.42-1.71)	1.57 ±0.14 (1.42-1.85)	1.56 ± 0.12 (1.42-1.85)
VFH	1.53 ± 0.13 (1.42-1.85)	2.85 ± 0.11 (2.71-3.00)	3.10 ± 0.27 (2.71-3.42)	3.24 ± 0.21 (3.00-3.57)	3.30 ± 0.38 (2.85-4.28)
IOD	3.05 ± 0.28 (2.70-3.60)	5.06 ± 0.26 (4.70-5.30)	5.77 ± 0.29 (5.30-6.10)	5.91 ± 0.36 (5.30-6.50)	5.86 ± 0.36 (5.10-6.70)
IND	1.49 ± 0.12 (1.33-1.66)	2.48 ± 0.08 (2.40-2.60)	2.73 ± 0.04 (2.66-2.80)	2.83 ± 0.10 (2.73-3.00)	2.85 ± 0.09 (2.66-3.06)
ESD	1.58 ± 0.19 (1.20-1.86)	2.51 ± 0.12 (2.33-2.60)	2.76 ± 0.17 (2.60-3.06)	3.05 ± 0.22 (2.60-3.33)	3.01 ± 0.18 (2.60-3.20)
NSD	0.47 ± 0.06 (0.40-0.53)	0.66 ± 0.00 (0.66-0.66)	0.66 ± 0.04 (0.60-0.73)	0.80 ± 0.10 (0.60-1.00)	0.93 ± 0.50 (0.60-1.00)
ED	0.87 ± 0.08 (0.80-1.00)	1.44 ± 0.03 (1.40-1.46)	1.70 ± 0.05 (1.60-1.73)	1.75 ± 0.07 (1.60-1.86)	1.80 ± 0.05 (1.66-1.86)
ND	0.14 ± 0.02 (0.13-0.20)	0.33 ± 0.00 (0.33-0.33)	0.30 ± 0.03 (0.26-0.33)	0.28 ±0.03 (0.26-0.33)	0.32 ± 0.03 (0.26-0.40)
ODD	1.33 ± 0.14 (1.15-1.55)	1.93 ± 0.04 (1.90-2.00)	2.40 ± 0.11 (2.25-2.55)	2.38 ± 0.09 (2.15-2.50)	2.45 ± 0.08 (2.25-2.65)
LJSL	0.41 ± 0.07 (0.22-0.46)	0.78 ± 0.07 (0.71-0.88)	0.83 ± 0.08 (0.77-0.97)	0.86 ± 0.05 (0.77-0.95)	0.82 ± 0.07 (0.71-0.95)
LJSH	0.05 ± 0.01 (0.02-0.06)	0.09 ± 0.01 (0.08-0.11)	0.08 ± 0.00 (0.06-0.08)	0.08 ± 0.01 (0.06-0.11)	0.08 ± 0.01 (0.06-0.13)
UJSL	0.51 ± 0.08 (0.31-0.57)	1.00 ± 0.02 (0.97-1.04)	1.10 ± 0.07 (1.02-1.22)	1.12 ± 0.06 (1.02-1.22)	1.14 ± 0.05 (1.04-1.22)
UJSH	0.07 ± 0.01 (0.04-0.08)	0.13 ± 0.00 (0.13-0.13)	0.14 ± 0.02 (0.11-0.17)	0.14 ± 0.01 (0.11-0.15)	0.13 ± 0.01 (0.11-0.15)

Table 2. Comparison of external morphological characteristics between tadpoles of species from the genus *Pithecopus*.

Species	Stage	Labial tooth row formula	Ventral gap	Marginal papillae	Rows on lower lip	Submarginal papillae	Reference
Pithecopus ayeaye Lutz, 1966	31	2(2)/3	Absent	Uniserial, interrupted by a wide dorsal gap	-	Present	Lutz (1966)
	31	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap	Three	Present	Cruz (1982)
	37	2(2)/3(1)	Narrow	Uniserial, interrupted by a wide dorsal gap.	Two	Present	Pezzuti et al. (2009)
Pithecopus azureus (Cope, 1862)	38	2(2)/2(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Two	Absent	Cei (1980)
	34-38	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Two	Absent	Schulze et al. (2015)
Pithecopus centralis (Bokermann, 1965)	27-40	2(2)/3(1)	Absent	Uniserial, interrupted by a small to medium dorsal gap. Emarginated.	Two-Three	Absent	Brandão et al. (2009)
Pithecopus hypochondrialis (Daudin, 1800)	33-41	2(2)/3(1)	Present	Biserial, interrupted by a wide dorsal gap.	Two	Absent	Pyburn & Glidewell (1971)
	26-41	2(2)/3(1)	Absent	Biserial, interrupted by a wide dorsal gap.	Two	Absent	Martínez (1990)
Pithecopus megacephalus (Miranda-Ribeiro, 1926)	30	2(2)/3(1)	Narrow	Uniserial interrupted by a wide dorsal gap.	Two	Present	Cruz (1982)
Pithecopus nordestinus (Caramaschi, 2006)	37	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Single	Present	Cruz (1982)
Pithecopus oreades (Brandão, 2002)	37	2(2)/3(1)	Present	Biserial, interrupted by a wide dorsal gap.	Two	Absent	Brandão (2002)
Pithecopus palliatus (Peters, 1873)	37	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Two	Absent	Duellman (1978)
Pithecopus rohdei (Mertens, 1926)	36	2(2)/3(1)	Present	Uniserial, interrupted by a wide dorsal gap.	Two	Absent	Cruz (1982)
Pithecopus rusticus (Bruschi, Lucas, Garcia & Recco-Pimentel, 2014)	27	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Single	Absent	Present study
	37	2(2)/3(1)	Absent	Uniserial, interrupted by a wide dorsal gap.	Single	Present	

serrated; upper jaw sheath M-shaped; lower sheath V-shaped; upper jaw sheath wider than high (UJSL/UJSH: 8.76); lower jaw longer than high (LJSL/LJSH: 10.25); tooth row formula 2(2)/3(1); A1 and A2 with same length; P1 slightly longer than P2; P3 smaller than P1 and P2 (Figure 2). Body, tail and fins yellow; dorsal and lateral surface of the body, tail musculature and fin with scattered brown dots, more evident along tail musculature and ventral fin; tail tip and flagellum transparent; irregular longitudinal stripes along the middle and ventral margin of tail musculature; lateral dark brown strip, from oral disc to eye, sometimes continuing beyond eye (Figure 3A); iris spotted with golden coloration; bright metallic peritoneum, ranging from gold, orange and yellowish-green to violet. In fixed tadpoles yellow turns cream; peritoneum turns faded violet; iris turns black (Figure 1). The color pattern of the metamorphosing specimens is the same as that of the froglet and adult (Figure 3B).

Morphological variation. The length of the labial tooth row of three tadpoles at Gosner stage 37 (20% of the lot) varied with A1 > A2. A single individual at stage 37 (6.66% of the lot) exhibited P1 and P2 interrupted and another exhibited only P1 interrupted. Also, at stage 37, submarginal papillae of five tadpoles (33.33% of the lot) were absent, and in another five tadpoles (33.33% of the lot) the upper jaw sheaths were arc-shaped. The tadpoles of *P. rusticus* showed ontogenetic variation (Table 2). The vent tube of six tadpoles at stage 27 (75% of the lot) was dextral, directed downward, while in two tadpoles (25% of the lot), the vent tube was like that at stage 37, dextral and directed posteriorly. At stage 27, the dorsal fin started in the middle third of the body. The lateral line system was visible at stage 27, exhibiting two lines on the side of the body, one line over the body bordering part of the tail muscle, and another surrounding the eyes and nostrils. The labial tooth row formula of all tadpoles at stage 27 (100% of the lot) was 2(2)/3(1), with A1 longer than A2, P1 longer than P2 and P3 smaller than P1 and P2, but P3 was tiny, where it could be confused with it being absent. However, in four tadpoles (50% of the lot), P3 was absent. Submarginal papillae of all tadpoles at stage 27 (100% of the lot) were absent and upper jaw sheath was arc-shaped (Table 2).

Natural history and conservation notes. Adults of *P. rusticus* deposit eggs on individual leaves of *Senecio bonariensis* Hook & Arn. (Asteraceae), along the margins of the pond, approximately 23.5 ± 6.41 cm (15-36 cm; n = 16) above the water surface. We did not observe the tadpoles in the natural environment. In the laboratory, *P. rusticus*

Figure 1. Tadpole of *Pithecopus rusticus* at Gosner stage 36 (Lot CAUC 003) in lateral view (top) and dorsal view (down). Scale: 10 mm.

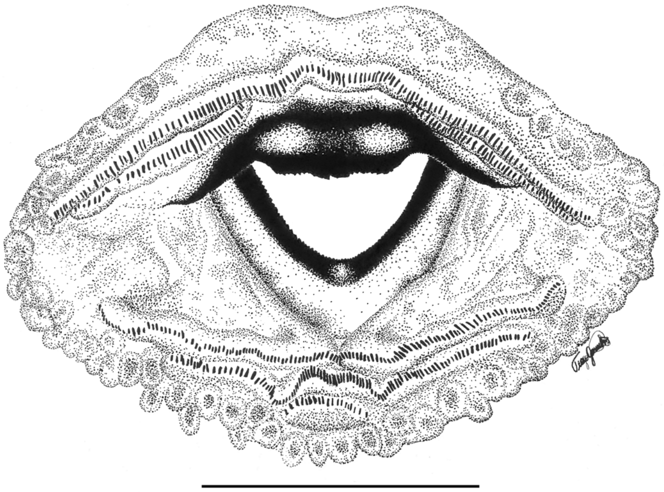

Figure 2. Oral disc from of the tadpole of *Pithecopus rusticus* at Gosner stage 37 (Lot CAUC 004). Scale: 1 mm.

tadpoles did not aggregate, and were observed at mid-water, floating with quick and short movements of their tail tips, with their bodies inclined upwards at an angle of approximately 90° with the water surface; when disturbed, they promptly fled to deeper regions. The tadpoles of *P. rusticus* belong to the suspension-rasper guild (McDiarmid & Altig 1999). Tadpoles of *Dendropsophus minutus* (Peters, 1872), *D. sanborni* (Schmidt, 1944) and *Physalaemus cuvieri* Fitzinger, 1826, and potential predators such as *Phrynops williamsi* Rhodin & Mittermaier, 1983, *Erythrolamprus miliaris* (Linnaeus, 1758) and aquatic insects (Belostomatidae and Odonata), were observed in the same pond inhabited by *P. rusticus*.

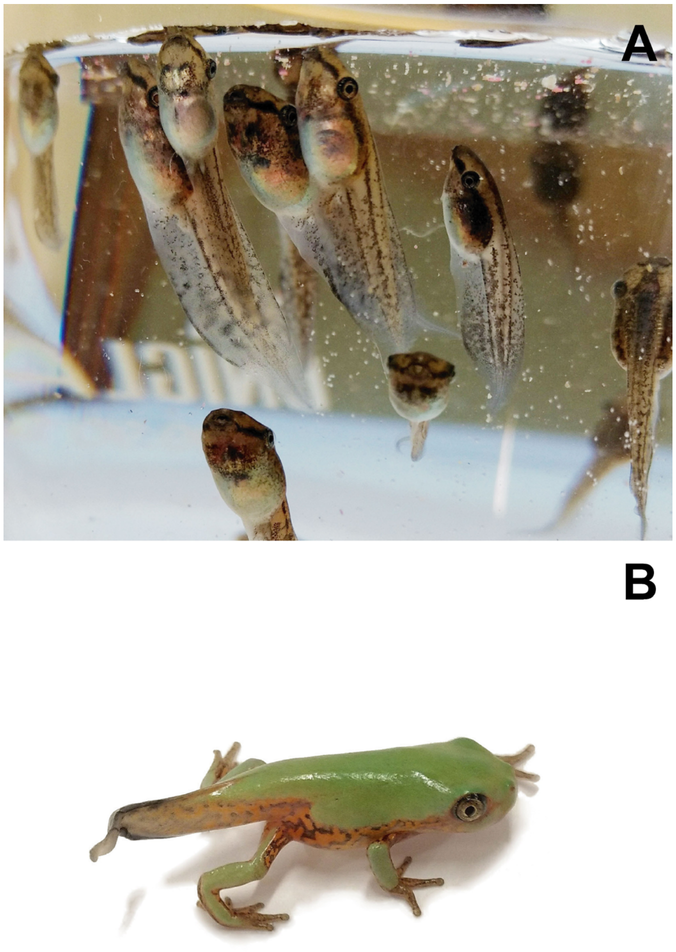

Figure 3. Tadpoles of *Pithecopus rusticus* at Gosner stage 36 in life (A, Lot CAUC 004) and froglet at Gosner stage 44 (B, CAUC 2396, SVL 16.14 mm).

Discussion

Tadpoles of *Pithecopus rusticus* are similar to those of other species of the genus, regarding most of the external morphology characters, such as the oval shape of the body in dorsal view (except *P. oreades*, in which the body is trapezoidal), anteroventral oral disc surrounded by a row of marginal papillae interrupted by a wide dorsal gap, vent tube dextral, short and attached to the ventral fin, ventral fin higher than dorsal fin, and labial tooth row formula 2(2)/3(1), with P3 smaller than P1 and P2 (Pyburn & Glidewell 1971, Duellman 1978, Cruz 1982, Brandão 2002, Brandão et al. 2009, Pezzuti et al. 2009, Schulze et al. 2015). Tadpoles of *P. rusticus* can be diagnosed from those of the remaining species of the genus by the following combination of oral disc characteristics: uniserial marginal papillae being alternate on lower lip and lateral portion of oral disc and interrupted by a wide dorsal gap, oral disc not emarginate, submarginal papillae laterally aggregate in the oral disc, not forming rows, upper jaw sheath M-shaped and lower jaw sheath V-shaped. *Pithecopus ayeaye* has two rows of marginal papillae on the lower lip and lateral portion, with a narrow ventral gap (Lutz 1966, Cruz 1982, Pezzuti et al. 2009); *P. azureus* has uniserial to irregularly aligned biserial rows on lower lip and no submarginal papillae (Cei 1980, Schulze et al. 2015); *P. centralis* has an emarginate oral disc, a single row of marginal papillae on each side of the rostral gap, extending into two and three rows of papillae, with numerous papillae on the lateral margins and lower lip and no submarginal papillae (Brandão et al. 2009); *P. hypochondrialis* has two rows of marginal papillae and no submarginal papillae (Pyburn & Glidewell 1971, Martínez 1990); *P. megacephalus* has two rows of marginal papillae on the lower lip and lateral portion, with a narrow ventral gap (Cruz 1982); *P. nordestinus* differs only by non-alternate marginal papillae (Cruz 1982); *P. oreades* has two rows of papillae on posterior margin, one row of small papillae on the anterior margin, small and scattered lateral papillae in the transverse mouth midline and no submarginal papillae (Brandão 2002); *P. palliatus* has two rows of marginal papillae ventrally and no submarginal papillae (Duellman 1978); *P. rohdei* has two rows of marginal papillae on the lower lip and lateral portion and no submarginal papillae (Cruz 1982); *P. ayeaye* and *P. centralis* are the only species in the genus that have the upper jaw sheath M-shaped, while the others have it arc-shaped; *P. azureus* is the only species with the lower jaw sheath U-shaped, while the others have it V-shaped. Giaretta et al. (2007) pointed out that the narrow ventral gap characteristic could vary ontogenetically in *P. ayeaye* tadpoles. Accordingly, morphological variation in larvae of *P. rusticus* (size of third lower row and presence/absence of submarginal papillae) was related to total length of the body.

The species phylogenetically closest to *P. rusticus* are *P. ayeaye*, *P. centralis* and *P. oreades* (Bruschi et al. 2014). In addition to differences in oral disc (see above), *P. rusticus* tadpole differs from these species by nostrils positioned anterolaterally (dorsolaterally positioned in *P. ayeaye*, *P. centralis* and *P. oreades*), eyes positioned laterally in *P. rusticus* and *P. ayeaye* (dorsolaterally positioned in *P. centralis* and *P. oreades*),and spiracle ventral with free opening, located on the left side in *P. rusticus* and *P. ayeaye* (spiracle positioned over the horizontal midline in *P. centralis* and *P. oreades*). Yet, the M-shaped upper jaw sheath is similar to those of *Pithecopus* species, except *P. oreades*, in which it is arc-shaped (Lutz 1966, Cruz 1982, Brandão 2002, Brandão et al. 2009, Pezzuti et al. 2009). The tadpole of *P. rusticus* is morphologically closer to that of *P. ayeaye* than *P. centralis* and *P. oreades*, and the same pattern was shown by Bruschi et al. (2014) for adults.

Pithecopus rusticus lays eggs attached to vegetation above the water surface of ponds. Each leaf nest is composed of just one leaf, and eggs hatch into tadpoles, which drop into the lentic water (reproductive mode 24 of Haddad & Prado 2005). This reproductive mode is also reported to *P. azureus*, *P. hypochondrialis*, *P. megacephalus*, *P. nordestinus*, *P. palliatus* and *P. rohdei* (Pyburn & Glidewell 1971, Duellman 1978, Cruz 1982, Martínez 1990, Schulze et al. 2015). However, it differs in *P. ayeaye*, *P. centralis* and *P. oreades* (Lutz 1966, Cruz 1982, Brandão 2002, Brandão et al. 2009, Pezzuti et al. 2009), which deposit eggs above streams in nests composed of just one folded leaf and whose larvae complete their development in creek pools and backwaters (reproductive mode 25, Haddad & Prado 2005). Tadpoles of all *Pithecopus* species show a characteristic position, nearly vertical, with the mouth toward the surface, with small movements of their tail tips (Lutz 1966, Pyburn & Glidewell1971, Duellman 1978, Cruz 1982, Martínez 1990, Brandão 2002, Brandão et al. 2009, Pezzuti et al. 2009, Schulze et al. 2015).

In summary, tadpoles of *P. rusticus* are similar to those of other species of the genus *Pithecopus*, especially *P. ayeaye*. Interestingly, *P. rusticus* has a distribution that is isolated from all other species of the genus, where it is known to occur at present only at its type locality with a restricted distribution in the high plateaus of western state of Santa Catarina, in the region known as Campos de Palmas (i.e. natural grassland landscape), in southern Brazil. The inclusion of *P. rusticus* at the species list of amphibians in grasslands of Paraná state (Crivellari et al. 2014) was based on the record of this species at the type locality (i.e. Água Doce, state of Santa Catarina, Brazil; Crivellari in personal communication), which is placed in the surrounds of Palmas Wildlife Refuge. Sampling efforts related to anuran surveys carried out by unrelated herpetologist teams at the region during approximately the last ten years failed to detect this species in additional localities (EML unpublished data and personal communication from L. Pedroso and L. Crivellari). In addition, this region has suffered intense anthropic changes due to the transformation of large grassland areas into monoculture of *Pinus* sp. and accelerated expansion of agriculture, especially soybeans and potatoes (Overbeck et al. 2009, Pillar & Vélez 2010). Unfortunately, the breeding habitat of *P. rusticus* is now under high anthropogenic pressure, since exotic fishes were introduced to the pond and because a dam construction project has been stated in the pond surroundings (involving removal of soil and vegetation in the whole area). Thus, according to current knowledge, *P. rusticus* can be considered a microendemic and possibly endangered species due to its extremely restricted distribution and the continuous destruction of its habitat. Our results are important to clarify the systematics and the knowledge about the taxonomy and life history of the genus *Pithecopus*. Since the larvae of all species of *Pithecopus* are known, a comprehensive phylogenetic analysis would be welcome to help us understand the evolutionary relationships and systematics of larval characters in the genus.

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Authors' Contributions

Veluma Ialú Molinari De Bastiani: 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.

Joana Priscilla Boschetti: 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.

Tiago Gomes dos Santos: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Elaine Maria Lucas: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of interest

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

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Morphological diversity of springtails (Hexapoda: Collembola) as soil quality bioindicators in land use systems

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Abstract: The aim of this work was to evaluate the soil quality of native forest, eucalyptus plantations, pasture, integrated crop-livestock, and no-tillage systems, correlating the morphological diversity of springtails with physical and chemical soil properties. Springtail samples were captured from soils of the southern plateau of the State of Santa Catarina in Brazil, during winter and summer, by using Pitfall traps, using a 3 × 3 point grid. The morphotyping of springtails consisted of the observation of five traits and for each one a partial value of the eco-morphological index was assigned to obtain the modified Soil Quality Index. A correlation of the morphotype abundance and diversity with physical (soil moisture, bulk density, biopores, microporosity, and macroporosity) and chemical (pH in water, calcium/magnesium ratio and total organic carbon content) soil properties was studied, describing all results by variance and multivariate analyses. The springtail abundance and diversity were influenced by the different land use systems. Higher soil quality index was determined in native forest followed by eucalyptus plantations, pasture, no-tillage system and integrated crop-livestock, in the winter. Moreover, higher soil quality index was found in native forest followed by integrated crop-livestock, eucalyptus plantations, no-tillage system and pasture, in the summer. Therefore, the quality index of a soil can be evaluated by the springtail morphological traits in correlation with the physical and chemical properties such as calcium/magnesium ratio, total organic carbon contents, biopores, macroporosity, microporosity, soil moisture, bulky density and pH.
Keywords: *Biodiversity; Morphotype; Soil Mesofauna; Soil Quality Index.*

Diversidade morfológica de colêmbolos (Hexapoda: Collembola) como bioindicadora de qualidade do solo em sistemas de uso

Resumo: O objetivo deste trabalho foi avaliar a qualidade do solo de florestas nativas, reflorestamento de eucalipto, pastagem, sistemas de integração lavoura-pecuária e plantio direto, correlacionando a diversidade morfológica de colêmbolos com propriedades físicas e químicas do solo. Amostras de colêmbolos foram coletadas de solos do planalto sul do Estado de Santa Catarina no Brasil, durante o inverno e o verão, usando armadilhas de queda, em grade amostral de 3 × 3. A morfotipagem dos colêmbolos consistiu na observação de cinco características e para cada uma foi atribuído um valor parcial do índice eco-morfológico para a obtenção do Índice de Qualidade do Solo modificado. As avaliações foram da abundância e diversidade de morfotipos e de atributos físicos (umidade do solo, densidade do solo, bioporos, microporosidade e macroporosidade) e químicos do solo (pH em água, relação cálcio/magnésio e carbono orgânico total), descrevendo os resultados por análises de variância e multivariada. A abundância e a diversidade de colêmbolos foram influenciadas pelos diferentes sistemas de uso do solo. Maior índice de qualidade do solo foi determinado em floresta nativa seguida de reflorestamento de eucalipto, pastagem, plantio direto e integração lavoura-pecuária, no inverno. Além disso, encontrou-se maior índice de qualidade do solo em floresta nativa seguida de integração lavoura-pecuária, reflorestamento de eucalipto, plantio direto e pastagem, no verão. Portanto, o índice de qualidade do solo pode ser avaliado pelas características morfológicas de colêmbolos em correlação com as propriedades físicas e químicas, como razão cálcio/magnésio, teores de carbono orgânico total, bioporos, macroporosidade, microporosidade, umidade do solo, densidade do solo e pH.
Palavras-chave: *Biodiversidade; Morfotipos; Mesofauna do Solo; Índice de Qualidade do Solo.*

Introduction

The different land use systems alter the edaphic organism natural habitats, decreasing their abundance and biodiversity (Huerta & van der Wal 2012, Rosa et al. 2015, Keesstra et al. 2016). The edaphic organism communities in soils are important for the ecosystem due to their ecological functions and relations with plants, soils, animals, and water (Bardgett & Van Der Putten 2014). Edaphic organisms are also very important for understanding the dynamic of certain crop production systems, in addition, to be used as soil environmental quality natural bioindicators (Lavelle et al. 2006, Lima et al. 2013, Baretta et al. 2014).

Springtails (Hexapoda: Collembola) are an important and abundant soil microarthropod as interacts with ecosystem physiochemical processes, decomposing and mineralizing significant organic matter amount by the litterfall fragmentation and fecal production (Madej et al. 2011, Ruggiero et al. 2015). Moreover, the presence of springtails in soil is used for predicting and controlling primary decomposer actions such as bacteria and fungi and secondary decomposer actions such as nematodes and protozoa (Filser 2002, Petersen 2002, Jeffery et al. 2010, Paul et al. 2011, Chang et al. 2013). Springtails are frequently abundant and sensitive to changes in the physical and chemical soil properties and natural micro-habitat characteristics after agricultural and forestry activities. This sensitivity can be used for indicating pollution levels in different land use systems (Eaton et al. 2004, Larsen et al. 2004, Sousa et al. 2006, Baretta et al. 2008, Rieff et al. 2014). In this sense, different springtails communities have been widely used as soil environmental quality bioindicators in studies of environmental pollution related with the land use systems (Parisi et al. 2005, Ponge et al. 2006, Oliveira Filho et al. 2016, Winck et al. 2017, Santos et al. 2018). The springtails communities are divided in edaphic, hemiedaphic and epigeic organisms. Edaphic and epigeic organisms are generally found in soils that contain litterfall and deeper layer, respectively, whereas hemiedaphic organisms are generally found in both surface and deeper layers as an intermediate vertical distribution (Gisin 1943, Petersen 2002, Querner et al. 2013, Oliveira Filho & Baretta 2016). This vertical stratification is important to predict the soil environmental quality level since the decrease of litterfall affects much more the epigeic organisms than edaphic organisms.

Different springtails morphotypes in a region can be correlated with the physical and chemical soil properties and different land use systems. Moreover, it can be used to predict the relations between springtails and ecosystem (Oliveira Filho et al. 2016). The morphological classification of different springtails species in a region can be used for fast evaluation of this biodiversity compared to classification by the traditional taxonomy method. Moreover, initial springtails biodiversity evaluation by morphotype classification covers much more soils and areas than the traditional taxonomy method (Reis et al. 2016). For example, a sustainable land use system in Italy has been defined by the presence of different microarthropod groups in soil, using the organism biodiversity and speciation indexes as parameters for predicting the soil environmental quality (Parisi 2001, 2005, Gardi et al. 2002, Menta et al. 2018a, 2018b). The greater the soil environmental quality, higher the number of microarthropods adapted to this soil, indicating lower environmental pollution indexes. The different springtails and Coleoptera morphotype classifications in soils have been used as environmental tools for studying sustainable land use systems (Vandewalle et al. 2010, Oliveira Filho et al. 2016, Reis et al. 2016, Silva et al. 2016, Pompeo et al. 2017, Santos et al. 2018). In this case, the soil use was correlated with the organism morphotypes and soil biological quality index (*Qualità Biologica del Suelo* - QBS).

The aim of this work was to study the springtails community structure in different land use systems by measuring the abundance, morphological diversity and QBS index, correlating these informations with physical and chemical soil properties. The correlation between springtails morphotype diversity and physical and chemical properties explained the soil vertical stratification and its environmental quality. Evaluating the environmental quality of soils by using morphological classifications of edaphic, hemiedaphic and epigeic organism groups is not common around the world. Therefore, this new methodology could be an effective tool for defining sustainable land intense use systems by determining the significant correlations between springtails morphological diversity and physical and chemical soil properties in order to avoid the environmental pollution.

Material and Methods

1. *Description of the study site*

The experimental studies were accomplished in the southern plateau of the state of Santa Catarina in Brazil, including the counties of Campo Belo do Sul and Lages with Nitisol (*Nitossolo Bruno*) and Otacílio Costa with Humic Cambisol (*Cambissolo Húmico*) (WRB 2014; Brazilian classification: Embrapa, 2013). These regions were chosen due to the geographical characteristics of tepid climates with temperatures ranging from 18.0 to 22.0 ± 1.0 ºC according to the Köppen climate classification, Cfb (Alvares et al. 2013). Five land use systems (LUS) were chosen in each region with the aim of establishing the anthropological action gradients in native forest (NF), eucalyptus plantations (EP), pasture (PA), integrated crop-livestock (ICL), and no-tillage (NT) systems. Environmental and soil management characteristics in the sampling regions in each county of the southern plateau of the state of Santa Catarina are displayed in Table 1.

2. *Soil samples*

Soil samples were collected in the months of July (winter) and January (summer). Samples were collected using a 3 × 3 point grid, with sampling points at a distance of 30 m from each other and surrounded by a 20-m border, covering 1 ha for each LUS (Bartz et al. 2014b, Rosa et al. 2015). Five soil samples were collected in each sampling point at depths of 0 to 20 cm due to the high amount of springtails in this stratification, totaling 150 samples.

3. *Springtails samples*

The springtails samples were collected by using 500 mL pitfall traps with 8 cm cylindrical diameters. These traps containing 200 mL of 3:1 detergent aqueous solution were put on the soil surface for 72 h (Bartz et al. 2014a). Next, the traps were removed from the soil and the springtails samples separated for fixation in 99.5% ethanol. Specimens of this survey are housed at the Collection of the Centro de Educação Superior do Oeste, Santa Catarina State University.

Table 1. Environmental and soil management characteristics in the sampling regions in each county of the southern plateau of the state of Santa Catarina.

Attributes	Native Forest (NF)	Eucalyptus plantations (EP)	Pasture (PA)	Integrated crop-livestock (ICL)	No-Tillage (NT)
			County of Campo Belo do Sul		
Altitude (m)	1016	989	1004	947	923
UTM (J22 zone)	533895.8	534356.2195	534167.5	534356.22	534167.46
	6915778.881	6915002.151	6917279.118	6915002.2	6917279.1
Size (ha)	5	1	6.9	4	1.1
Age (years)	-	20	12	25	11
Vegetation and Management	Mixed ombrophilous forest. Secondary forest. Entrance of people and cattle.	Native grassland previously. Entrance of people and cattle.	Native grass. Controled burning every two years	No-tillage with crop rotation (soybean, corn, wheat, oat). Use of herbicides, insecticides, and fungicides.	No-tillage (soybean, corn, wheat and fallow). Use of herbicides, insecticides, and fungicides.
			County of Lages		
Altitude (m)	895	852	858	873	883
UTM (J22 zone)	539823.7	539289.7715	539402.3	539289.77	539402.33
	6924955.115	6925346.417	6925122.656	6925346.4	6925122.7
Size (ha)	3.5	2.8	7.6	14	4
Age (years)	-	7	15	10	7
Vegetation and Management	Mixed ombrophilous forest. Secondary forest. Entrance of cattle.	Entrance of cattle.	Native grass	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides (physiological), and fungicides.	No-tillage (soybean, corn, wheat). Use of herbicides, insecticides (physiological), and fungicides.
			County of Otacílio Costa		
Altitude (m)	919	855	900	902	879
UTM (J22 zone)	613615.7	603859.8085	612712.1	603859.81	612712.11
	6947179.474	6951380.279	6944460.548	6951380.3	6944460.5
Size (ha)	3	1.5	6.3	11.3	5.5
Age (years)	-	21	-	12	10
Vegetation and Management	Mixed ombrophilous forest. Secondary forest. Entrance of cattle.	-	Native grass. Annual controlled burning.	No-tillage with crop rotation (soybean, corn, oat, ryegrass). Use of herbicides, insecticides, and fungicides.	No-tillage (soybean, corn, oat, ryegrass). Use of herbicides, insecticides, and fungicides.

4. *Morphological characterization*

The collected springtails samples were counted and distributed according to their morphotypes by using a stereoscopic microscopic with magnifications from 0 to 50 times. Each springtails morphotype was identified by determining the eco-morphological index as described by Parisi (2001, 2005). The springtails adaptations and codifications were studied according to the methodologies described by Vandewalle et al. (2010), Parisi (2001, 2005), Oliveira Filho et al. (2016), Reis et al. (2016), Silva et al. (2016) and Santos et al. (2018). In this case, five traits were evaluated for each springtail including antennal length, size of furca, presence of ocelli (the authors mentioned above used the presence or absence, regardless of the number, which makes the evaluation faster), pigmentation, and presence of hairs and/or scales along the body (considered only macro hairs/scales). Each trait was identified by the eco-morphological index (EMI) value as displayed in Table 2. The EMI final values varied from 0 to 20 which were used for describing the springtails morphotypes (Table 3). The EMI value is important to separate the springtails samples according to adaptation degree in soil. In this case, the higher the EMI values the lower the capacities of morphotype dispersions, facilitating their adaptation on the soil profile. The springtails morphotypes were classified as edaphic, hemiedaphic and epigeic depending on the total EMI values. Total EMI values ranging from 14 to 20 indicated the presence of edaphic organisms due to lower dispersion and better adaptation in soil. For hemiedaphic organisms, the total EMI values ranged from 8 to 12 and for epigeic organisms, the total EMI values ranged from 0 to 6.

Table 2. Springtails morphological characteristics and eco-morphological index (EMI) values

Characteristics (trait)	Codification	EMI
Ocelli	Present	0
	Absent	4
Antenna length	Antenna length > body length	0
	Antenna length > 0.5x body length	2
	Antenna length < 0.5x body length	4
Furca	Present (fully developed)	0
	Present but reduced/short	2
	Absent	4
Hairs and/or Scales	Present	0
	Absent	4
Pigmentation	Colored and with patterns	0
	Colored but not uniformly pigmented	2
	Absent (white color or transparent)	4

5. *Soil biological quality index*

In the present study, an adaptation was used to calculate the QBS index (QBS-adapt; Parisi 2001, 2005), because it is a specific study for the Collembola group. The QBS-adapt tries to cover all the morphotypes found in an area and does not consider only the edaphic ones (higher EMI). In QBS-adapt, the abundance of springtails of a determined morphotype is multiplied by its respective final value of EMI, after which the sum of this multiplication is added to all the morphotypes found in a given area. In this way, it is possible to obtain more reliable information related with the adaptations of springtails in the environment and on the conditions of soil quality, since all the individuals that occur in a place are considered.

6. *Soil properties*

The physical and chemical properties collected from NF, EP, PA, ICL, and NT were determined by using fifteen samples of different points of each soil (Table 4). All fifteen samples from each soil were homogenized with the aim of obtaining a representative mixed sample for the determination of pH in water, available Ca^{2+} and Mg^{2+} and total organic carbon contents according to methodologies described by Tedesco et al. (1995). Soil moisture and bulk density, biopores, microporosity, and macroporosity were determined by using samples with preserved structures collected in a 5.0 cm diameter and 5.0 cm height steel cylinder according to procedures described by Embrapa (2011).

7. *Statistical analyses*

The abundance, richness, and Margalef Index were calculated by using software PAST 2.17c (Hammer et al. 2001). Data (without outliers) were transformed ($\sqrt{x+1}$) to comply with the requirements of analysis of variance (ANOVA; normal distribution and homoscedasticity of error variances). The normality of data was checked with Kolmogorov–Smirnov's and homogeneity of variances was tested using Bartlett's test. Analysis of variance was calculated by using software Statistic 7.0 (StatSoft 2004) with the aim of comparing the organism abundances in traps, total abundances, and land use system indexes. All analyses were accomplished for soil samples collected from NF, EP, PA, ICL, and NT considering as reliable replicates those calculated by multiplying triplicate samples by number of municipalities. All results were presented as a mean ± standard deviation, comparing these media by using Newman Keuls test ($p < 0.05$) (Keuls 1952). Means presented in the text and figures were calculated using non-transformed data (± standard deviation). Similarity percentages (SIMPER) analyses were run to determine dissimilarity between treatments and morphotypes contributions to the observed collembolan assemblage structure under the different LUS, using PRIMER version 5.2.6 (Clarke, 1993).

Morphotype abundances and physical and chemical soil properties were also determined by principal component analysis (PCA) by using software Canoco 4.0 (Ter Braak & Šmilauer 2002). Morphotype abundances were studied as response variables and physical and chemical soil properties as explanatory environmental variables. Forward selection was applied for successive redundant analyses by using Monte-Carlo test ($p < 0.002$), removing information that has presented collinearity and using variables that explained the variations of data ($p \leq 0.05$). Therefore, the significant explanatory environmental variables from redundant tests were used for plotting the PCA graphics with the aim of explaining all changes observed for the springtails morphotypes.

Results

1. *Abundance, richness, and diversity of springtails in the land use systems*

A total of 21,151 individuals were collected and morphotyped, of which 12,259 individuals were collected in the summer and 8,892 individuals in the winter. The abundances of springtails in the summer were higher in the NF followed by EP, NT, PA, and ICL (Table 5). Moreover, these abundances in the winter were higher in the NF followed by ICL, NT, EP, and PA (Table 5). Forty-five springtails morphotypes were determined (Table 3) including E8 with 7,997 individuals, H4 with 6,396 individuals and E3 with 2,951 individuals.

The mean richness was not significantly different among the land use systems during the winter, however was significantly different during the summer. There were not common standards for the biodiversity in the different land use systems. The diversity index in the winter was not significantly different by using Margalef test.

The diversity index based on the Margalef test during the summer was significantly different for soils from areas of PA was 1.70 compared to the richness of morphotypes in NF. The presence of certain morphotypes (E3, E8, E20, H4, H16, H24, H25, H37, H46, H50), indicated by SIMPER analysis, showed that were responsible for 50.5% of the dissimilarity between NF and PA; 50.7% between EP and PA; 42.1% between PA and ICL; 42.9% PA and NT; 48.6% between NF and EP; 46.3% between NF and ICL; 47.1% between EP and ICL; 45.5% between NF and NT; 45.9% between EP and NT; 30.2% between ICL and NT. Thus, these morphotypes may come to be considered as the most important in these LUS.

Table 3. Standard morphological classification of collembola

Partial eco-morphological index values					EMI Final	Eco-morphological groups	Sampled morphotypes
Ocelli	Antenna length	Furca	Hairs/scales	Pigmentation			
0-4	0-2-4	0-2-4	0-4	0-2-4			
4	4	4	4	4	20	Edaphic	E1
4	4	4	4	2	18	Edaphic	E2
4	4	2	4	4	18	Edaphic	E3
4	2	4	4	4	18	Edaphic	E4
4	4	4	0	4	16	Edaphic	E6
4	4	2	4	2	16	Edaphic	E7
4	4	0	4	4	16	Edaphic	E8
0	4	4	4	4	16	Edaphic	E12
4	4	2	0	4	14	Edaphic	E15
4	4	0	4	2	14	Edaphic	E16
4	2	4	0	4	14	Edaphic	E18
4	2	2	4	2	14	Edaphic	E19
4	2	0	4	4	14	Edaphic	E20
0	4	4	4	2	14	Edaphic	E23
0	4	2	4	4	14	Edaphic	E24
0	2	4	4	4	14	Edaphic	E25
4	4	2	0	2	12	Hemiedaphic	H2
4	4	0	4	0	12	Hemiedaphic	H3
4	4	0	0	4	12	Hemiedaphic	H4
4	2	0	4	2	12	Hemiedaphic	H8
4	0	0	4	4	12	Hemiedaphic	H12
0	4	4	0	4	12	Hemiedaphic	H14
0	4	2	4	2	12	Hemiedaphic	H15
0	4	0	4	4	12	Hemiedaphic	H16
0	2	2	4	4	12	Hemiedaphic	H18
4	4	0	0	2	10	Hemiedaphic	H21
4	2	0	0	4	10	Hemiedaphic	H25
4	0	0	4	2	10	Hemiedaphic	H29
0	4	2	0	4	10	Hemiedaphic	H32
0	4	0	4	2	10	Hemiedaphic	H33
0	2	2	4	2	10	Hemiedaphic	H36
0	2	0	4	4	10	Hemiedaphic	H37
4	2	0	0	2	8	Hemiedaphic	H42
4	0	0	0	4	8	Hemiedaphic	H46
0	4	2	0	2	8	Hemiedaphic	H48
0	4	0	4	0	8	Hemiedaphic	H49
0	4	0	0	4	8	Hemiedaphic	H50
0	2	4	0	2	8	Hemiedaphic	H51
0	2	0	4	2	8	Hemiedaphic	H54
0	0	0	4	4	8	Hemiedaphic	H58
0	4	0	0	2	6	Epigeic	EP5
0	2	0	0	4	6	Epigeic	EP9
0	0	0	4	2	6	Epigeic	EP13
0	2	0	0	2	4	Epigeic	EP17
0	0	0	0	4	4	Epigeic	EP21

Table 4. Soil properties in native forest (NF), eucalyptus plantations (EP), pasture (PA), integrated crop-livestock (ICL), and no-tillage (NT) systems in the southern plateau of the state of Santa Catarina during winter and summer.

Soil properties		**NF**	**EP**	**PA**	**ICL**	**NT**
			Winter			
TOC	(g kg^{-1})	57.2	33.7	40.8	36.6	35.8
pH in water		4.4	4.7	4.8	5.4	5.6
Calcium (Ca)	(cmol$_c$ dm^{-3})	4.3	2.3	2.2	7.4	7.6
Magnesium (Mg)	(cmol$_c$ dm^{-3})	1.5	2.1	1.4	3.8	3.9
Ca/Mg ratio		2.6	1.2	1.5	2.4	2.0
Soil moisture	(%)	56.7	40.8	49.8	39.0	37.8
Bulk density	(g cm^{-3})	0.89	0.99	0.95	1.01	1.01
Macroporosity	(m^3 m^{-3})	0.08	0.14	0.17	0.19	0.20
Microporosity	(m^3 m^{-3})	0.56	0.51	0.49	0.46	0.44
Biopores	(m^3 m^{-3})	0.03	0.02	0.02	0.01	0.01
			Summer			
TOC	(g kg^{-1})	69.0	40.9	48.6	39.9	39.7
pH in water		4.4	4.7	4.8	5.4	5.6
Calcium (Ca)	(cmol$_c$ dm^{-3})	4.3	2.3	2.2	7.4	7.6
Magnesium (Mg)	(cmol$_c$ dm^{-3})	1.5	2.1	1.4	3.8	3.9
Ca/Mg ratio		2.6	1.2	1.5	2.4	2.0
Soil moisture	(%)	52.8	35.2	40.0	32.5	35.8
Bulk density	(g cm^{-3})	0.89	0.99	0.95	1.01	1.01
Macroporosity	(m^3 m^3)	0.08	0.14	0.17	0.19	0.20
Microporosity	(m^3 m^3)	0.56	0.51	0.49	0.46	0.44
Biopores	(m^3 m^3)	0.03	0.02	0.02	0.01	0.01

TOC = Total organic carbon; Calcium/Magnesium ratio in soils.

Table 5. Abundance, richness, diversity of springtails morphotypes (mean ± standard deviation), and soil biological quality-adapt (QBS-adapt) in native forest (NF), eucalyptus plantations (EP), pasture (PA), integrated crop-livestock (ICL), and no-tillage (NT) systems of the southern plateau of the state of Santa Catarina (n = 15).

LUS	**Abundance (Organism trap^{-1})**	**Richness (Number of morphotypes)**	**Margalef Index**	**QBS-adapt**
		Winter		
NF	235a (±53)	10^{ns} (±1.1)	1.4^{ns} (±0.8)	53,242
EP	145ab (±40)	8 (±0.7)	1.54 (±0.5)	33,294
PA	81b (±20)	9 (±1.2)	1.96 (±0.8)	19,320
ICL	46b (±8)	9 (±1.0)	2.10 (±0.8)	9,524
NT	86b (±24)	8 (±0.9)	1.85 (±0.6)	17,476
		Summer		
NF	307a (±82)	8a (±0.8)	1.22b (±0.1)	66,442
EP	107b (±38)	6ab (±07)	1.23b (±0.1)	25,316
PA	72b (±25)	7a (±0.7)	1.70a (±0.2)	15,690
ICL	207ab (±58)	5bc (±0.5)	0.97bc (±0.2)	40,550
NT	125b (±48)	4c (±0.5)	0.67c (±0.1)	23,400

^{ns} without significant difference.

2. *Analysis of springtails community composition*

The springtails morphotype abundances were applied as response variables and physical and chemical soil properties as explanatory environmental variables. Based on multivariate analysis, different land use systems were identified with NF different of PA, EP, NT, and ICL. The morphotype abundances and diversities were mainly related with the physical and chemical soil properties, changing with the alterations of porosity, soil moisture, acidity and fertility of the soils from the land use systems.

The variability of the experimental data was explained by the first (21.0%) and second (16.9%) principal component during the winter (Figure 1A). Higher morphotype abundances and distributions were observed in NF compared to different eco-morphological groups. Moreover, significant correlations of hemiedaphic morphotypes were observed in the ICL and NT. A correlation among the calcium/magnesium ratio, total organic carbon, biopores, microporosity, and soil moisture contributed for the explanation of the morphotype abundances in NF whereas macroporosity explained the association among morphotypes in ICL and NT. The microporosity was used to explain the morphotype abundances in PA and EP.

The variability of the experimental data was explained by the first (22.1%) and second (17.7%) principal component during the summer (Figure 1B). Similar results were obtained for the abundances, distributions, and adaptations of morphotypes in NF during winter and summer. Higher abundances of epigeic organisms were determined in NT. However, significant correlations between hemiedaphic organisms were similarly observed in ICL during the summer. Important correlations were also observed during the summer between springtails and physical and chemical soil properties in PA and EP. Total carbon content and microporosity contributed for the explanation of the morphotype abundances in NF whereas pH, biopores, and bulk density explained the morphotype association among ICL and NT.

3. *Soil biological quality index*

The soil biological quality-adapt (QBS-adapt) index is used to define the quality of a specific soil based on the microarthropod abundance and sensibility (Parisi et al. 2005). Higher QBS-adapt index was determined in the winter for soils with less anthropological actions. The higher the QBS-adapt index the more complex is the land use system. High QBS-adapt index was determined in soils from NF at both seasons due mainly to the increase of the organic matter contents on the soil surface which are food sources for different springtails morphotypes (Table 5).

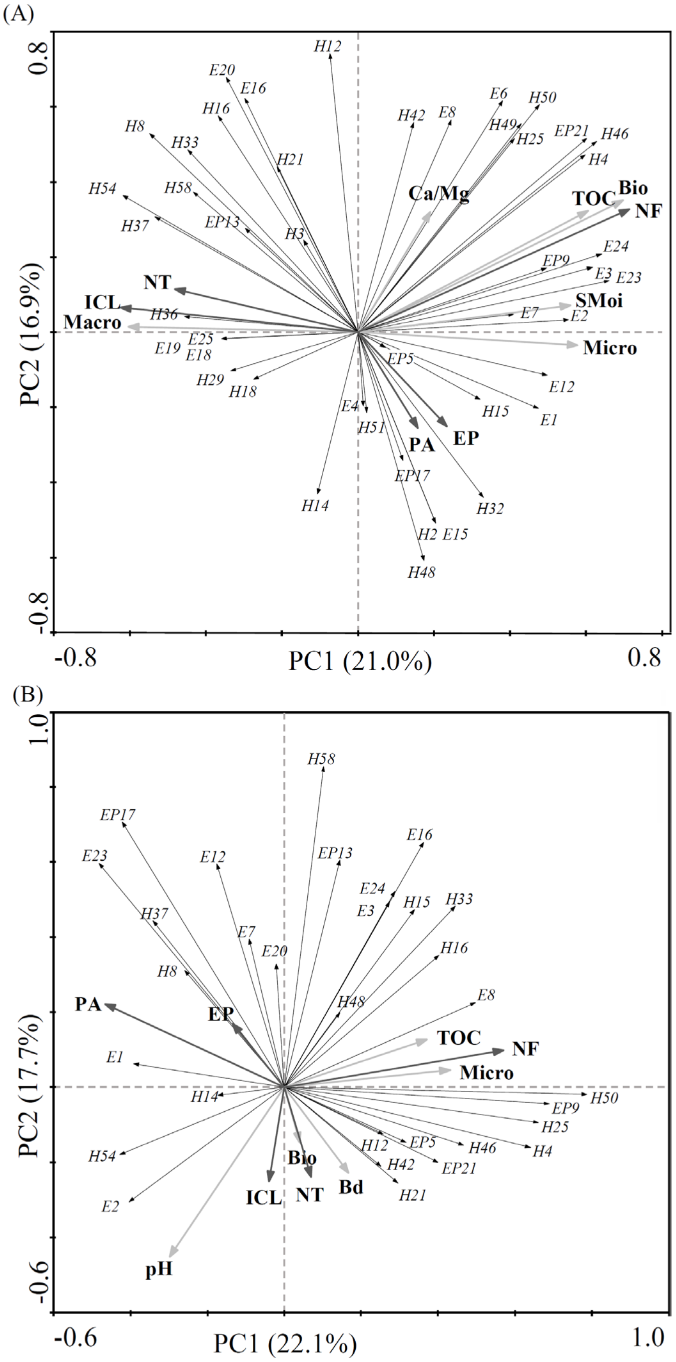

Figure 1. Principal component analysis (PCA) for springtails morphotypes and physiochemical attributes in native forest (NF), eucalyptus plantations (EP), pasture (PA), integrated crop-livestock (ICL) and no-tillage (NT) systems during winter (A) and summer (B) for land use systems in the southern plateau of the state of Santa Catarina. E: Edaphic; H: Hemiedaphic; EP: Epigeic. Bio: Biopores; Macro: macroporosity; Micro: microporosity; Bd: Bulk density; SMoi: Soil moisture; TOC: Total organic carbon; Ca/Mg: Calcium/Magnesium ratio; pH: acidity.

Discussion

1. *Community composition of springtails*

The intense land use systems and landscape differences influenced the abundance and diversity of springtails morphotypes. In this sense, the springtails community structure can be used for identify changes in the land use systems (Loring et al. 1981, Paul et al. 2011, Chang et al. 2013). The abundance and diversity of springtails in land use systems are also altered due to region fauna, soil density, microorganism activities, climate conditions, micro-habitat structures, agricultural practices, physiochemical properties of soils such as soil type and structure, pH and organic matter content (Hopkin 1997, Pflug & Wolters 2002, Schröder 2008, Rożen et al. 2010). Higher springtails abundances were determined in the land use systems without anthropological actions and soil management due to the better ecological equilibrium. It increases the food sources for organisms by the significant litterfall deposition and root exudation on/in soils (Bandyopadhyaya et al. 2002, Paul et al. 2011).

The similar values of richness and Margalef index during the winter can be associated to the climate conditions in the southern plateau of the state of Santa Catarina (Alvares et al. 2013) and different soil management processes that contain vegetal permanent covers (Rosa et al. 2015). According to Sousa et al. (2006), pasture areas are more important for the development and growth of springtails than agricultural areas due to anthropological actions during the planting of different crops. Accordingly, lower abundance and richness of springtails were experimentally determined for agricultural soils from the southern plateau of the state of Santa Catarina (Santos et al. 2018), indicating that intense land use systems increase the impacts on the environment. However, the springtails biodiversity increases in intense agro-ecosystems containing perennial pastures without using agrochemicals (Gardi et al. 2002).

Higher values for the Margalef index were determined in regions containing pastures, indicating higher springtails diversity compared to others areas as observed elsewhere (Santos et al. 2018). In general, the Margalef index were lower than 2.0 in the southern plateau of the state of Santa Catarina as observed in the east plateau studied by Santos et al. (2018), indicating low organism diversity and potential environmental impact. On the contrary, the Margalef index and total richness of springtails in the southwest plateau were higher than those determined in the east plateau, demonstrating that the springtails morphotype diversities can intrinsically or naturally be lower in all study areas in the southern plateau (Santos et al. 2018).

2. *Community distribution of springtails and their relationships with soil properties*

The principal component analysis was efficient for explaining and describing environmental variables and the springtails presence in each land use system. Lower soil porosity influenced the springtails abundances in soils for all land use systems. There is a close inter-correlation between springtails, soil macroporosity (pore sizes higher than 0.08 mm) and soil biopores (pore sizes higher than 1.0 mm) since these organisms are not morphologically capable of either digging tunnels or galleries into soils (Hopkin 1997, Beylich et al. 2010). Therefore, survival rates of springtails morphotypes depend on their body diameters and soil porosities in land use systems (Larsen et al. 2004). A decrease in the springtails abundance was also observed with the decrease of the soil surface macroporosity in NT and ICL due to lower organic matter concentration which is responsible by the channel formation in soils from land use systems (Bot & Benites 2005, Cavalieri et al. 2009, He et al. 2011).

A significant correlation between the calcium/magnesium ratio and total organic carbon contents in NF was determined as observed elsewhere (Chagnon et al. 2000, 2001, Cassagne et al. 2003, Oliveira Filho et al. 2016). Another correlation between eco-morphological groups and total organic carbon contents should be occurring which favor the growing of springtails morphotypes that contain fungivorous habits (Scheu & Folger 2004, Sawahata 2006). Some springtails morphotypes can act as immobilizers of nutrients in either soils or plants during the planting of different crops (Eaton et al. 2004). The immobilization of nutrients in either soils or plants also increases the springtails growth rates (Ineson et al. 1982, Filser 2002, Culliney 2013).

Main correlations were determined among pH, biopores, and bulk density with ICL and NT. The limestone application in agricultural soils increases the pH and nutrient bioavailability, generating a multivariate statistic correlation as experimentally observed (Frazão et al. 2008). Changes in the soil pH and bulk density alter the springtails communities (Chagnon et al. 2000, 2001, Ponge 2000), although these soil properties did not influence the edaphic organism communities and root growth in the crops of the different studied land use systems. This was attributed to the soil biopores and structure in the depths ranging from 0.00 to 20.0 cm. A correlation between total organic carbon contents and NF was observed in the two seasons as indicated in Figures 1A and 1B due to the absence of anthropological actions in this land use system. This was not observed in the other land use systems due to soil enrichment processes that change the total organic contents (Paustian et al. 1997). Depending on the land use system, it can be determined by a decrease in the organic matter decomposition rates in soil (Jastrow et al. 1996, Six et al. 1998, Ogle et al. 2005) which increase with the presence of springtails morphotypes (Yang et al. 2012, Verma et al. 2014).

3. *Adapted Soil Biological Quality Index (QBS-adapt)*

Higher QBS-adapt index is associated with wooded regions which improve the environmental conditions and increase the microarthropod diversities (Madej et al. 2011). The QBS-adapt index determined during the winter indicated higher intensification in the land use systems, confirming that the more complex and structured the system the higher the amount and diversity of springtails morphotypes. It indicates a higher soil quality. Lands mixed by plow tend to have lower QBS-adapt index than pasture and forest soils as experimentally observed (Gardi et al. 2008). A high QBS-adapt index value was determined during the summer in ICL due to animal removal from this region and planting of soybean, improving the survival conditions for the springtails morphotypes. Lower QBS-adapt index values were determined in PA, indicating the influence of the climate changes, burned and types of land use systems (Baretta et al. 2005, Bartz et al. 2014b, Rosa et al. 2015). The burned processes of plants and forests as those that occurred in the southern plateau of the state of Santa Catarina increase the phosphorus concentrations and stimulate the seed germination (Bot & Benites 2005). However, it can be harmful for the edaphic organism fauna since fire destroys most of the organic matter present on the soil surface which is an important food source and natural habitat for these organisms (DeBano et al. 1998, Malmström 2012).

The QBS-adapt index for eco-morphological groups in PA was higher for edaphic and hemiedaphic organisms compared to epigeic organisms in the different seasons. Some morphology and physiology results of the studied springtails morphotypes suggest that a more dynamic lifestyle strategy was adopted by the edaphic organisms whereas a more conservative lifestyle strategy was adopted by the edaphic organisms, preferring more stable and protective environments (Petersen 2002). As observed in this study and according to (Loring et al. 1981), the different land use systems affected significantly the springtails survival rates and their morphological characteristic in soils as studied elsewhere. However, the high QBS-adapt index values found for edaphic and hemiedaphic organisms pointed out for a non-polluted environment. For non-polluted environments, the springtails morphotypes were not affected by the variation of the physical and chemical soil properties or different land use systems (Hopkin 1997).

Conclusions

Evaluating the springtails communities by monitoring their morphological characteristics was efficient for comparing the different land use systems. Native forests, eucalyptus plantations, pasture, integrated crop-livestock, and no-tillage systems were studied, describing the different land use systems by correlations with collembola communities and physical and chemical soil properties. Preserved land use systems were more appropriated and non-toxic for the determined collembola morphotypes. Significant correlations were determined between collembola morphotypes and physical and chemical soil properties during the winter, including biopores, calcium/magnesium ratio, and total organic carbon content in soil, macroporosity, microporosity, and soil moisture. Significant correlations were also determined during the summer between springtails morphotypes and biopores, total organic carbon content, soil bulk density, pH, and microporosity. The QBS-adapt index was an efficient and sensitive tool for the distinction of different land use systems for different seasons. Correlations among springtails morphotypes, QBS-adapt index, and physical and chemical soil properties generated practical and relevant informations for a fast evaluation of the soil quality. The diversity of the collembola communities could be also evaluated with low-cost and without taxonomy analysis.

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Author Contributions

Julia da Silva Machado: responsible for data collection, contribution to data analysis and interpretation, contribution to critical revision, adding intellectual content.

Luís Carlos Iuñes de Oliveira Filho: contribution to data analysis and interpretation, contribution to critical revision, adding intellectual content.

Julio Cesar Pires Santos and Alexandre Tadeu Paulino: contribution to critical revision, adding intellectual content.

Dilmar Baretta: responsible for sampling of springtails and for data collection, substantial contribution in the concept and design of the study, contribution to data analysis and interpretation, contribution to manuscript preparation, contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Inventory

Phanerogamic flora of the Catimbau National Park, Pernambuco, Brazil

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Abstract: Specimens of phanerogamic plants from the Catimbau National Park, located in northeastern Brazil, were collected during extensive fieldwork and analyzed together with information gathered from the specialized literature. A total of 613 species was recorded, belonging to 366 genera and 85 families. The largest families were Fabaceae, Poaceae, Euphorbiaceae, Asteraceae, Convolvulaceae, Malvaceae, Myrtaceae, Apocynaceae, Malpighiaceae, and Cyperaceae, comprising almost 60% of the species. The level of endemism found was below that projected in the literature, as only four known species are unique to the study area. The area is, however, home to a varied flora with high richness and numerous rare or threatened species, demonstrating the importance of the National Park for conserving the regional flora. Additionally, 34 new registrations are reported here for Pernambuco State.
Keywords: *Conservation, diversity, flora, Northeastern Brazil.*

Flora fanerogâmica do Parque Nacional do Catimbau, Pernambuco, Brasil

Resumo: Espécimes de plantas fanerogâmicas do Parque Nacional do Catimbau, localizado no nordeste do Brasil, foram coletados durante um extenso trabalho de campo e analisados em conjunto com informações obtidas da literatura especializada. Foi registrado um total de 613 espécies pertencentes a 366 gêneros e 85 famílias. As maiores famílias foram: Fabaceae, Poaceae, Euphorbiaceae, Asteraceae, Convolvulaceae, Malvaceae, Myrtaceae, Apocynaceae, Malpighiaceae e Cyperaceae, compreendendo quase 60% das espécies. O nível de endemismo encontrado foi abaixo do projetado na literatura, pois apenas quatro espécies conhecidas são exclusivas da área de estudo. A área é, no entanto, o lar de uma flora variada com alta riqueza e numerosas espécies raras ou ameaçadas, demonstrando a importância do Parque Nacional para a conservação da flora regional. Além disso, 34 novos registros são reportados aqui para o estado de Pernambuco.
Palavras-chave: *Conservação, diversidade, flora, Nordeste do Brasil.*

Introduction

The Catimbau National Park (CNP) is considered to be an area of extreme biological importance in the Brazilian Northeast as it has high numbers of endemic and rare species associated with a diverse flora and various phytophysiognomies, justifying its high conservation priority (MMA 2002). One of the most important aspects of the park is its mosaic of vegetation, which includes rocky savanna ("campo rupestre"), upland forests ("brejos de altitude"), evergreen subshrub vegetation, neotropical savanna (Cerrado), dryland Caatinga, and vegetation refuges. The diversity and floristic richness associated with those different vegetation types are related to its geomorphology and a paleoclimate that strongly influenced the area (Ab'saber 1974). Caatinga dryland vegetation is the most representative phytophysiognomy in the region (Rodal et al. 1998, Sales et al. 1998).

The Caatinga is a unique Brazilian ecosystem growing in a semiarid climate with high solar radiation and evapotranspiration levels, determining a deciduous thorny vegetation that varies from shrubby and open, to closed forests (Sampaio 1995, Pennington et al. 2009). The caatinga in Northeastern Brazil is one of twelve disjunct groups of Neotropical dry forests (DRYFLOR 2016). The dry forests of the caatinga, together with those of central Brazil, have strong floristic affinities and share almost 700 species (DRYFLOR 2016). Although the Caatinga is the only exclusive Brazilian domain, with an unparaleled biological heritage, it is considered the least known vegetation form in the country and has not yet had its importance fully recognized by the government (Maciel 2010).

Due to the high human population density in the region, the floristic diversity there is highly influenced by anthropogenic pressures. The loss of plant diversity is a real and alarming threat that has intensified mainly due to the impacts of human activities on the natural habitat, climate change, and the intense exploitation of natural resources, pollution, and the voluntary or involuntary introduction of exotic species (Lobo et al. 2011). The discharacterization of the original vegetation has led to habitat fragmentation in Pernambuco, leaving natural vegetation remnants interspersed among plantations and cities. No other Brazilian biome has been as neglected as the caatinga, which can be seen in its low numbers of conservation areas. Conservation Areas (CAs) have been established to ensure the preservation of the biological diversity of Brazil (Leão et al. 2011), with a total of 328 CAs being found in northeastern region of the country, of which 123 are established in the Caatinga biome. Pernambuco has 78 state level CAs, but only four areas (Vale do CNP, Serra Negra, Negreiros, and Chapada do Araripe) under federal responsibility (CPRH 2014). Only six of the state CAs protect caatinga sites.

Various studies of the flora and vegetation of the CNP have been developed with taxonomic (e.g., Sales et al. 1998, Santos et al. 2013, Melo 2012, Delgado-Júnior et al. 2014, Ferreira et al. 2015, Vasconcelos & Melo 2016, Costa & Melo 2017, Delgado-Júnior & Alves 2017), floristic, or phytosociological focuses (e.g., Andrade et al. 2004, Serafim-Filho 2014).

National Parks with touristic vocations, such as the CNP, require detailed floristic inventories for planning aimed at the conservation and rational use of their natural resources. In that context, the present work sought to provide a species list of the Angiosperms found in the CNP as a way of guiding its sustainable utilization.

Material and Methods

The present study was conducted in the Catimbau National Park (created in 2002), situated in the Ipanema Valley in the central region of Pernambuco State (Northeastern Brazil) (8°24’00” to 8º36’35”S and 37º09’30” to 37º14’40”W) (DOU 2002). The Park covers 62,300 ha and includes parts of the municipalities of Buíque, Ibimirim, and Tupanatinga (MMA 2002; Sampaio et al. 2002) (Figure 1). It is geologically part of the Jatobá sedimentary basin, and is characterized topographically by low mountainous elevations (800 to 1000 m a.s.l.) and by open valleys with abrupt slopes (Rodal et al. 1998, SIGEP 2010). The predominant climate in the region is type BShs’ with an average annual rainfall is approximately 1,100 mm and a rainy season between January and June (Alvares et al. 2014). The vegetation there is typical of Caatinga, but also shows influences of other Brazilian ecosystems (Atlantic rainforest and Cerrado), with physiognomies such as dense arboreal Caatinga and shrubby spineless vegetation growing on deep sandy soils with sandstone rock outcrops (Sales et al. 1998, IBAMA 2009).

Data from specimen collections were compiled using the specialized literature focusing on the flora of the CNP (or part of it), and on some of the municipalities included in the Park, such as Buíque. Additional data was obtained from extensive collections carried out by a team led by Dr. Margareth Sales since the turn of the recent century, and others funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - “Diversidade e Conservação do Parque Nacional do Vale do Catimbau, Buíque, Pernambuco” with periodic collections between September/2011 and August/2014 during the plants’ reproductive phases. The collection material was pressed and dried following Mori et al. (1989), and incorporated into the “Professor Vasconcelos Sobrinho” Herbarium (PEUFR) at the Federal Rural University of Pernambuco (UFRPE). In addition to the specimens deposited in the herbarium, specimens belonging to other Pernambuco herbarium collections, such as HST, HVASF, IPA, and UFP (acronyms according to Thiers 2018) were consulted. The species were identified by comparisons with collections, by using identification keys and the specialized literature (e.g. Queiroz 2006, Laurênio & Sales 2008, Silva et al. 2009, Delgado-Júnior et al. 2017, Delgado-Júnior & Alves 2017) and, when necessary, by consultations with specialists. The floristic list is based on the Angiosperm Phylogeny Group APG IV (2016) classification system, with exception of Turneraceae (= Passifloraceae). The present study is registered in the National System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) under number AE11C7F.

The new records detected in the state of Pernambuco were indicated in the table (Tab. 1) with asterisks (*).

Results

This work provides an inventory of angiosperms in the CNP and includes 613 species belonging to 85 families (Table 1). The highest percentage (78.9%) of families of flowering plants were in the clade of eudicots, followed by monocotyledons (17.6%), and magnolids (3.5%) (Figures 2 to 5). The most species rich families were: Fabaceae (109 spp.), Poaceae (49 spp.), Euphorbiaceae (40 spp.), Asteraceae (36 spp.), Convolvulaceae (35 spp.), Malvaceae (22 spp.), Myrtaceae (19 spp.), Apocynaceae (19 spp.), Malpighiaceae (18 spp.), and Cyperaceae (18 spp.) (Figure 6). The ten richest families represented almost 60% of the total species identified in the study area. Cactaceae and Bromeliaceae, traditionally associated with the caatinga phytophysiognomy, are well-represented in the region, with 10 and 12 species respectively. Twenty-seven families were represented by only one species (Aizoaceae, Alstroemeriaceae, Arecaceae, Asparagaceae, Balanophoraceae, Brassicaceae, Burseraceae, Hydroleaceae, Krameriaceae, Lentibulariaceae, Loasaceae, Marantaceae, Molluginaceae, Moraceae, Ochnaceae, Olacaceae, Phyllanthaceae, Phytolaccaceae, Piperaceae, Plumbaginaceae, Salicaceae, Santalaceae, Schoepfiaceae, Simaroubaceae, Trigoniaceae, Urticaceae, and Velloziaceae).

Of the 366 genera of Angiosperms registered for the CNP, fifteen (4.1%) showed high numbers of species: *Croton* L. (18 spp.), *Ipomoea* L. (18 spp.), *Chamaecrista* Moench (16 spp.), *Mimosa* L. (14 spp.), *Senna* Mill. (10 spp.), *Cyperus* L. (10 spp.), *Jacquemontia* Choisy (7 spp.), *Erythroxylum* P. Browne (7 spp.), *Polygala* L. (7 spp.), *Sida* L. (7 spp.), *Tillandsia* L. (6 spp.), *Evolvulus* L. (6 spp.), *Dioscorea* L. (6 spp.), *Eugenia* L. (5 spp.), and *Myrcia* DC. (5 spp.) (Table 1).

The most representative habitat in the study area was herbaceous, represented by 202 species (33% of the total), followed by shrubs with 99 species (16.1%), and vines/climbers with 95 species (15.5%) (Fig. 7). As such, the flora of the area was predominantly herbaceous-

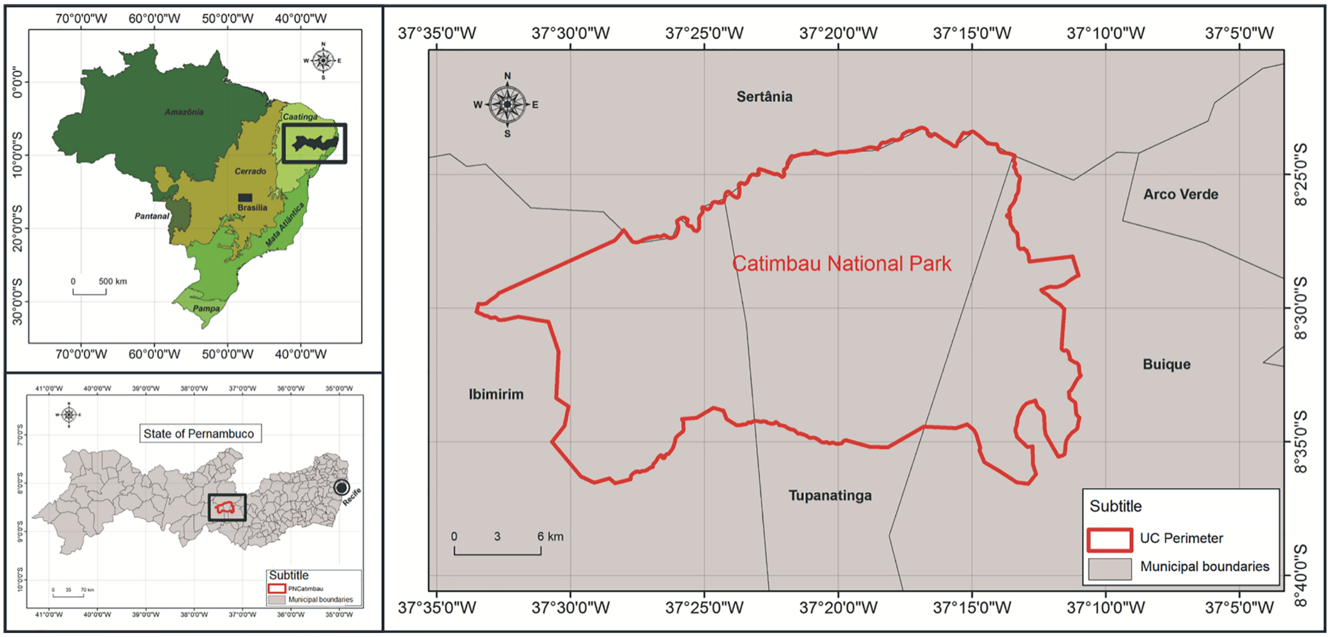

Figure 1. Map of the Catimbau National Park located in the state of Pernambuco in the Northeast region of Brazil.

Table 1. Angiosperms of the Catimbau National Park, Brazil. H = Herb; SU = Subshrub; S = Shrub; T = Tree; V = Vine; C = Climber; P = Palm tree. The new records detected in the state of Pernambuco were indicated with asterisks (*).

Taxa	Habit	Voucher(s)
Acanthaceae		
1. *Harpochilus neesianus* Mart.	SU, S	A. Laurênio 439
2. *Justicia aequilabris* (Nees) Lindau	H	O. Cano 810
3. *Ruellia asperula* (Nees) Lindau	H	L.S. Figueiredo 216
4. *Ruellia bahiensis* (Nees) Morong	H	O. Cano 806
5. *Ruellia paniculata* L.	H	O. Cano 805
6. *Thyrsacanthus ramosissimus* Lindau	S	M.F. Sales 381
Aizoaceae		
7. *Sesuvium portulacastrum* L.	H	F. Gallindo 43389
Alstroemeriaceae		
8. *Alstroemeria longistaminea* Mart. ex Schult. & Schult. f.	H	A.M. Miranda s.n.
Amaranthaceae		
9. *Alternanthera brasiliana* (L.) Kuntze	H	O. Cano 720
10. *Amaranthus spinosus* L.	H, SU	R. Pereira 2712
11. *Froelichia humboldtiana* Roem. & Schult.	H	Menezes 47
12. *Gomphrena demissa* Mart.	H	O. Cano 705
13. *Gomphrena vaga* Mart.	H	M.J.N. Rodal 626
14. *Pfaffia acutifolia* (Moq.) O. Stützer *	H	O. Cano 816
Amaryllidaceae		
15. *Habranthus sylvaticus* Herb.	H	M. Oliveira 5436
16. *Griffinia gardneriana* (Herb.) Ravenna	H	A. Alves-Araújo 66
17. *Hippeastrum stylosum* Herb.	H	C.R.S. Oliveira 170
Anacardiaceae		
18. *Anacardium occidentale* L.	T	E. Freire 03
19. *Myracrodruon urundeuva* Allemão	T	J.I.S. Barbosa et al. 65
20. *Schinopsis brasiliensis* Engl.	T	M.C. Tschá 563

Continuation Table 1.

Taxa	Habit	Voucher(s)
21. *Spondias tuberosa* Arruda	T	A.P.S. Gomes 377
Annonaceae		
22. *Annona leptopetala* (R.E. Fries) H. Rainer	S	C.R.S. Oliveira 292
23. *Oxandra reticulata* Mass. *	S	K. Andrade 24
Apocynaceae		
24. *Allamanda blanchetii* A.DC.	SU, S	M.F.A. Lucena 165
25. *Asclepias curassavica* L.	SU	F. Gallindo et al. CFPE695
26. *Aspidosperma pyrifolium* Mart.	T	M.C. Tschá 561
27. *Blepharodon manicatum* (Decne) Fontella	C	Andrade-Lima 8070
28. *Blepharodon pictum* (Vahl) W.D. Stevens	V	E. Inácio 39
29. *Calotropis procera* (Ait.) Ait. f.	SU	A. Laurênio 399
30. *Ditassa capillaris* E.Fourn.	H, V	A. Laurênio 322
31. *Ditassa crassifolia* Decne.	V	M.J.N. Rodal 291
32. *Ditassa hastata* Decne.	V	A.C.G. Costa 23
33. *Ditassa oxyphylla* Turcz.	H, V	K. Andrade 119
34. *Ditassa rotundifolia* (Decne.) Baill. ex K. Schum.	C, SU	A.M. Miranda 1780
35. *Mandevilla catimbauensis* Souza-Silva et al.	C	G.C. Delgado-Junior 627
36. *Mandevilla scabra* Hoffmann ex Roem.	V	F.M.O. Villarouco 3
37. *Mandevilla tenuifolia* (J.C. Mikan) Woodson	H	M.C. Tschá 618
38. *Marsdenia hilariana* E. Fourn.	S	Andrade-Lima 61-3997
39. *Matelea ganglinosa* (Vell.) Rapini	V	K. Andrade 120
40. *Schubertia multiflora* Mart.	C	G.C. Delgado-Júnior 719
41. *Skytanthus hancornifolius* (A. DC.) Miers	C	G.C. Delgado-Júnior 351
42. *Temnadenia violacea* (Vell.) Miers	V	M.F. Sales 509
Araceae		
43. *Anthurium affine* Schott	H	R. Pereira 2117
44. *Anthurium petrophilum* K.Krause	H	A.M. Miranda et al. 1803
45. *Philodendron acutatum* Schott.	H	C.R.S. Oliveira
Arecaceae		
46. *Syagrus coronata* (Mart.) Becc.	P	C.R.S. Oliveira 51
Asparagaceae		
47. *Agave sisalana* Perrine	H	J.V.A. Ferreira 177
Asteraceae		
48. *Acanthospermum hispidum* DC.	H	R. Pereira 1037
49. *Achyrocline satureioides* (Lam.) DC. *	SU	L.S. Figueiredo 183
50. *Acmella uliginosa* (Sw.) Cass.	H	R. Pereira 2784
51. *Acritopappus buiquensis* H.P. Bautista & D.J.N. Hind	SU	K. Andrade 41
52. *Ageratum conyzoides* L.	H	R. Pereira 2785
53. *Aspilia martii* Baker	H	O. Cano 763
54. *Baccharis serrulata* (Lam.) Pers.	SU	A. Viana 05
55. *Bidens pilosa* L.	H	O. Cano 714
56. *Blainvillea acmella* (L.) Philipson	H	R. Pereira 2775
57. *Centratherum punctatum* Cass.	H	L.S. Figueiredo 132
58. *Chresta pacourinoides* (Mart. ex DC.) C.M. Siniscalchi & B. Loeuille	H	A. Bocage 1065

Continuation Table 1.

Taxa	Habit	Voucher(s)
59. *Chrysanthellum indicum* DC.	H	R. Pereira 1034
60. *Conocliniopsis prasiifolia* (DC.) R.M. King & H.Rob.	SU	K. Andrade 96
61. *Cyrtocymura scorpioides* (Lam.) H. Rob.	SU	A.P.S. Gomes 70
62. *Dasyphyllum sprengelianum* (Gard.) Cabrera	SU, S	M.F. Sales 364
63. *Eclipta prostrata* L.	H	O. Cano 804
64. *Egletes viscosa* (L.) Less.	H	R. Pereira 1033
65. *Elephantopus mollis* Kunth	H	R. Pereira 1039
66. *Emilia fosbergii* Nicolson	SU	K. Andrade 123
67. *Emilia sonchifolia* (L.) DC.	H	R. Pereira 2766
68. *Erechtites hieraciifolius* (L.) Raf. ex DC.	H	R. Pereira 66439
69. *Eremanthus arboreus* (Gard.) MacLeisch *	S	L.P. Félix 7457
70. *Eremanthus capitatus* (Spreng.) MacLeish	S	M.F. Sales 424
71. *Lepidaploa chalybaea* (Mart.ex DC.) H. Rob	SU, S	A.P.S. Gomes 41
72. *Moquiniastrum oligocephala* (Gard.) G. Sancho	SU, S	E. Freire 20
73. *Paralychnophora reflexoauriculata* (G.M. Barroso) MacLeish	SU, S	A. Laurênio 354
74. *Platypodanthera melissifolia* (DC.) R.M. King & H.Rob.	H	M.J.N. Rodal 259
75. *Pluchea sagittalis* (Lam.) Cabrera	H, SU	R. Pereira 1036
76. *Synedrella nodiflora* (L.) Gaertn.	H	R. Pereira 2771
77. *Tagetes minuta* L.	H	R. Pereira 2786
78. *Tilesia baccata* (L.) Pruski	S	K. Andrade 78
79. *Trichogonia heringeri* R.M. King & H. Rob.	H	O. Cano 723
80. *Trichogonia salviifolia* Gard.	SU	Andrade – Lima 8068
81. *Tridax procumbens* L.	H	M.F. Sales 628
82. *Verbesina macrophylla* (Cass.) S.F. Blake	SU	R. Pereira 1150
83. *Wedelia vilosa* Gardner	SU	K. Andrade 159
Balanophoraceae		
84. *Langsdorffia hypogaea* Mart.	H	L. Figueiredo 147
Begoniaceae		
85. *Begonia grisea* DC.	H	R. Pereira 2733
86. *Begonia pernambucensis* Brade	H	Andrade - Lima 61-4001
Bignoniaceae		
87. *Anemopaegma chamberlaynii* (Sims.) Bureau & K. Schum.*	V	A.P.S. Gomes s.n
88. *Anemopaegma laeve* DC.	V	M.F. Sales et al. 518
89. *Bignonia ramentacea* (Mart. ex DC.) L.G. Lohmann	S	A.T. Carvalho 05
90. *Cuspidaria argentea* (Wawra) Sandwith	V	L.S. Figueiredo 110
91. *Fridericia dichotoma* (Jacq.) L.G. Lohmann	S	L.S. Figueiredo 233
92. *Fridericia limae* (A. H. Gentry) L.G. Lohmann	V	A. Laurênio 63
93. *Fridericia parviflora* (Mart. ex DC.) L.G. Lohmann	S	J.I.S. Barbosa 55
94. *Handroanthus chrysotrichus* (Mart. ex DC.) Standl.	T	M.J.N. Rodal 423
95. *Handroanthus impetiginosus* (Mart. ex DC.) Mattos	S	A.C.G. Costa 45
96. *Jacaranda rugosa* A. Gentry	S	M.J.N. Rodal 429
97. *Mansoa difficilis* (Cham.) Bur. & K. Schum.	V	E. Inácio 56
98. *Mansoa paganuccii* M.M.Silva-Castro	C	A. Laurênio 24
99. *Pyrostegia venusta* (Ker Gawl.) Miers	V	M.F. Sales 382
100. *Tabebuia aurea* (Silva Manso) Benth. & Hook.	T	A.M. Miranda et al. 2297

Continuation Table 1.

Taxa	Habit	Voucher(s)
Boraginaceae		
101. *Cordia rufescens* A.DC.	S	H.B. Oliveira et al. 4
102. *Cordia trichotoma* (Vell.) Arrab. ex Steud.	S	R. Pereira 2709
103. *Euploca humilis* (L.) Feuillet	H	A.M. Miranda et al. 1827
104. *Euploca procumbens* (Mill.) Diane & Hilger	SU	G.D. Alcântara 29
105. *Heliotropium angiospermum* Murray	S	A. Laurênio 11
106. *Heliotropium elongatum* (Lehm.) I.M. Johnst.	H	O. Cano 841
107. *Heliotropium indicum* L.	H	G.D. Alcântara 32
108. *Myriopus rubicundus* (Salzm. ex DC.) Luebert	S	G.D. Alcântara et al. 30
109. *Myriopus salzmannii* (DC.) Diane & Hilger	S, C	A. Bocage et al. 1067
110. *Varronia curassavica* Jacq.	S	D.N. Silva et al. 23
111. *Varronia dardani* (Taroda) J.S. Mill.	S	J.I.M. Melo 452
112. *Varronia globosa* Jacq.	S	M.C. Tschá 562
113. *Varronia leucocephala* (Moric.) J. S. Mill.	S, SU	A. Laurênio 314
Brassicaceae		
114. *Lepidium bonariense* L.	H	L.S. Figueiredo 161
Bromeliaceae		
115. *Aechmea leptantha* (Harms) Leme & J.A.Siqueira	H	A.C.G. Costa 8
116. *Billbergia porteana* Brongn.	H	K. Andrade 98
117. *Bromelia laciniosa* Mart. ex Schult. & Schult. f.	H	C.R.S. Oliveira 295
118. *Dyckia limae* L.B. Sm.	H	M.C. Tschá 25
119. *Encholirium spectabile* Mart. ex Schult. f.	H	R. Pick 155
120. *Neoglaziovia variegata* (Arruda) Mez.	H	A. Laurênio 266
121. *Tillandsia catimbauensis* Leme, W. Till & J.A. Siqueira	H	C.R.S. Oliveira 76
122. *Tillandsia loliacea* Mart. ex Schult. f.	H	E.A. Rocha 1551
123. *Tillandsia recurvata* (L.) L.	H	R. Pimentel 96
124. *Tillandsia streptocarpa* Baker	H	Andrade-Lima 60-3528
125. *Tillandsia tricholepis* Baker	H	R. Pereira 2108
126. *Tillandsia usneoides* L.	H	R. Pimentel 95
Burseraceae		
127. *Commiphora leptophloeos* (Mart.) J.B.Gillett	T	A. Laurênio 429
Cactaceae		
128. *Cereus albicaulis* (Britton & Rose) Luetzelb.	S	E.A. Rocha 1198
129. *Cereus jamacaru* DC.	S, T	A.P.S. Gomes 385
130. *Harrisia adscendens* (Gurke) Britton & Rose	S, SU	L.S. Figueiredo 267
131. *Melocactus zehntneri* (Britton & Rose) Luetzelb.	H	E.A. Rocha 1704
132. *Pilosocereus chrysostele* (Vaupel) Byles & G.D. Rowley	T	E.A. Rocha 1490
133. *Pilosocereus pachycladus* F. Ritter	SU, S	L.S. Figueiredo 60
134. *Pilosocereus pachycladus* subsp. *pernambucensis* (F. Ritter) Zappi	S	L.S. Figueiredo 102
135. *Pilosocereus tuberculatus* (Werderm.) Byles & G.D. Rowley	SU, S	A.P.S. Gomes 383
136. *Rhipsalis baccifera* (J.S. Muell.) Stearn	H	O. Cano 736
137. *Tacinga inamoena* (K. Schum.) N.P. Taylor & Stuppy	H, S	A.P.S. Gomes 381
138. *Tacinga palmadora* (Britton & Rose) N.P. Taylor & Stuppy	S	A. Laurênio 420
Capparaceae		
139. *Colicodendron yco* Mart.	S, T	A. Laurênio 271
140. *Cynophalla flexuosa* (L.) J.Presl	S	E.A. Rocha 1324

Continuation Table 1.

Taxa	Habit	Voucher(s)
141. *Neocalyptrocalyx longifolium* (Mart.) Cornejo & Iltis	S	M.J.N. Rodal 548
Celastraceae		
142. *Maytenus imbricata* Mart. ex Reissek	S	A.P.S. Gomes 291A
143. *Monteverdia rigida* (Mart.) Biral	S, T	A.P.S. Gomes 363
Chrysobalanaceae		
144. *Hirtella ciliata* Mart. & Zucc.	S	E. Freire 18
145. *Hirtella racemosa* (Willd. ex Roem. & Schult.) Prance	SU, S	A.M. Miranda et al. 2265
Cleomaceae		
147. *Tarenaya diffusa* (Banks ex DC.) Soares-Neto & Roalson	H	A.P.S. Gomes 346
146. *Tarenaya microcarpa* (Ule) Soares-Neto & Roalson	H	A.M. Miranda et al. 4467
148. *Tarenaya spinosa* (Jacq.) Raf.	H	R. Pereira 2782
Clusiaceae		
149. *Clusia hilariana* Schltdl.	T	M.F. Sales 1059
150. *Clusia nemorosa* G. Mey.	S, T	M.F. Sales 420
Combretaceae		
151. *Buchenavia tetraphylla* (Aubl.) R.A. Howard	S, T	G.D. Alcântara et al. 21
152. *Combretum hilarianum* D. Dietr.	V	A. Laurênio 49
Commelinaceae		
153. *Commelina erecta* L.	H	Andrade-Lima 8102
154. *Commelina obliqua* Vahl.	H	R. Pereira 2768
155. *Tradescantia ambigua* Mart.	H	E.A. Rocha 1481
Convolvulaceae		
156. *Daustinia montana* (Moric.) Buril & A.R. Simões	C	A.C. Lacerda et al. s.n. (HST10114)
157. *Evolvulus daphnoides* Moric.	H	M.T. Vital et al. 21
158. *Evolvulus elegans* Moric.	H, SU	M.F. Sales 506
159. *Evolvulus filipes* Mart.	H	A.G. Silva 1665
160. *Evolvulus frankenioides* Moric.	H	L.S. Figueiredo 64
161. *Evolvulus glomeratus* Nees & Mart.	H, SU	M.F. Sales 375
162. *Evolvulus sericeus* Sw.	V	L.S. Figueiredo 14
163. *Ipomoea asarifolia* (Desr.) Roem. & Schult.	H	G.C. Delgado-Junior 624
164. *Ipomoea bahiensis* Willd.	C	G.C. Delgado-Junior et al. 808
165. *Ipomoea brasiliana* (Choisy) Meissn.	V	L.S. Figueiredo 109
166. *Ipomoea carnea* (Mart. ex Choisy) D.F.Austin	SU	E. Freire 26
167. *Ipomoea grandifolia* (Dammer) O'Donell	C	G.C. Delgado-Junior 722
168. *Ipomoea hederifolia* L.	C	R. Pereira 2833
169. *Ipomoea indica* (Burm. f.) Merr.	H	R. Pereira 2777
170. *Ipomoea longeramosa* Choisy	C	G.C. Delgado-Junior 695
171. *Ipomoea marcellia* Meissn.	V	L.S. Figueiredo 111
172. *Ipomoea nil* (L.) Roth	C	G.C. Delgado-Junior 678
173. *Ipomoea philomega* (Vell.) House	H	O. Cano 794
174. *Ipomoea pintoi* O'Donell	V	L.S. Figueiredo 108
175. *Ipomoea piurensis* O'Donell	V	G.C. Delgado-Junior 691
176. *Ipomoea rosea* Choisy	C	G.C. Delgado-Junior 668
177. *Ipomoea setosa* Huber	V	R. Pereira 2702
178. *Ipomoea subalata* Hassl.	C	G.C. Delgado-Junior 364
179. *Ipomoea subincana* (Choisy) Meissn.	V	K. Andrade 348

Continuation Table 1.

Taxa	Habit	Voucher(s)
180. *Ipomoea triloba* L.	H	O. Cano 835
181. *Jacquemontia bahiensis* O'Donell	V	L.S. Figueiredo 117
182. *Jacquemontia chrysanthera* Buril	C	A.C.G. Costa et al.17
183. *Jacquemontia corymbulosa* Benth.	C	A. Araújo 02
184. *Jacquemontia evolvuloide*s (Moric.) Meissn.	V	M.F. Sales 431
185. *Jacquemontia multiflora* (Choisy) Hallier f.	V	M.J.N. Rodal 366
186. *Jacquemontia nodiflora* Meisn.	V	A. Laurênio 60
187. *Jacquemontia pentanthos* (Jacq.) G. Don	V	A.C.P. Oliveira 3150
188. *Merremia aegyptia* (L.) Urb.	V	E.A. Rocha 1568
189. *Merremia cissoides* (Vahl) Hallier f.	V	K. Andrade 55
190. *Turbina cordata* (Choisy) D.F. Austin & Staples	V	R. Pereira 2844
Cucurbitaceae		
191. *Apodanthera congestiflora* Cogn.	H	G.C. Delgado-Junior 630
192. *Apodanthera glaziovi*i Cogn.	C	G.C. Delgado-Junior 701
193. *Cayaponia martiana* (Cogn.) Cogn.	V	R. Pereira 2713
194. *Cayaponia tayuya* (Vell.) Cogn.	C	A.M. Miranda et al. 2828
195. *Citrullus lanatus* (Thunb.) Matsum. & Nakai	H	J.V.A. Ferreira 170
196. *Cucumis anguria* L.	H	R. Pereira 2776
197. *Momordica charantia* L.	C	G.C. Delgado-Junior 654
Cyperaceae		
198. *Bulbostylis capillaris* (L.) C.B. Clarke	H	O. Cano 824
199. *Bulbostylis junciformis* (Kunth) C.B. Clarke	H	E.A. Rocha 1548
200. *Bulbostylis scabra* (J. Presl & C. Presl) C.B. Clarke	H	E.A. Rocha 1210
201. *Cyperus aggregatus* (Willd.) Endl.	H	M.B. Costa & Silva 3657
202. *Cyperus amabilis* Vahl	H	M.B. Costa & Silva 3651
203. *Cyperus cuspidatus* Kunth	H	E.A. Rocha 1547
204. *Cyperus haspan* L.	H	R. Pereira 1078
205. *Cyperus hermaphroditus* (Jacq.) Standl.	H	M.B. Costa & Silva 3658
206. *Cyperus laxus* Lam.	H	O. Cano 797
207. *Cyperus meyenianus* Kunth	H	O. Cano 829
208. *Cyperus schomburgkianus* Nees	H	Andrade - Lima 8130
209. *Cyperus subsquarrosus* (Muhl.) Bauters	H	E.C. Tenório 1115
210. *Cyperus surinamensis* Rottb.	H	E.A. Rocha 1543
211. *Eleocharis elegans* (Kunth) Roem. & Schult.	H	R. Pereira 1030
212. *Eleocharis filiculmis* Kunth	H	R. Pereira 1044
213. *Eleocharis flavescens* (Poir.) Urb.	H	R. Pereira 1043
214. *Rhynchospora cephalotes* (L.) Vahl	H	M. Luceño 316
215. *Rhynchospora ridleyi* C.B. Clarke	H	A. Laurênio 356
Dioscoreaceae		
216. *Dioscorea campestris* Griseb.	V	Andrade – Lima 75-8117
217. *Dioscorea cinnamomifolia* Hook.	C	G.C. Delgado-Junior et al. 312
218. *Dioscorea leptostachya* Gardn.	C	L.B. Oliveira 174
219. *Dioscorea piperifolia* Humb. & Bonpl. ex Willd.	C	G.C. Delgado-Junior et al. 681
220. *Dioscorea polygonoides* Humb. & Bonpl. ex Willd.	V	M.J.N. Rodal 519
221. *Dioscorea subhastata* Vell.	V	G.C. Delgado-Junior 702

Continuation Table 1.

Taxa	Habit	Voucher(s)
Eriocaulaceae		
222. *Paepalanthus lamarckii* Kunth	H	R. Pereira 1014
223. *Paepalanthus myocephalus* (Mart.) Körn.	H	A.M. Miranda 2752
224. *Paepalanthus subtilis* Miq. *	H	M.B. Costa & Silva 3681
225. *Paepalanthus tortilis* (Bong.) Mart.	H	O. Cano 793
Erythroxylaceae		
226. *Erythroxylum betulaceum* Mart. *	S	M.J.N. Rodal 503
227. *Erythroxylum caatingae* Plowan	S, T	E. Freire 19
228. *Erythroxylum* cf. *distortum* Mart.	SU, S	L.S. Figueiredo 22
229. *Erythroxylum loefgrenii* Diogo	S	M. Oliveira 4995
230. *Erythroxylum nummularium* Peyr.	S	A. Laurênio 335
231. *Erythroxylum pungens* O.E.Schulz	T	M.J. Hora 67
232. *Erythroxylum revolutum* Mart.	S	A. Laurênio 368
Euphorbiaceae		
233. *Acalypha brasiliensis* Müll. Arg. *	S	R. Pereira 2846
234. *Acalypha multicaulis* Müll. Arg.	SU	K. Andrade 267
235. *Astraea lobata* (L.) Klotzsch	SU	L.S. Figueiredo 308
236. *Cnidoscolus bahianus* (Ule) Pax & K. Hoffm.	S	M.J.N. Rodal 323
237. *Cnidoscolus pubescens* Pohl *	S, T	A. Laurênio 398
238. *Cnidoscolus quercifolius* Pohl ex Baill.	S, T	A. Laurênio 313
239. *Cnidoscolus urens* (L.) Arthur	S, T	A. Laurênio 395
240. *Cnidoscolus urens* var. *neglectus* (Pohl) Lourteig	S, T	A. Laurênio 461
241. *Cnidoscolus vitifolius* (Mill.) Pohl	SU, S	L.S. Figueiredo 197
242. *Croton adamantinus* Müll. Arg.	S	S.I. Silva 400
243. *Croton argyrophyllus* Kunth	S	S.I. Silva 399
244. *Croton blanchetianus* Baill.	SU	J.A. Siqueira-Filho 2921
245. *Croton campestris* A. St. –Hill.	SU	M. Correia 179
246. *Croton echioides* Baill.	S	A.P.S. Gomes 1106
247. *Croton glandulosus* L.	H	S.I. Silva 751
248. *Croton grewioides* Baill.	H	M.C. Tschá 633
249. *Croton heliotropiifolius* Kunth	SU	K. Andrade 33
250. *Croton hirtus* L'Hér.	S	R. Pereira 2769
251. *Croton janeirensis* Radd.-Sm. & Govaerts. *	S	M.J.N. Rodal 499
252. *Croton lundianus* (Didr.) Mull. Arg.	H, SU	S.I. Silva 291
253. *Croton nummularius* Baill.	S	A. Laurênio 351
254. *Croton pedicellatus* Kunth *	SU, S	A. Laurênio 123
255. *Croton rudolphianus* Müll. Arg.	SU, S	A.P.S. Gomes 1124
256. *Croton sonderianus* Müll. Arg.	S	E. Inácio 175
257. *Croton tricolor* Klotzsch ex Baill.	S	J.S. Silva 347
258. *Croton velutinus* Baill.	S	G. Webster 25729
259. *Croton virgultosus* Müll. Arg.	S	J.S. Silva s.n.
260. *Dalechampia schenckiana* Pax & K. Hoffm.	V	A. Laurênio 321
261. *Euphorbia comosa* Vell.	H, SU	A. Laurênio 69
262. *Euphorbia sarcodes* Boiss.	S	Andrade-Lima 8063
263. *Euphorbia thymifolia* L.	H	M.F. Sales 629

Continuation Table 1.

Taxa	Habit	Voucher(s)
264. *Euphorbia tirucalli* L.	SU	A. Bocage et al. 1076
265. *Jatropha mollissima* (Pohl) Baill.	S	A.P.S. Gomes 368
266. *Jatropha mutabilis* (Pohl) Baill.	SU, S	A.P.S. Gomes 323
267. *Jatropha ribifolia* (Pohl) Baill.	SU, S	A.P.S. Gomes 81
268. *Manihot* cf. *dichotoma* Ule	SU, S	M.J.N. Rodal 508
269. *Microstachys corniculata* (Vahl) Griseb.	S	O. Cano 769
270. *Microstachys hispida* (Mart.) Govaerts	SU	M.J.N. Rodal 520
271. *Tragia friesii* Pax & K. Hoffm.	C	G.C. Delgado-Junior et al. 688
272. *Sapium argutum* (Müll. Arg.) Huber	S	R. Pereira 2759
273. *Stillingia trapezoidea* Ule	S	S.M.A. Souza 13
Fabaceae		
274. *Aeschynomene histrix* var. *densiflora* (Benth.) Rudd	SU	M.F. Sales 417
275. *Aeschynomene marginata* Benth.	SU	Andrade-Lima 8069
276. *Aeschynomene martii* Benth.	S	Andrade-Lima 61-3979
277. *Albizia polycephala* (Benth.) Killip ex Record	T	E.B. Ferraz 293A
278. *Anadenanthera colubrina* var. *cebil* (Griseb.) Reis	T	A.G. da Silva 1360
279. *Ancistrotropis peduncularis* (Kunth) A. Delgado	V	G.C. Delgado-Júnior 699
280. *Apuleia leiocarpa* (Vogel) J.F. Macbr.	T	A. Laurênio 339
281. *Bauhinia acuruana* Moric.	S	A. Laurênio 117
282. *Bauhinia pentandra* (Bong.) D. Dietr.	S	A. Laurênio 394
283. *Bionia pedicellata* (Benth.) L.P. Queiroz	S	Andrade-Lima 60-3519
284. *Bowdichia virgilioides* Kunth	T	K. Andrade 319
285. *Cajanus cajan* (L.) Millsp.	S	M.J.N. Rodal 500
286. *Calliandra aeschynomenoides* Benth.	SU, S	A.P.S. Gomes 403
287. *Canavalia brasiliensis* Mart. ex Benth.	C	G.C. Delgado-Junior 693
288. *Centrosema arenarium* Benth.	V	R.A. Pick 236
289. *Centrosema brasilianum* (L.) Benth.	C	R. Pereira 3704
290. *Centrosema coriaceum* Benth.	V	G.C. Delgado-Júnior et al. 460
291. *Centrosema pubescens* Benth. *	C	Rocha et al. 1262
292. *Centrosema virginianum* (L.) Benth.	V	A. Laurênio 84
293. *Chaetocalyx scandens* var. *pubescens* (DC.) Rudd.	V	A. Laurênio 66
294. *Chamaecrista absus* (L.) H.S. Irwin & Barneby	H	R. Pereira 3711
295. *Chamaecrista brachystachy*a (Benth.) Conc.	S, T	A.M. Miranda et al. 4468
296. *Chamaecrista brevicalyx* (Benth.) H.S. Irwin & Barneby	S	A. Laurênio 13
297. *Chamaecrista calycioides* (DC. ex Collad.) Greene	S	R.A. Pick 244
298. *Chamaecrista cytisoides* (DC. Collad.) H.S. Irwin & Barneby *	S	A.P.S. Gomes 302
299. *Chamaecrista desvauxii* (Collad.) Killip	S	M. Campelo 13
300. *Chamaecrista diphylla* (L.) Greene	H	R.A. Pick 245
301. *Chamaecrista flexuosa* (L.) Greene	SU, S	A.P.S. Gomes 22
302. *Chamaecrista hispidula* (Vahl) H.S. Irwin & Barneby	H	R.A. Pick 281
303. *Chamaecrista nictitans* (L.) Moench	SU	K. Andrade 162
304. *Chamaecrista pilosa* var. *luxurians* (Benth.) H.S. Irwin & Barneby	H	K. Andrade 122
305. *Chamaecrista ramosa* var. *ramosa* (Vogel) H.S. Irwin & Barneby	SU, S	A. Araújo 7
306. *Chamaecrista repens* var. *multijuga* (Vogel) H.S. Irwin & Barneby	SU, S	E. Menezes 13
307. *Chamaecrista rotundifolia* (Pers.) Greene	V, SU	M.F. Sales 353
308. *Chamaecrista swainsonii* (Benth.) H.S. Irwin & Barneby	SU	K. Andrade 134

Continuation Table 1.

Taxa	Habit	Voucher(s)
309. *Chamaecrista zygophylloides* var. *colligans* (H.S. Irwin & Barneby) H.S. Irwin & Barneby	S	M.J.N. Rodal 463
310. *Chloroleucon foliolosum* (Benth.) G.P. Lewis	S	A. Laurênio 296
311. *Cratylia mollis* Mart. ex Benth.	S	A. Laurênio 127
312. *Crotalaria bahiensis* Windler & S.G.Skinner *	S	K. Andrade 48
313. *Crotalaria holosericea* Nees & Mart.	SU, S	A. Laurênio 89
314. *Dahlstedtia araripensis* (Benth.) M.J. Silva & A.M.G. Azevedo	T	G.D. Alcântara et al.10
315. *Dalbergia cearensis* Ducke	S, T	L.S. Figueiredo 52
316. *Dalbergia frutescens* var. *frutescens* (Vell.) Britton	S, C	E.B. Ferraz et al. 294
317. *Dioclea grandiflora* Mart. ex Benth.	V	M.C. Tschá 138
318. *Enterolobium contortisiliquum* (Vell.) Morong	T	A. Bocage 918
319. *Erythrostemon calycina* (Benth.) L.P. Queiroz	S	A.M. Miranda 5658
320. *Erythrina velutina* Willd.	T	R. Pereira 3702
321. *Galactia remansoana* Harms. *	V	L.S. Figueiredo 68
322. *Hymenaea courbaril* L.	T	L.S. Figueiredo 253
323. *Hymenaea cangaceira* R.B. Pinto, V.F. Mansano & A.M.G. Azevedo	T	E.A. Rocha et al. 1308
324. *Hymenaea stigonocarp*a Mart. ex Hayne	T	E.A. Rocha 1038
325. *Indigofera spicata* Forssk.	SU	L.B. Oliveira 165
326. *Indigofera suffruticosa* Mill.	SU, S	M.F.A. Lucena 164
327. *Inga laurina* (Sw.) Willd.	T	A. Bocage 1098
328. *Leptolobium dasycarpum* Vogel *	T	Andrade-Lima71-6559
329. *Libidibia ferrea* (Mart. ex Tul.) L.P. Queiroz	T	K. Andrade 302
330. *Libidibia ferrea* var. *leiostachya* (Benth.) L.P. Queiroz *	T	V. Sobrinho 708
331. *Macroptilium bracteatum* (Nees & Mart.) Maréchal & Baudet	C	G.C. Delgado-Junior 694
332. *Macroptilium gracile* (Poepp.) Urb.	SU	M.F. Sales 415
333. *Macroptilium martii* (Benth.) Maréchal & Baudet	C	G.C. Delgado-Juniore et al.689
334. *Mimosa adenophylla* var. *mitis* Barneby	SU, S	M.F. Sales 567
335. *Mimosa gemmulata* var. *adamantina* Barneby	S	A.P.S. Gomes 304
336. *Mimosa gemmulata* var. *gemmulata* Barneby *	S	A.P.S. Gomes 157
337. *Mimosa guaranitica* Chod. & Hassl. *	SU	M.F. Sales 433
338. *Mimosa hirsutissima* var. *hirsutissima* Mart.	H	K. Andrade 82
339. *Mimosa lewisii* Barneby	SU, S	A. Laurênio 65
340. *Mimosa misera* Benth.	SU	M.J.N. Rodal 530
341. *Mimosa modesta* var. *ursinoides* (Harms) Barneby	H	L.S. Figueiredo 98
342. *Mimosa ophtalmocentra* Mart. ex Benth.	T	M. Meiado 18
343. *Mimosa sensitiva* L.	SU, S	K. Andrade 239
344. *Mimosa setosa* Benth. *	SU, S	R. Pereira 3647
345. *Mimosa somnians* Humb. & Bonpl. ex Willd.	S, SU	M.J.N. Rodal 422
346. *Mimosa tenuiflora* (Wills.) Poir.	T	A.P.S. Gomes 153
347. *Mimosa ursina* Mart.	H	R.A. Pick 117
348. *Mimosa verrucosa* Benth.	S	M.F. Sales 567
349. *Myroxylon peruiferum* L. f. *	T	A.G. da Silva 1389
350. *Parapiptadenia zehntneri* (Harms) M.P.Lima & H.C.Lima	T	A.P.S. Gomes 80
351. *Peltogyne pauciflora* Benth.	T	A. Laurênio 362
352. *Periandra coccinea* (Schrad.) Benth.	H, SU	L.S. Figueiredo 248
353. *Periandra mediterranea* (Vell.) Taub.	SU, S	E. Menezes 23

Continuation Table 1.

Taxa	Habit	Voucher(s)
354. *Piptadenia stipulacea* (Benth.) Ducke	S, T	E. Inácio 47
355. *Pityrocarpa obliqua* (Pers.) Brenan	S	L.S. Figueiredo 48
356. *Pityrocarpa moniliformis* (Benth.) Luckow & R.W. Jobson	T	A.G. da Silva 1380
357. *Plathymenia reticulata* Benth.	T	G.D. Alcântara 36
358. *Platymiscium floribundum* Vogel	T	M.J.N. Rodal 441
359. *Poecilanthe ulei* (Harms) Arroyo & Rudd	T	O. Cano 815
360. *Poeppigia procera* C. Presl	S, T	A. Laurênio 366
361. *Poincianella microphylla* (Mart. ex G. Don) L.P. Queiroz	S, T	A. Laurênio 55
362. *Poincianella pyramidalis* (Tul.) L.P. Queiroz	S, T	A. Laurênio 12
363. *Pterogyne nitens* Tul.	T	Andrade-Lima 2036
364. *Senna acuruensis* (Benth.) H.S. Irwin & Barneby	S	A. Laurênio 360
365. *Senna angulata* (Vogel) H.S. Irwin & Barneby	S	A. Laurênio 194
366. *Senna cana* var. *cana* (Nees & Mart.) H.S. Irwin & Baneby	SU, S	A. Laurênio 316
367. *Senna macranthera* var. *micans* (Nees) H.S. Irwin & Barneby	S, T	G.M. Souza 40
368. *Senna macranthera* var. *pudibunda* (Collad.) H.S. Irwin & Barneby	S	L.S. Figueiredo 37
369. *Senna obtusifolia* (L.) Irwin & Barneby	H	K. Andrade 145
370. *Senna occidentalis* (L.) Link	SU, S	E.H. Rodrigues 15
371. *Senna rizzinii* H.S. Irwin & Barneby	S	A.P.S. Gomes 350
372. *Senna spectabilis* var. *excelsa* (DC.) H.S. Irwin & Barneby	S, T	A. Laurênio 358
373. *Senna splendida* var. *gloriosa* (Vogel) H.S. Irwin & Barneby	S, T	K. Andrade 45
374. *Senna trachypus* (Benth.) H.S. Irwin & Barneby	S, T	A. Laurênio 81
375. *Senegalia bahiensis* (Benth.) Seigler & Ebinger	S	R.A. Pick 151
376. *Senegalia piauhiensis* (Benth.) Seigler & Ebinger	SU, S	A.P.S. Gomes 14
377. *Senegalia polyphylla* (DC.) Britton & Rose	S	R.A. Pick 219
378. *Stylosanthes gracilis* Kunth	H	Z. Travassos 206
379. *Stylosanthes guianensis* var. *guianensis* (Aubl.) Sw.	H	Z. Travassos 207
380. *Stylosanthes macrocephala* M.B. Ferr. & Souza Costa	H	Z. Travassos 210
381. *Stylosanthes scabra* Vogel	H	E. Menezes 14
382. *Stylosanthes viscosa* Sw.	H, SU	E. Menezes 24
383. *Trischidium molle* (Benth.) H.E. Ireland	T	R.A. Pick 131
384. *Vachellia farnesiana* (L.) Wight & Arn.	S	R. Pereira 3689
385. *Zollernia ilicifolia* (Brongn.) Vogel	T	Andrade-Lima 71-6542
Gentianaceae		
386. *Chelonanthus purpuracens* (Aubl.) Struwe, S. Nilsson & V.A. Albert	H	M.F. Sales 428
387. *Schultesia guianensis* (Aubl.) Malme	H	R. Pereira 1009
Hydroleaceae		
388. *Hydrolea spinosa* L.	H	R. Pereira 1046
Krameriaceae		
389. *Krameria tomentosa* A. St.-Hill.	SU, S	O. Cano et al. 746
Lamiaceae		
390. *Aegiphila verticillata* Cham.	S	M.J. Campelo 26
391. *Eplingiella fruticosa* (Salzm. ex Benth.) Harley & J.F.B. Pastore	SU	K. Andrade 137
392. *Eriope* cf. *macrostachya* Mart. ex Benth.	S	M.J.N. Rodal 305
393. *Hypenia salzmannii* (Benth.) Harley	SU	M.J.N. Rodal 266
394. *Leonotis nepetifolia* (L.) R.Br.	H, SU	K. Andrade 160

Continuation Table 1.

Taxa	**Habit**	**Voucher(s)**
395. *Medusantha martiusii* (Benth.) Harley & J.F.B. Pastore	SU, S	A. Laurênio 46
396. *Mesosphaerum pectinatum* (L.) Kuntze	SU, S	M.J.N. Rodal 284
397. *Rhaphiodon echinus* (Nees & Mart.) Schauer	H	M.J.N. Rodal 271
Lauraceae		
398. *Cassytha filiformis* L.	H	A.P.S. Gomes 21
399. *Ocotea fasciculata* (Nees) Mez	S, T	E. Freire 30
400. *Ocotea nitida* (Meisn.) Mez	S	L.S. Figueiredo 213
401. *Ocotea xanthocalyx* (Nees) Mez	T	K. Andrade 144
Lentibulariaceae		
402. *Utricularia pusilla* Vahl	H	C.R.S. Oliveira 367
Loasaceae		
403. *Mentzelia aspera* L.	H	R. Pereira 2765
Loganiaceae		
404. *Spigelia gracilis* A.DC.	H	R. Pereira 2123
405. *Spigelia linarioides* DC.	H	O. Cano 768
406. *Strychnos gardneri* A.DC.	C	G.C. Delgado-Júnior 413
407. *Strychnos rubiginosa* DC.	SU, S	M.J. Hora 45
Loranthaceae		
408. *Psittacanthus cordatus* (Hoffmanns.) G. Don	H	A. Laurênio 370
409. *Struthanthus attenuatus* Eichl.	H	A. Bocage 1092
410. *Struthanthus concinnus* Mart.	H	A. Bocage 1091
411. *Struthanthus polyrhizus* Mart.	H	E. Freire 48
412. *Struthanthus syringifolius* Mart.	H	K. Andrade 26
Lythraceae		
413. *Cuphea ericoides* Cham. & Schltdl.	H, SU	E. Menezes 22
414. *Lafoensia glyptocarpa* Koehne	SU	K. Andrade 110
Marantaceae		
415. *Maranta zingiberina* (L.) Andersson	H	L. Figueiredo 27
Malpighiaceae		
416. *Banisteriopsis muricata* (Cav.) Cuatr.	V	R. Pereira et al. 1063
417. *Banisteriopsis schizoptera* Juss. (B. Gates)	S	M.J.N. Rodal 447
418. *Banisteriopsis stellaris* (Griseb.) B. Gates	V	K. Andrade 24
419. *Barnebya harleyi* W.R. Anderson & B. Gates	T	R.S. Pinho 58
420. *Bunchosia pernambucana* W.R. Anderson	SU	A. Laurênio 40
421. *Byrsonima cydoniifolia* A. Juss.	T	K. Andrade 260
422. *Byrsonima gardneriana* A. Juss.	S, T	A. Laurênio 47
423. *Byrsonima vacciniifolia* A. Juss.	SU, S	L.S. Figueiredo 16
424. *Carolus chasei* (W.R. Anderson) W.R. Anderson	V	E.A. Rocha 1229
425. *Diplopterys lutea* (A. Juss.) W.R. Anderson & C.C. Davis	V	L.S. Figueiredo 219
426. *Diplopterys pubipetala* (A. Juss.) W.R. Anderson & C.C. Davis	V	R.S. Pinheiro 38
427. *Heteropterys byrsonimifolia* A. Juss. *	V	A.P.S. Gomes 29
428. *Heteropterys caducibracteata* W.R. Anderson	C	A. Lima 75-8105
429. *Heteropterys trichanthera* A. Juss.	C	A.P.S. Gomes et al. 29
430. *Janusia anisandra* (Juss.) Griseb.	V	L.S. Figueiredo 135
431. *Mascagnia sepium* (A.Juss.) Griseb.	C	E.A. Rocha et al. 1529

Continuation Table 1.

Taxa	Habit	Voucher(s)
432. *Stigmaphyllon auriculatum* (Cav.) A. Juss.	V	M. J. Campelo 3
433. *Stigmaphyllon paralias* A. Juss.	SU, S	A. Laurênio 22
Malvaceae		
434. *Ceiba glaziovi*i (Kuntze) K. Schum.	T	E.A. Rocha 1506
435. *Helicteres baruensis* Jacq.	S	A. Bocage 353
436. *Helicteres brevispira* A. St.-Hil.	S	R. Pereira 2853
437. *Helicteres macropetala* A. St.-Hil.	SU	O. Cano et al. 845
438. *Helicteres velutina* K. Schum.	S	A. Laurênio 364
439. *Herissantia crispa* (L.) Brizicky	H, V	L.S. Figueiredo 17
440. *Melochia tomentosa* L.	S	R. Pereira 2723
441. *Pavonia blanchetiana* Miq.	SU, S	L.S. Figueiredo 171
442. *Pavonia cancellata* Cav.	SU, S	K. Andrade 155A
443. *Pavonia* cf. *glazioviana* Gurke	SU, S	C.S. Zickel 10
444. *Pavonia humifusa* A. St. -Hill.	H, V	K. Andrade 255
445. *Pavonia varians* Moric.	SU	O. Cano 699
446. *Sida angustissim*a A. St. –Hill.	H	E. Freire 56
447. *Sida cordifolia* L.	S	K. Andrade 17
448. *Sida galheirensi*s Ulbr.	H, SU	K. Andrade 263
449. *Sida glomerat*a Cav.	H	O. Cano 836
450. *Sida linifolia* Cav.	H	K. Andrade 107
451. *Sida rhombifolia* L.	H	R. Pereira 2789
452. *Sida ulei* Ulbr.	SU, S	E. Freire 62
453. *Sidastrum paniculatum* (L.) Fryxell	SU	L. Figueiredo 62
454. *Triumfetta semitriloba* Jacq.	H	O. Cano 779
455. *Waltheria indica* L.	H, SU	K. Andrade 114
Melastomataceae		
456. *Clidemia hirta* (L.) D. Don	S	K. Andrade et al. 76
457. *Comolia villosa* (Aubl.) Triana	H	O. Cano 754
458. *Marcetia taxifolia* (A. St.-Hil.) Cogn.	S	Andrade-Lima 60-3521
459. *Miconia caudigera* DC.	S	Andrade-Lima 61-3972
460. *Mouriri pusa* Gard. ex Gard. *	T	Andrade-Lima 61-3972
461. *Pleroma heteromallum* (D. Don) D. Don	S	Andrade-Lima 60-3516
462. *Pterolepis glomerat*a (Rottb.) Miq.	S	M.J.N. Rodal 279
463. *Pterolepis perpusilla* (Naudin) Cogn. *	H	M.F. Sales 348
Meliaceae		
464. *Guarea guidonia* (L.) Sleum.	T	O. Cano 831
465. *Trichilia hirta* L.	T	O. Cano 827
Molluginaceae		
466. *Mollugo verticillata* L.	H	A.M. Miranda et al. 1757
Moraceae		
467. *Ficus caatingae* R.M. Castro	T	L.P. Félix et al. 7447
Myrtaceae		
468. *Calyptranthes brasiliensis* Spreng.	T	A. Bocage et al. 1044
469. *Campomanesia aromatica* (Aubl.) Griseb.	SU, S	A.P.S. Gomes 296
470. *Campomanesia eugenioides* (Cambess.) D. Legrand ex Landrum	SU	R. Pereira 1016
471. *Eugenia brejoensis* Mazine	T	J.I.S. Barbosa 39

Continuation Table 1.

Taxa	Habit	Voucher(s)
472. *Eugenia candolleana* DC.	S, T	A. Laurênio 422
473. *Eugenia prasina* Kiaerstk.	S	K. Andrade 124
474. *Eugenia punicifolia* (Kunth) DC.	S, T	M.F. Sales 108
475. *Eugenia supraaxillaris* Vell. *	SU, S	E. Freire 51
476. *Marlierea clausseniana* (O. Berg) Kiaersk.	S	A. Bocage 1044
477. *Myrcia densa* (DC.) Sobral	S, T	Andrade-Lima 758055
478. *Myrcia guianensis* (Aubl.) DC.	S	M.F. Sales 1063
479. *Myrcia multiflora* (Lam.) DC.	S, T	A. Laurênio 29
480. *Myrcia splendens* (Sw.) DC.	S, T	A. Laurênio 14
481. *Myrcia tomentosa* (Aubl.) DC.	S, T	E. Freire 21
482. *Psidium brownianum* DC.	S	A. Bocage 1046
483. *Psidium myrtoides* O. Berg *	S	A. Laurênio 17
484. *Psidium oligospermum* DC.	T	A.M. Miranda 2751
485. *Psidium riparium* Mart. ex DC.	S	A.B. Marcon 8
486. *Psidium salutare* (Kunth) O. Berg *	T	E. Freire 111
Nyctaginaceae		
487. *Boungainvillea spectabilis* Willd.	S	R. Pereira 2764
488. *Guapira laxa* (Netto) Furlan	S, T	H.B. Oliveira et al. 19
Ochnaceae		
489. *Ouratea blanchettiana* (Planch.) Engl.	S, T	A.B. Marcon 7
Olacaceae		
490. *Ximenia americana* L.	S	C.R.S. Oliveira 161
Onagraceae		
491. *Ludwigia erecta* (L.) H. Hara	S, H, SU	R. Pereira 1079
492. *Ludwigia* cf. *octovalvis* (Jacq.) Raven	S, T	E. Menezes 19
Orchidaceae		
493. *Acianthera ochreata* (Lindl.) Pridgeon & M.W. Chase	H	A.M. Giulietti 8075
494. *Cyrtopodium gigas* (Vell.) Hoehne	H	E.C. Tenório 1142
495. *Vanilla palmarum* (Salzm. ex Lindl.) Lindl.	H	R. Pereira 1011
Oxalidaceae		
496. *Oxalis divaricata* Mart. ex Zucc.	H	R. Pereira 1019
497. *Oxalis frutescens* subsp. *frutescens* L.	H, SU	M.J.N. Rodal 462
498. *Oxalis glaucenses* Norlind	H	K. Randau 84010
499. *Oxalis psoraleiodes* Kunth	H	R. Pereira 2716
500. *Oxalis psoraleiodes* subsp. *insipida* (A. St.-Hil.) Lourteig	S	O. Cano 823
Passifloraceae		
501. *Passiflora cincinnata* Mast.	V	G.C. Delgado-Junior 327
502. *Passiflora foetida* L.	V	F.M.O. Oliveira 12
503. *Passiflora luetzelburgii* Harms	V	E. Freire 45
504. *Passiflora silvestris* Vell.	V	A. Laurênio 38
Phyllanthaceae		
505. *Phyllanthus klotzschianus* Müll. Arg.	SU	C.R.S. Oliveira 358
Phytolaccaceae		
506. *Microtea paniculata* Moq.	H	M.J.N. Rodal 286
Piperaceae		
507. *Peperomia blanda* (Jacq.) Kunth	H	C.R.S. Oliveira 277

Continuation Table 1.

Taxa	Habit	Voucher(s)
Plantaginaceae		
508. *Angelonia campestris* Nees & Mart.	C, H	R.A. Pick 250
509. *Angelonia cornigera* Hook. f.	H, SU	A.M. Miranda 1761
510. *Scoparia dulcis* L.	SU	E.A. Rocha 1282
511. *Stemodia foliosa* Benth.	H	A.M. Miranda et al. 1796
512. *Tetraulacium veroniciforme* Turcz.	H	O. Cano et al. 758
Plumbaginaceae		
513. *Plumbago scandens* L.	H	O. Cano 811
Poaceae		
514. *Anthephora hermaphrodita* (L.) Kuntze	H	M.B. Costa e Silva 3653
515. *Aristida adscensionis* L.	H	A.M. Giulietti 8145
516. *Aristida setifolia* Kunth	H	Andrade-Lima 8138
517. *Axonopus capillaris* (Lam.) Chase	H	O. Cano 744
518. *Axonopus compressus* (Sw.) P. Beauv.	H	M.B. Costa e Silva 3677
519. *Axonopus laxiflorus* (Trin.) Chase *	H	E.C. Tenório 1124
520. *Axonopus polydactylus* (Steud.) Dedecca	H	E. Inácio 38
521. *Cenchrus ciliaris* L.	H	M.B. Costa e Silva 3686
522. *Cenchrus echinatus* L. *	H	M.B. Costa e Silva 3706
523. *Chloris barbata* Sw.	H	M.B. Costa e Silva 3678
524. *Chloris orthonoton* Döll.	H	E.C. Tenório 1042
525. *Chloris pycnothrix* Trin.	H	M.B. Costa e Silva 3705
526. *Dactynoctenium aegyptium* (L.) Willd.	H	M.B. Costa e Silva 3684
527. *Digitaria bicornis* (Lam.) Roem. & Schult.	H	E.C. Tenório 1120
528. *Digitaria insularis* (L.) Fedde	H	Andrade-Lima 8136
529. *Digitaria tenuis* (Nees) Henrard	H	M.B. Costa e Silva 3652
530. *Eleusine indica* (L.) Gaertn.	H	M.B. Costa e Silva 3649
531. *Enteropogon mollis* (Ness) Clayton	H	M.B. Costa e Silva 3660
532. *Eragrostis acutiflora* (Kunth) Nees	H	M.B. Costa e Silva 3662
533. *Eragrostis articulata* (Schrank) Nees	H	E.C. Tenório 1109
534. *Eragrostis ciliaris* (L.) R. Br.	H	M.B. Costa e Silva 3656
535. *Eragrostis japonica* (Thunb.) Trin.	H	E.C. Tenório 1063
536. *Eragrostis maypurensis* (Kunth) Steud.	H	Andrade-Lima 8135
537. *Gymnopogon foliosus* (Willd.) Nees	H	E.C. Tenório 1104
538. *Ichnanthus glaber* (Raddi.) Hitchc.	H	E.C. Tenório 1111
539. *Ichnanthus leiocarpus* (Spreng.) Kunth *	H	E.C. Tenório 1131
540. *Megathyrsus maximum* (Jacq.) B.K. Simon & S.W.L. Jacobs	S, H	E.C. Tenório 1064
541. *Melinis repens* (Willd.) Zizka	H	D.N. Silva 1
542. *Panicum millegrana* Poir.	H	E.C. Tenório 1146
543. *Panicum trichoides* Sw.	H	E.C. Tenório 1144
544. *Pappophorum mucronulatum* Nees	H	E.C. Tenório 1138
545. *Pappophorum pappiferum* (Lam.) Kuntze	H	M.B. Costa e Silva 3650
546. *Paspalum arenarium* Schrad.	H	Andrade-Lima 8128
547. *Paspalum conjugatum* Bonpl.	H	P. Luetzelburg 26304
548. *Paspalum decumbens* Sw. *	H	M.B. Costa e Silva 3674
549. *Paspalum maritimum* Trin.	H	E.C. Tenório 1128
550. *Paspalum scutatum* Nees ex Trin.	H	M.B. Costa e Silva 3648

Continuation Table 1.

Taxa	**Habit**	**Voucher(s)**
551. *Setaria parviflora* (Poir.) Kerguélen	H	L. Figueiredo 13
552. *Setaria scabrifolia* (Nees) Kunth	H	M.B. Costa e Silva 3683
553. *Setaria setosa* (Sw.) P. Beauv.	H	E.C. Tenório 1137
554. *Setaria tenax* (Rich.) Desv.	H	E. Inácio 4
555. *Setaria vulpiseta* (Lam.) Roem. & Schult.	H	E.C. Tenório 1126
556. *Steinchisma laxum* (Sw.) Zuloaga	H, C	E.C. Tenório 1134
557. *Streptostachys asperifolia* Desv.	H	O. Cano 745
558. *Tragus berteronianus* Schult.	H	M.B. Costa e Silva 3659
559. *Urochloa mollis* Morrone & Zuloaga	H	M.B. Costa e Silva 3671
560. *Urochloa paucispicata* (Morong) Morrone & Zuloaga	H	M.B. Costa e Silva 3667
Polygalaceae		
561. *Asemeia ovata* (Poir.) J.F.B. Pastore & J.R. Abbott	H	R. Pereira 2830
562. *Monnina insignis* A.W. Benn.	H	I. Machado 84461
563. *Polygala appendiculata* Vell.	H	Y. Marinho 82586
564. *Polygala boliviensis* A.W. Benn.	H	J.I. Barbosa 84
565. *Polygala galioides* Poir.	T	L.S. Figueiredo 106
566. *Polygala glochidata* Kunth	H	O. Cano 830
567. *Polygala longicaulis* Kunth	H, S	L.S. Figueiredo 178
568. *Polygala paniculata* L.	H	J.I. Barbosa 89
569. *Polygala trichosperma* Jacq.	H	A. Bocage 352
570. *Securidaca coriacea* Bonpl. *	V, T	R. Pereira 1072
571. *Securidaca diversifolia* (L.) S.F.Blake	C	G.S. Baracho et al. 271
Polygonaceae		
572. *Ruprechtia laxiflora* Meisn.	T	C.R.S. Oliveira 411
573. *Triplaris gardneriana* Wedd.	T	M.J.N. Rodal 426
Portulacaceae		
574. *Portulaca elatior* Mart. ex Rohrb.	H	C.R.S. Oliveira 63
575. *Portulaca grandiflora* Hook. *	H	M.F. Sales 563
576. *Portulaca halimoides* L.	H	R. Pereira 2788
577. *Portulaca mucronata* Link	H	A.G. da Silva 1365
578. *Portulaca pilosa* L.	S	L.S. Figueiredo 114
Rhamnaceae		
579. *Colubrina cordifolia* Reisseck	S, T	Andrade-Lima 61-4003
580. *Gouania colurnifolia* Reisseck	C	M. Oliveira 5006
581. *Ziziphus cotinifolia* Reisseck	T	O. Cano 822
582. *Ziziphus joazeiro* Mart.	T	A.P.S. Gomes 354
Rubiaceae		
583. *Declieuxia fruticosa* (Willd. ex Roem. & Schult.) Kuntze	S, SU	A.M. Miranda et al. 1824
584. *Manettia cordifolia* Mart.	C	M.F. Sales 416
585. *Richardia grandiflora* (Cham. & Schtdl.) Steud.	SU, H	A.M. Miranda et al. 1759
586. *Staelia virgata* (Link ex Roem. & Schult.) K.Schum.	SU, H	K. Andrade 333
587. *Tocoyena formosa* (Cham. & Schltdl.) K. Schum.	SU, H	A.P.S. Gomes 16
Rutaceae		
588. *Balfourodendron molle* (Miq.) Pirani	T	A.P.S. Gomes 331
589. *Zanthoxylum stelligerum* Turcz.	T	Rita Pereira et al. 2867

Continuation Table 1.

Taxa	Habit	Voucher(s)
Salicaceae		
590. *Casearia sylvestris* Sw.	S, T	D.N. Silva et al. 22
Sapindaceae		
591. *Allophylus quercifolius* (Mart.) Radlk.	T, S	C. Farias-Fonseca 285
592. *Cardiospermum corindum* L.	C	E.A. Rocha et al. 1510
593. *Dodonaea viscosa* (L.) Jacq.	S, T, SU	M.J.N. Rodal 470
594. *Serjania glabrata* Kunth	C	A.M. Miranda et al. 2487
595. *Serjania lethalis* A.St.-Hil.	C	G.C. Delgado-Junior 480
596. *Serjania marginata* Casar	C	G.C. Delgado-Junior 437
597. *Serjania pernambucensis* Radlk.	C	A.M. Miranda et al. 2740
598. *Urvillea ulmacea* Kunth	C	A.C. Lacerda et al. s.n
Sapotaceae		
599. *Manilkara rufula* (Miq.) H.J. Lam.	T	A.M. Miranda et al. 2799
600. *Sideroxylon obtusifolium* (Roem. & Schult.) T.D. Penn.	S, T	M.F. Sales 351
Santalaceae		
601. *Phoradendron tunaeforme* (DC.) Eichl.	H	A.C.G. Costa et al. 62
Schoepfiaceae		
602. *Schoepfia brasiliensis* A. DC.	S, T	A.M. Miranda et al. 1809
Simaroubaceae		
603. *Simarouba amara* Aubl.	T	K. Andrade 227
Smilacaceae		
604. *Smilax campestris* Griseb.	C	G.C. Delgado-Junior 321
605. *Smilax cissoides* Mart. ex Griseb.	C	E. Freire 29
Solanaceae		
606. *Datura stramonium* L.	S	M.J.N. Rodal 313
607. *Solanum rhytidoandrum* Sendtn.	S	M.J.N. Rodal 546
608. *Solanum stipulaceum* Willd.	T, S	G.D. Alcântara et al. 15
609. *Solanum thomasiifolium* Sendtn. *	S	M.J.N. Rodal 534
Trigoniaceae		
610. *Trigonia nivea* Cambess.	C	A.M. Miranda et al. 1712
Turneraceae		
611. *Piriqueta duarteana* (Cambess.) Urb.	H, SU	M.J. Campelo 63
612. *Piriqueta sidifolia* Cambess. var. *multiflora* Urb.	S	M.J.N. Rodal 368
613. *Turnera diffusa* Willd. ex Schult.	S, SU	R. Pick 257
Urticaceae		
614. *Pilea hyalina* Fenzl	H	R. Pereira 2701
Velloziaceae		
615. *Vellozia cinerascens* (Mart. ex Schult. & Schult. f.) Mart. ex Seub	H	E.A. Rocha et al. 1489
Verbenaceae		
616. *Lantana camara* L.	S	A. Bocage et al. 1070
617. *Lantana canescens* Kunth	S	J.A. Siqueira-Filho 2925
Vitaceae		
618. *Cissus blanchetiana* Planch.	C	G.C. Delgado-Júnior 680
619. *Cissus verticillata* (L.) Nicolson & C.E.Jarvis	C	Rita Pereira 2700

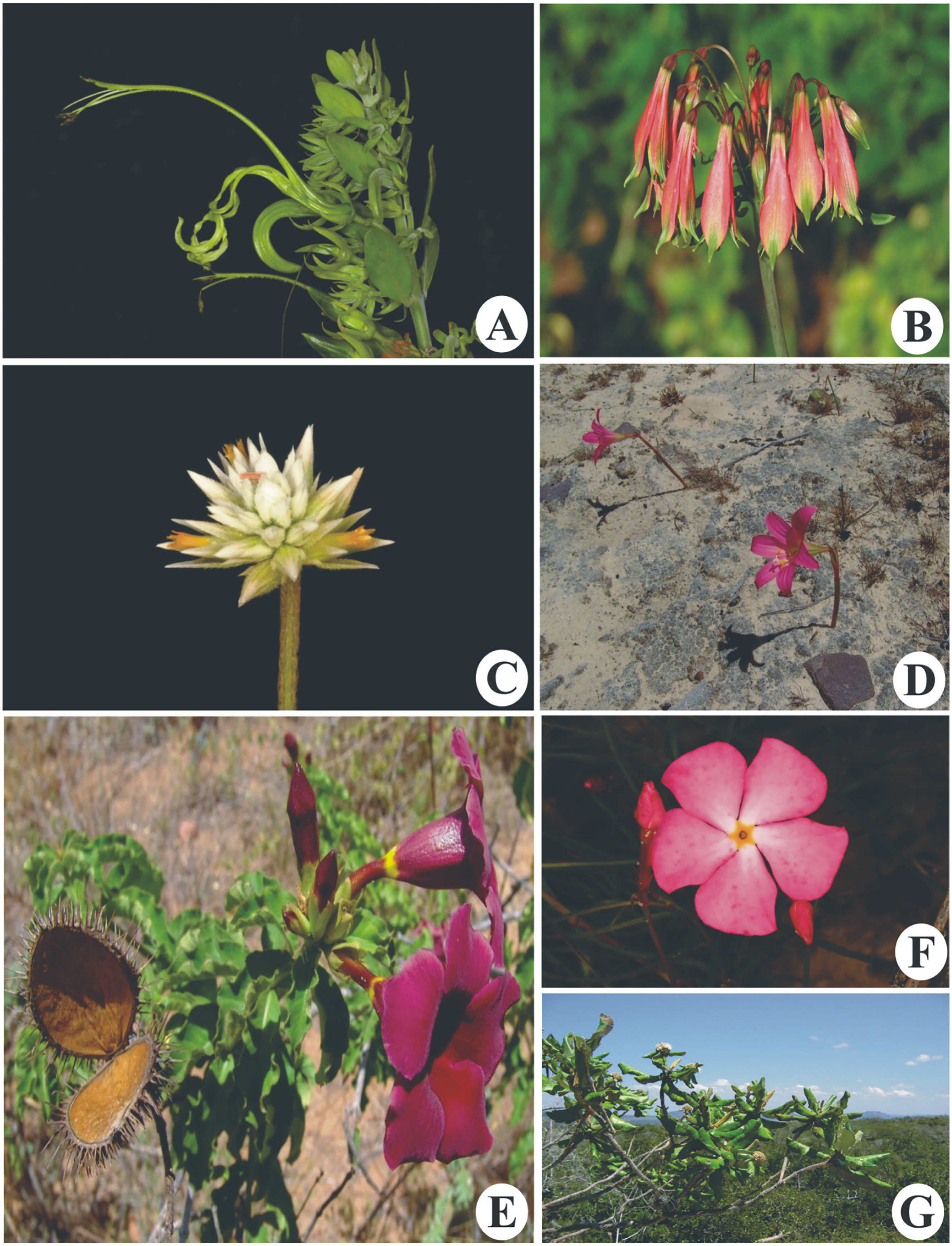

Figure 2. A. *Harpochilus neesianus* Mart. **B.** *Alstroemeria longistaminea* Mart. ex Schult. & Schult. f. **C.** *Gomphrena vaga* Mart. **D.** *Habranthus sylvaticus* Herb. **E.** *Allamanda blanchetii* A.DC. **F.** *Mandevilla tenuifolia* (J.C. Mikan) Woodson. **G.** *Paralychnophora reflexoauriculata* (G.M. Barroso) MacLeish.

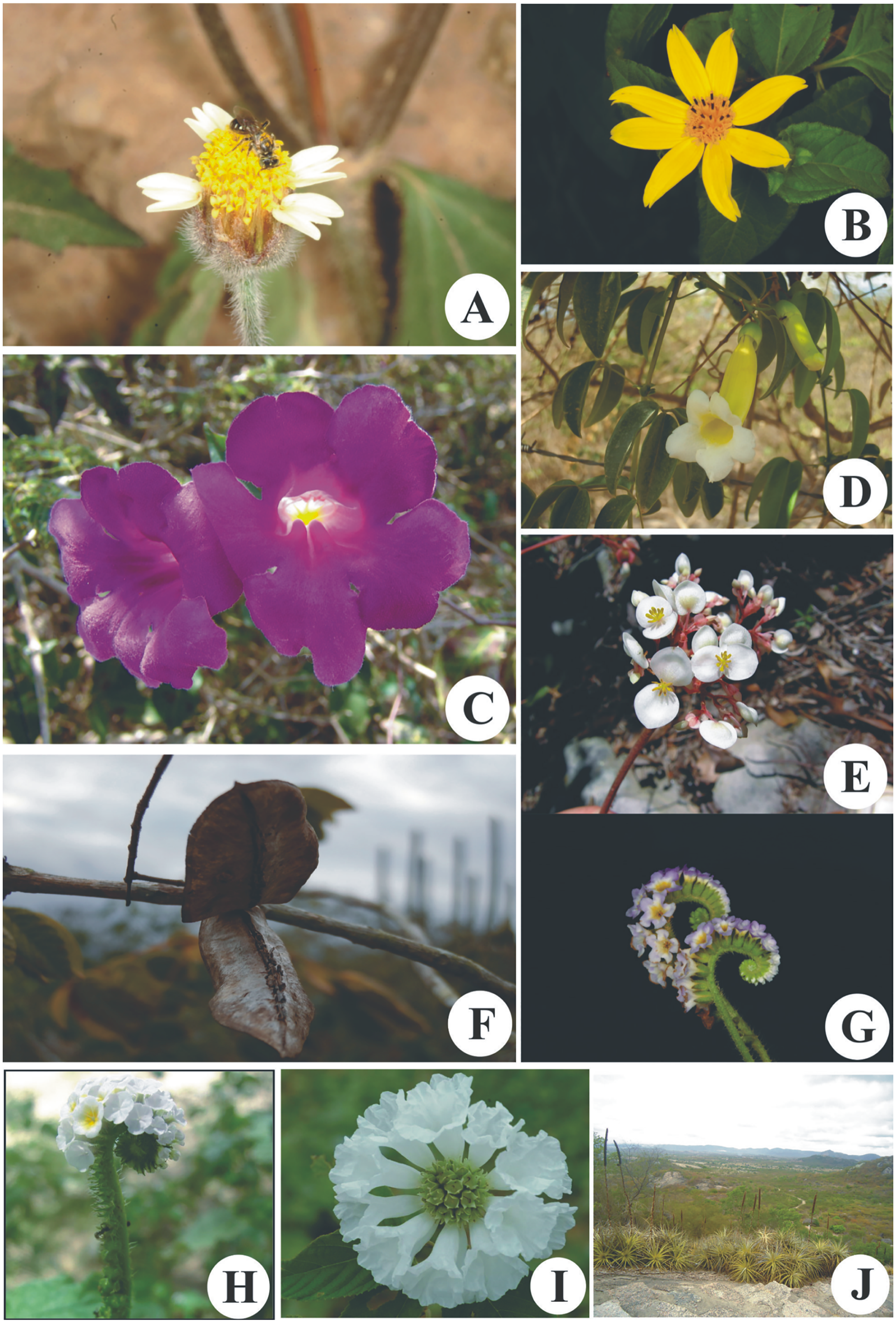

Figure 3. A. *Tridax procumbens* L. **B.** *Wedelia vilosa* Gardner. **C.** *Fridericia limae* (A.H. Gentry) L.G. Lohmann. **D.** *Anemopaegma laeve* DC. **E.** *Begonia grisea* DC. **F.** *Jacaranda rugosa* A. Gentry. **G.** *Heliotropium angiospermum* Murray. **H.** *Heliotropium elongatum* (Lehm.) I.M. Johnst. **I.** *Varronia leucocephala* (MORO c.) J. S. Mill. **J.** *Encholirium spectabile* Mart. ex Schult. f.

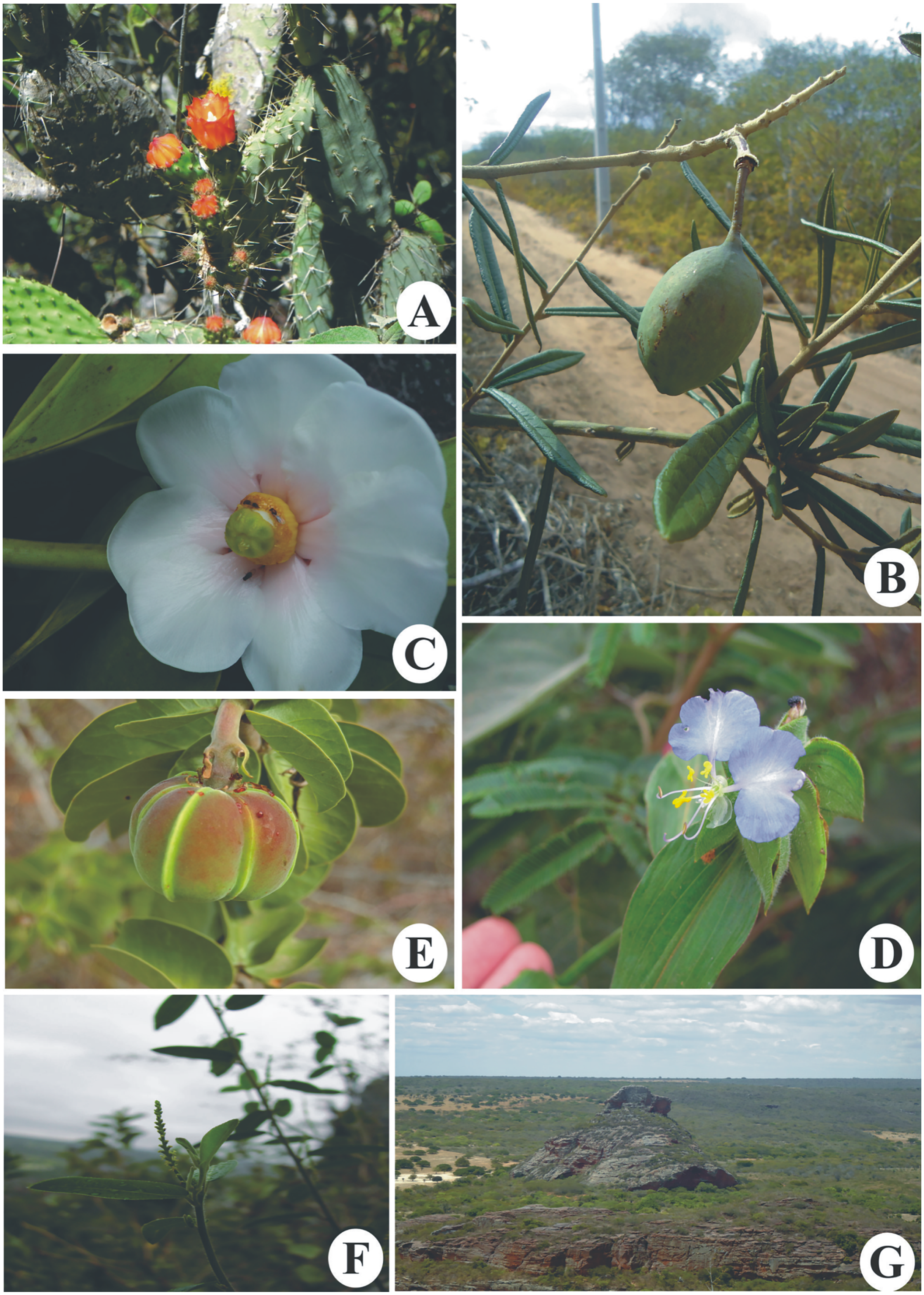

Figure 4. A. *Tacinga palmadora* (Britton & Rose) N.P. Taylor & Stuppy. **B.** *Colicodendron yco* Mart. **C.** *Clusia nemorosa* G. Mey. **D.** *Commelina erecta* L. **E.** *Jatropha molissima* (Pohl) Baill. **F.** *Microstachys corniculata* (Vahl) Griseb. **G.** Partial view of the Catimbau National Park.

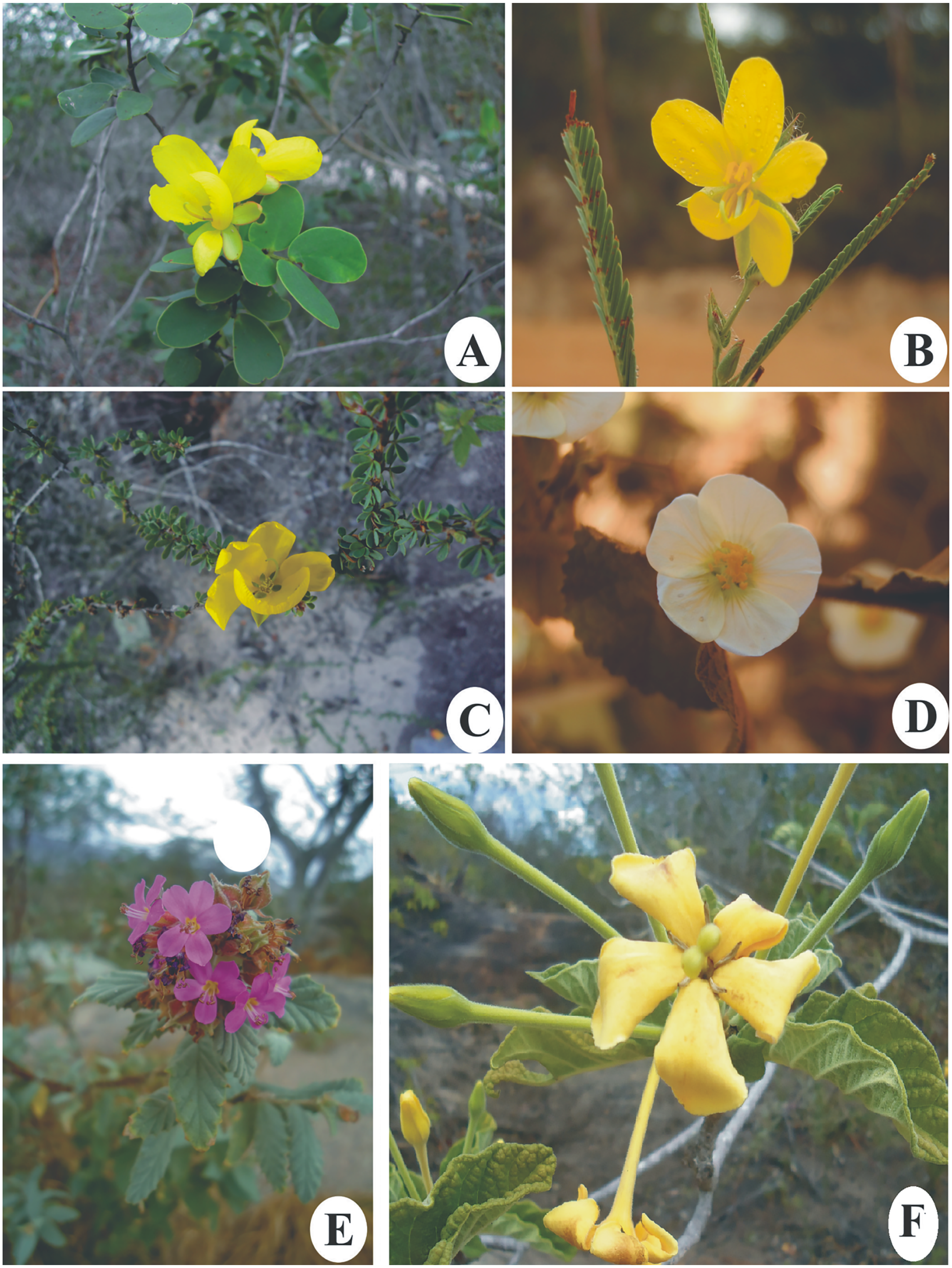

Figure 5. A. *Chamaecrista cytisoides* (DC. Collad.) H.S. Irwin & Barneby. **B.** *Chamaecrista flexuosa* (L.) Greene. **C.** *Chamaecrista ramosa* var. *ramosa* (Vogel) H.S. Irwin & Barneby. **D.** *Herissantia crispa* (L.) Brizicky. **E.** *Melochia tomentosa* L. **F.** *Tocoyena formosa* (Cham. & Schltdl.) K. Schum.

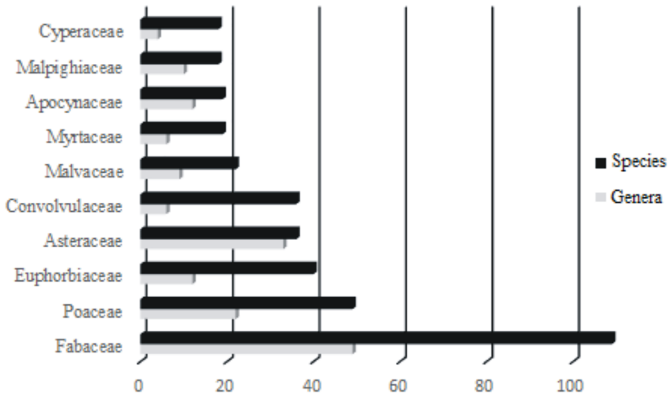

Figure 6. Number of species and genus in the ten most representative families in the Catimbau National Park.

shrub (49%), with a very prominent presence of vines/climbers (15.6%) (Figure 7). The families Poaceae, Asteraceae, Cyperaceae, Bromeliaceae, and Fabaceae stood out in terms of their herbaceous species, with Poaceae and Asteraceae representing 7.5% and 3.1% of all of the species recorded. The families that stood out in terms of the numbers of shrub representatives were Fabaceae (26 species) and Euphorbiaceae (17 species).

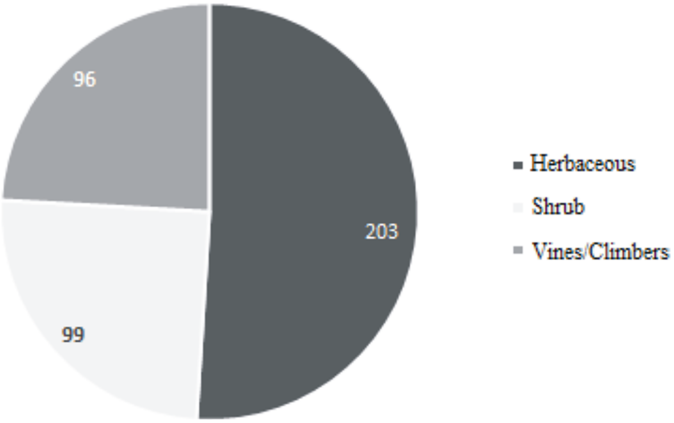

Figure 7. Number of species in the Catimbau National Park considering the habit.

Among vines/climbers, the most diverse families were Convolvulaceae (25 spp.), Fabaceae (12 spp.), Malpighiaceae (11 spp.), Apocynaceae (10 spp.), Bignoniaceae (7 spp.), and Dioscoreaceae and Sapindaceae (6 species each).

Among the 613 species listed for the CNP, 34 are new records for Pernambuco State (see table 1). Some of the species found in the CNP occur in most Brazilian states (e.g., *Alternanthera brasiliana* (L.) Kuntze, *Anacardium occidentale* L., *Asclepias curassavica* L., *Chamaecrista flexuosa* (L.) Greene, *Bidens pilosa* L., *Commelina erecta* L., *Croton glandulosus* L., *Emilia fosbergii* Nicolson, *Ipomoea nil* (L.) Roth, and *Lantana camara* L.). On the other hand, four are endemic to the CNP (*Acritopappus buiquensis* D.J.N. Hind & Bautista, *Dyckia limae* L.B. Sm., *Mandevilla catimbauensis* Souza-Silva, Rapini & J.F. Morales, and *Tillandsia catimbauensis* Leme, W. Till & J.A. Siqueira). The majority of species listed for the Park are native to Brazil, although (*Agave sisalana* Perrine, *Cajanus cajan* (L.) Millsp., *Calotropis procera* (Ait.) Ait. f., *Citrullus lanatus* (Thunb.) Matsum. & Nakai, *Cucumis anguria* L., *Euphorbia tirucalli* L., *Megathyrsus maximum* (Jacq.) B.K. Simon & S.W.L. Jacobs, *Melinis repens* (Willd.) Zizka, *Momordica charantia* L.) are exotic (BFG 2015). Among the species recorded for the Park (87 spp.), many show restricted geographical distributions, occurring only in the Caatinga domain [e.g., *Acritopappus buiquensis*, *Alstroemeria longistaminea* Mart. ex Schult. & Schult f., *Apodanthera congestiflora* Cogn., *A. glaziovii* Cogn., *Chresta pacourinoides* (Mart. ex DC.) C.M. Siniscalchi & B. Loeuille, *Cnidoscolus quercifolius* Pohl ex Baill., *Crotalaria bahiensis* Windler & S.G. Skinner, *Cuspidaria argentea* (Wawra) Sandwith, *Dioclea grandiflora* Mart. ex Benth., *Dyckia limae* L.B. Sm., *Encholirium spectabile* Mart. ex Schult. f., *Fridericia limae* (A.H. Gentry) L.G. Lohmann, *Harpochilus neesianus* Mart., *Ipomoea marcellia* Meissn., *I. pintoi* O'Donell, *Jacaranda rugosa* A. Gentry, *Mandevilla catimbauensis*, *Mansoa paganuccii* M.M. Silva-Castro, *Paralychnophora reflexoauriculata* (G.M. Barroso) MacLeish, *Pilosocereus pachycladus* F. Ritter, *Piptadenia stipulacea* (Benth.) Ducke, *Ruellia asperula* (Nees) Lindau, *R. bahiensis* (Nees) Morong, *Senna rizzinni* H.S. Irwin & Barneby, *Stillingia trapezoidea* Ule, *Tillandsia catimbauensis*, *Trichogonia heringeri* R.M. King & H. Rob., *Thyrsacanthus ramosissimus* Lindau and *Varronia dardani* (Taroda) J.S. Mill.) (Tölke et al. 2011, Giulietti et al. 2002, *Flora do Brasil 2020* (2018)]. The remaining 527 species occur in more than one Brazilian domain, with 70 occurring in both caatinga and Cerrado [e.g., *Allamanda blanchetii* A. DC., *Allophylus quercifolius* (Mart.) Radlk., *Aspilia martii* Baker, *Chamaecrista swainsonii* (Benth.) H.S. Irwin & Barneby, *Chrysanthellum indicum* DC., *Ditassa capillaris* E.Fourn., *Froelichia humboldtiana* Roem. & Schult., *Ipomoea brasiliana* (Choisy) Meissn., *Manilkara rufula* (Miq.) H.J. Lam., *Mimosa ursina* Mart., *Schinopsis brasiliensis* Engl., *Tradescantia ambigua* Mart.]; 35 are found exclusively in the Caatinga and the Atlantic rainforest [e.g., *Croton pedicellatus* Kunth, *Ditassa oxyphylla* Turcz., *Eleocharis flavescens* (Poir.) Urb., *Heliotropium angiospermum* Murray, *Ichnanthus glaber* (Raddi.) Hitchc., *Macroptilium martii* (Benth.) Maréchal & Baudet, *Monteverdia rigida* (Mart.) Biral, *Varronia globosa* Jacq.].

Discussion

An inventory of Brazilian seed plants (BFG 2015) listed 32,086 Angiosperms species, with 3,133 species in Pernambuco State, including 69 endemic species (2.2% of the total number of species). The Caatinga has been ranked as the biome with the fourth highest number of Angiosperm species (4,657) and the highest number of endemic species (913). According to Tabarelli & Vicente (2004), information is still lacking in relation to the composition of 41% of the caatinga area, and therefore the actual number of species must certainly be underestimated.

In a floristic and phytogeographical study of semideciduous vegetation of the São José Plateau (near the CNP), the most representative families were Fabaceae (29 spp.), Euphorbiaceae (19 spp.), and Myrtaceae (10 spp.) (Gomes et al. 2006). That Fabaceae is the most representative group in the Caatinga has been corroborated in various floristic lists (Rodal and Melo 1999, Queiroz 2002). The family Myrtaceae, together with Cactaceae, have the largest numbers of endemic species in that ecosystem (Queiroz 2002, Taylor & Zappi 2002). According to the catalogue of vascular plants of the Caatinga (Moro et al. 2014), the most diverse families there are Fabaceae (292 spp.), Euphorbiaceae (103 spp.), Malvaceae (82 spp.), and Asteraceae (67 spp.). Ours results are quite similar to other surveys carried out in caatinga vegetation, which showed Fabaceae, Euphorbiaceae, and Convolvulaceae to be the most diverse (e.g., Rodal 1992, Sales et al. 1998, Araújo et al. 1995, Rodal & Melo 1999, Alcoforado Filho et al. 2003, Barbosa et al. 2005, Santos & Melo 2010, Moro et al. 2014). It is worth noting that the seven most diverse families in the CNP coincide with the ten richest families in Brazil (regardless of the type of ecosystem) (BFG 2015), except for Convolvulaceae, which does not appear on the BFG list. The BFG (2015) list for the caatinga cites Fabaceae, Poaceae, Asteraceae, Euphorbiaceae, Rubiaceae, Cyperaceae, Malvaceae, Apocynaceae, Melastomataceae, and Orchidaceae as the most numerous families.

The diversity of families (approximately 20) in the CNP was significantly higher than average when compared to other surveys conducted in caatinga sites (e.g., Barbosa et al. 2005, Andrade et al. 2002, Amorim et al. 2005, Costa et al. 2009, Santos & Melo 2010). The large number of botanical families found in the study area probably reflects the diversity of regional vegetation types in a varied mosaic of environments. The preservation the Park for at least 16 years could also contribute to the species richness found there.

Among the fifteen most diverse genera of the CNP, nine coincide with the genera listed by Moro et al. (2014) as most representative of Caatinga vegetation (*Croton* - 37 spp., *Mimosa* - 28 spp., *Ipomoea* - 28 spp., *Chamaechrista* - 24 spp., *Erythroxylum* - 24 spp., *Senna* - 21 spp., *Cyperus* - 20 spp., *Eugenia* - 19 spp. and *Sida* - 17 spp.). *Croton* was also found to be the most diversified genus in terms of the number of species in other floristic surveys carried out in areas of caatinga (Guerra et al. 2008, Santos & Melo 2010). The CNP holds 51% and 50% of the species of *Croton* (35) and *Ipomoea* (36) recorded in caatinga vegetation in all of Pernambuco State.

Our data indicated that most genera (175) comprise only one species, while 137 genera comprise more than one. Other surveys carried out in areas of Caatinga (e.g., Rodal 1992, Araújo et al. 1995, Ramalho et al. 2009) likewise showed a tendency of low diversity within the genera, corroborating our results.

Some of the most well-represented families in the region (e.g., Convolvulaceae, Euphorbiaceae, and Fabaceae) have been the subject of reviews (Delgado-Júnior et al. 2014, Gomes et al. 2006, Melo 2013), with extensive collections and identification updates in regional herbaria.

Myrtaceae is generally poorly represented in the surveys of woody components in areas associated with the *sertaneja* depression of the central *sertão* region of Pernambuco (Rodal 1992), although *Eugenia* (with five species) was included in the present study among the fifteen most diverse genera. Moro et al. (2014) ranked Myrtaceae as the eleventh largest family in the caatinga (with 42 species) and highlighted the genus *Eugenia* (with 19 species) as the eighth most diverse taxon in that ecosystem. Ferraz (1998) observed that the Myrtaceae family stood out as the most representative (with nine species) in humid forests located at altitudes ≥1,100 m in the uplands forests of Pernambuco. That family was listed as the sixth richest (with six species) at 900 m a.s.l., with a predominance of species typical of the caatinga. In the survey carried out by Lyra (1982) in montane forests in the municipality of Bituri (Pernambuco), Myrtaceae also stood out as the family with the greatest number of species (9). Upland forests, where the greatest fraction of the representatives of *Eugenia* was found, represent one of the phytophysiognomies that compose the intricate vegetation complex in the study area. Additionally, Myrtaceae species are more frequently found in sedimentary areas, which would help to explain the richness of the family in the study area (Rodal et al. 1999, Lemos & Rodal 2002). *Anadenanthera colubrina* (Vell.) Brenan (Fabaceae) was registered in eight of the twelve floristic groups of the dry forest categories established by DRYFLOR (2016), and is considered one of the most widely disseminated species in the Caatinga and in central Brazil. Our floristic listing included one of its varieties: *Anadenanthera colubrina* var. *cebil* (Griseb.) Reis, which can be found in several regions of Brazil (northeast, central-western, and southeast) and in several of its phytogeographical domains (caatinga, savanna, and Atlantic Forest) (*Flora do Brasil 2020* 2018).

In terms of plant habits, Poaceae was referred to as the family with the highest herbaceous species richness in other studies conducted in the Caatinga (e.g., Costa et al. 2009, Araújo et al. 2002, Alcoforado-Filho et al. 2003). Generally, high numbers of grasses are associated with anthropic intervention and disturbance (Albuquerque & Bandeira 1995).

Delgado-Júnior & Alves (2017) reported results similar to ours in the same study area. According to those authors, Convolvulaceae, Apocynaceae, Fabaceae, Malpighiaceae, and Bignoniaceae had the highest number of species with a climbing habit. Other authors (e.g., Rodal et al. 1999, Figueiredo et al. 2000, Costa et al. 2009) reported high diversities of climbing plants in vegetation types associated with sedimentary basins and mountains in the semiarid region of Brazil, which could explain the high number of climbing species encountered in the Park.

Dyckia limae and *Tillandsia catimbauensis* are restricted to the Park and represented by just a few irregularly distributed populations in the area (Fabricante et al. 2014). Those authors reported that due to their extents of occurrence and areas of occupation, both species were classified as critically endangered (CR) according the criteria of IUCN (2010). *Mandevilla catimbauensis* has a number of records in the park, but has not been evaluated in terms of its threatened status (Delgado-Júnior & Alves 2017, *Flora do Brasil 2020* 2018). The specimen labels of *A. buiquensis* do not accurately indicate its collection locations within the Park, and the species was therefore classified as Data Deficient (DD) (Hind & Bautista 2009). The low percentage of exclusive species in the Park reflects the large number of species shared with neighboring floristic groups, and our results contradict reports of high rates of endemism in the CNP (MMA 2002). That fact does not reduce the importance of the area, however, as it shows high diversity and floristic richness, and includes rare and endangered species; it also comprises a mosaic of vegetation forming unique landscapes in the region.

In addition to endemic species, the study area comprises species such as *Bunchosia pernambucana* W.R. Anderson (Malpighiaceae), *Griffinia gardneriana* (Herb.) Ravenna (Amaryllidaceae), and *Jacaranda rugosa* A.H. Gentry (Bignoniaceae) that fall into the endangered category (EN) (IUCN 2010, *Flora do Brasil 2020* 2018). According Amorim et al. (2013), *B. pernambucana* is known from only a few collections and has so far been recorded in only one other conservation area, the Serra Negra Biological Reserve (which has become increasingly degraded due to a lack of supervision). *Setaria parviflora* (Poir.) Kerguélen (Poaceae) was classified as critically endangered; *Apuleia leiocarpa* (Vogel) J.F. Macrb. was classified as

Vulnerable (VU), probably due to its economic importance (Lima et al. 2013); *Jacquemontia chrysanthera* Buril (Convolvulaceae) was also classified as Vulnerable (VU) by Buril (2011).

Considering the data available in the species lists of the *Flora do Brasil 2020* (2018), the Centro Nacional de Conservação da Flora (CNCFlora 2018), and in floristic studies conducted in Pernambuco (e.g., Rodal et al. 1998, Almeida-Júnior et al. 2007, Silva et al 2009, Nascimento et al. 2012, Delgado-Júnior & Alves 2017), 34 new occurrences for Pernambuco State were recorded in the present study. Most of the species reported for the first time in Pernambuco are known to occur in several states in Northeastern Brazil [*Acalypha brasiliensis* Müll. Arg., *Anemopaegma chamberlaynii* (Sims.) Bureau & K. Schum., *Cenchrus echinatus* L., *Cnidoscolus pubescens* Pohl, *Crotalaria bahiensis* Windler & S.G.Skinner, *Croton pedicelatus* Kunth., *Erythroxylum betulaceum* Mart., *Heteropterys byrsonimifolia* A. Juss., *Ichnanthus leiocarpus* (Spreng.) Kunth, *Leptolobium dasycarpum* Vogel, *Mimosa guaranitica* Chod. & Hassl., *M. setosa* Benth., *Mouriri pusa* Gard. ex Gard. *Myroxylon peruiferum* L. f., *Paepalanthus subtilis* Miq., *Psidium myrtoides* O. Berg., *Securidaca coriacea* Bonpl., and *Solanum thomasiifolium* Sendtn.].

The other new records for Pernambuco consist of species currently known to be endemic to Bahia State [*Axonopus laxiflorus* (Trin.) Chase, *Chamaecrista cytisoides* (DC. Collad.) H.S. Irwin & Barneby, *Croton janeirensis* Radd.-Sm. & Govaerts., *Eugenia supraaxillaris* Vell., *Galactia remansoana* Harms., *Libidibia ferrea* var. *leiostachya* (Benth.) Ducke, *Mimosa gemmulata* var. *gemmulata* Barneby, *Paspalum decumbens* Sw., *Portulaca grandiflora* Hook. and *Psidium salutare* (Kunth) O. Berg), Ceará State (*Centrosema pubescens* Benth. and *Eremanthus arboreus* (Gard.) MacLeisch), and Sergipe State (*Pterolepis perpusilla* (Naudin) Cogn.) (*Flora do Brasil 2020* 2018)].

The lack of knowledge of those taxa in Pernambuco probably reflects low collection efforts, and the high numbers of new records for the state encountered demonstrate the importance of the park area. The information generated by the present can be used to subsidize the proper management of the Catimbau National Park and help guide policies to ensure its sustainability.

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Authors Contributions

Sarah M. Athiê-Souza: Conceived the idea and structured the manuscript; contributed to the writing of the text, and the interpretation of the results. Conducted the fieldwork.

José Iranildo Miranda de Melo: Conceived the idea and structured the manuscript; contributed to the writing of the text, and the interpretation of the results.

Luan Pedro da Silva: Reviewed the species list, prepared the maps, the plates of photographs, and the table.

Leidiana Lima dos Santos: Conducted the fieldwork.

Juliana Silva dos Santos: Conducted the fieldwork.

Luciana dos Santos Dias de Oliveira: Conducted the fieldwork.

Margareth Ferreira de Sales: Conducted the fieldwork. Designed the study and has contributed to the correction and discussion of the results, and to research funding.

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

Community structure and species composition of a periodically flooded Restinga forest in Caraguatatuba, São Paulo, Brazil

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Abstract: The aim of this study was to characterize the structure and composition of the tree and shrub community in a 77-ha fragment of Restinga forest in Caraguatatuba, São Paulo, Brazil. In this fragment, forty 20 × 20-m plots (1.6 ha) were systematically allocated and all trees with a stem diameter at breast height (DBH) ≥4.8 cm were sampled. Sixteen plots were randomly chosen to also sample individuals with a DBH of between 1 and 4.8 cm. All individuals were tagged, identified to species level, and their diameters and heights were measured. A total of 2587 individuals (1616 ind./ha) from 119 species and 42 families were found with a DBH >4.8 cm, and 2659 individuals (4154 ind./ha) from 125 species and 38 families were found with a DBH >1 cm. The richest families were Myrtaceae, Lauraceae, and Fabaceae, and the most abundant species were *Diospyros brasiliensis* (Ebenaceae), *Anaxagorea dolichocarpa* (Annonaceae), and *Euterpe edulis* (Arecaceae). The structure of the studied site was similar to that of other Restinga forest sites, but the forest composition was quite distinct. Implications for the conservation and restoration of Restinga forests in this region are discussed in light of the results obtained.
Keywords: *white sandy soil, coastal sandy forest, waterlogging, coastal plain vegetation, Atlantic Forest.*

Estrutura e composição de espécies de uma floresta de Restinga periodicamente alagada em Caraguatatuba, São Paulo, Brasil

Resumo: O objetivo deste estudo foi caracterizar a estrutura e composição da comunidade arbórea e arbustiva em um fragmento de 77 ha de floresta de Restinga em Caraguatatuba, São Paulo. Neste fragmento, 40 parcelas de 20 × 20 m (1,6 ha) foram distribuídas sistematicamente e todas as árvores com diâmetro do tronco na altura do peito (DAP) ≥4,8 cm foram amostradas. Além disso, 16 parcelas foram escolhidas aleatoriamente para amostrar também todos os indivíduos com DAP entre 1 e 4.8 cm de DAP. Todos os indivíduos foram marcados, identificados ao nível de espécie e tiveram seu diâmetro e altura medidos. Para indivíduos acima de 4,8 cm de DAP, foi amostrado um total de 2587 indivíduos (1616 ind./ha) em 119 espécies e 42 famílias. Para indivíduos acima de 1 cm de DAP, foi amostrado um total de 2659 indivíduos (4154 ind./ha) em 125 espécies e 38 famílias. As famílias mais ricas foram Myrtaceae, Lauraceae e Fabaceae. As espécies mais abundantes foram *Diospyros brasiliensis*, *Anaxagorea dolichocarpa* e *Euterpe edulis*. Quando comparado com outros locais de florestas de Restinga, a estrutura florestal do fragmento estudado é semelhante a outras florestas de Restinga. No entanto, a composição da floresta é bastante distinta, evidenciando a heterogeneidade das florestas de Restinga ao longo da costa do Brasil. Implicações para a conservação e restauração das florestas de Restinga são destacadas.
Palavras-chave: *solo arenoso, floresta sobre areia, alagamento, vegetação da planície costeira, Mata Atlântica.*

Introduction

The Atlantic Forest domain harbors a great diversity of forest types, whose characteristics are defined primarily by climatic and topographic factors. However, soils are also an important factor for determining some forest types, such as Restinga forests (also called coastal sandy forests), which are established on Quaternary marine sand deposits. In such edaphic communities, variations in soil properties (e.g., soil texture, nutrient levels, and aluminum content) and groundwater dynamics largely determine variations in forest structure and composition (Scarano 2002, Magnago et al. 2012, Oliveira et al. 2014). As the water table is usually shallow in Restinga forests, tree roots are often subjected to some level of waterlogging (Maun 1994, Scarano 1998). Restinga forests subjected to long-lasting or permanent flooding tend to have low species richness and can show patterns of high species dominance (Galvão et al. 2002, Kurtz et al. 2013), while forests with intermittent flooding regimes tend to harbor more species (Guedes et al. 2006, Marques et al. 2009, Magnago et al. 2012). Although these diversity patterns are well known, there are uncertainties regarding some aspects of Restinga forest structure, such as stem density (Guedes et al. 2006, Marques et al. 2009, Magnago et al. 2012, Oliveira et al. 2014), patterns of species abundance, and the distribution and identity of the most abundant species (Galvão et al. 2002, Martins et al. 2008, Menezes et al. 2010, Assis et al. 2011, Oliveira et al. 2014). Therefore, new inventories are required to improve our understanding of Restinga forest structural and compositional variation.

In the state of São Paulo, Brazil, more surveys have been conducted on rainforests on clayey soils than on Restinga forests (Lima et al. 2015), so information gaps of the Restinga forest still remain along the 400-km coastline (Marques et al. 2011, Lima et al. 2015 and references therein). The south coast of São Paulo has been the most studied (Carvalhaes 1997, Sugiyama 1998, Sztutman & Rodrigues 2002, Carrasco 2003, Lima et al. 2011, Oliveira et al. 2014), while studies in central and northern regions have concentrated on the municipalities of Bertioga (Guedes et al. 2006, Lopes 2007, Martins et al. 2008) and Ubatuba (Cesar & Monteiro 1995, Assis 1999, Assis et al. 2011). Although Caraguatatuba is the largest coastal plain in the northern region, only one study has been conducted in this municipality (Mantovani 1992).

Since the 1970s, the Caraguatatuba region and surroundings municipalities have been converted into urban areas, livestock farms, and more recently, industrial areas. Currently, 63% of the Caraguatatuba coastal plain has been deforested, and most of the forest remnants are small and scattered across the landscape (Souza 2006, Souza & Luna 2008). Knowledge of the different types of vegetation in this region is fundamental to future conservation and restoration strategies. In this study, the structure and composition of a forest fragment in this poorly studied region of the coastal plain were investigated, and the importance of the area for regional conservation strategies was highlighted.

Material and Methods

1. Study site

The study was conducted in a ~77-ha Restinga forest fragment on the sandy coastal plain of Caraguatatuba on the north coast of São Paulo state, Brazil (23°38′22″S, 45°26′59″W (WGS-84); Figure 1). The study siteIt is located on property owned by a local resident, and is surrounded by a heterogeneous matrix of urban occupation eastward, pastures on its southern and western limits, and low-altitude rainforests on the northern boundary. The terrain is almost flat, and the elevation varies between 6 and 8 m above sea level. Due to variations in microtopography, the lowest areas are more susceptible to flooding, and are flooded for between a few days to over a month during rainy periods, particularly the summer. Even in the driest period of the year, the water table is shallow (~30–50 cm), so the area floods after occasional heavy rain. Only a small proportion of our study site is established on sand ridges with well-drained soils. Evidence of palm-heart harvesters, hunting, and domestic animals were found in our study site. Although no evidence of selective wood extraction was found during the fieldwork, it is possible that *Tabebuia cassinoides* was extracted in the past for its traditional use in handicrafts and oar-making (Borges & Peixoto 2009). However, our study site seems to be relatively well-conserved, because there were no signs that any major disturbances had occurred there, as observed in aerial photographs taken from 1967 to 2016, except for a small-scale deforestation event that occurred in a 100-m strip in 1990. Information regarding the secondary vegetation in this strip is provided as Supplementary Material.

The dominant soils in the region are Quartzarenic Neosols and Spodosols, according to Oliveira et al. (1999). These soils are sandy to sandy-clayey, very acidic, infertile, and have a high aluminum content (Table 1). The climate is tropical rainforest (*Af*, following the Köppen classification), with hot, wet summers and cool, dry winters. The average annual temperature is 24.9 °C, and the average temperatures of the hottest and coldest months are 26.5 °C and 20 °C, respectively. The average annual precipitation is 1758 mm. The dry season runs from April to September, and the driest month is July (average of 62.5 mm of precipitation). The wet season runs from October to March and the wettest month is January, with an average of 251.6 mm of precipitation (CEPAGRI 2016).

2. Data collection and analysis

Forty 20×20-m plots (total of 1.6 ha) were sampled from January 2013 to July 2014. The plots were 80 m apart, systematically distributed in the area (Figure 1), and at least 30 m from the edge of the fragment. Only trees with a stem diameter at breast height (DBH) ≥4.8 cm were included in all 40 plots. Individuals with a DBH of between 1 and 4.8 cm, which included shrub species, were additionally sampled in 16 randomly taken plots (Figure 1). All trees and shrubs were tagged and had their diameters measured and their heights estimated. For multi-stemmed individuals, all stems were measured if at least one of them reached the DBH cutoff. After collection, plant specimens were identified by referring to the literature, checking herbarium specimens, and/or consulting experts. Easily identified species, such as *Calophyllum brasiliense*, *Euterpe edulis*, and other palms, were identified in the field. Vouchers of the species identified by experts and/or after herbarium checks were deposited in the herbarium of the Universidade de São Paulo, Campus Piracicaba (ESA) under the collection numbers of M.P. Pansonato. Some unicates that were not deposited in the ESA are available at the Laboratório de Ecologia de Florestas Tropicais of the Universidade de São Paulo. The nomenclature followed that of the Angiosperm Phylogeny Group IV (APG IV 2016).

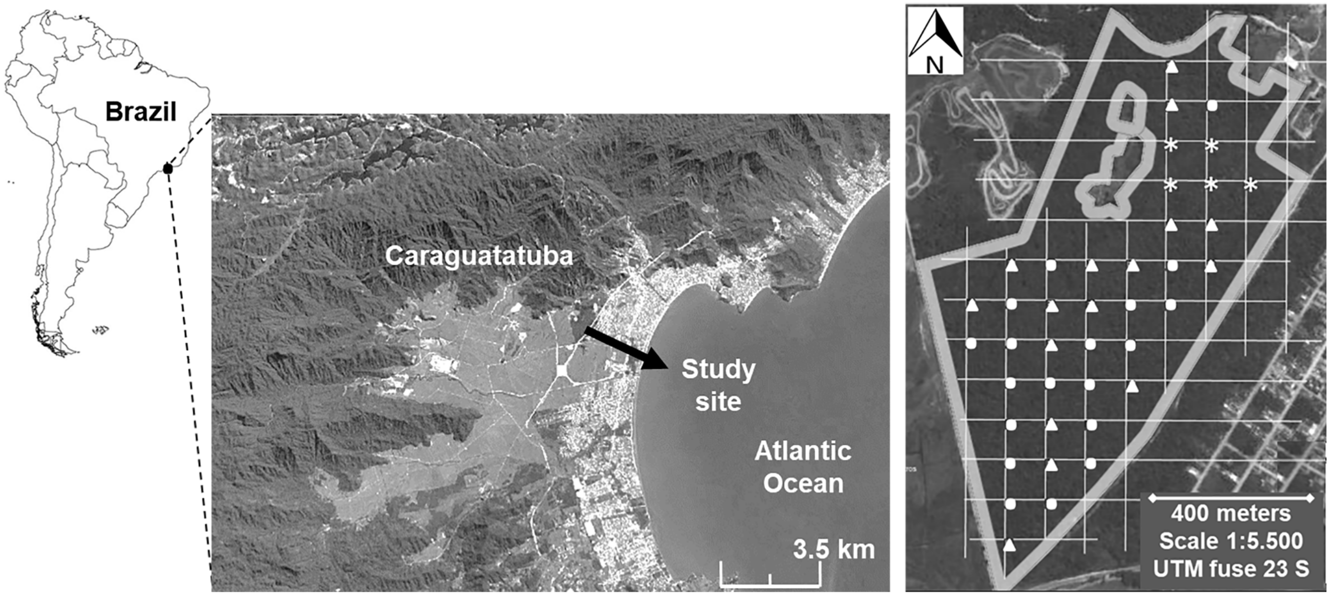

Figure 1. Location of the study site on the coastal plain of Caraguatatuba, SP, Brazil. In the central image, note the continuous forest cover (dark grey) surrounding the study site. In the right image, plots distributed systematically along the Restinga forest fragment are shown. Dots are plots sampled for DBH ≥ 4.8 cm; Triangles are plots sampled for DBH ≥ 1 cm; Asterisks represents the five plots located on degraded strip (see supplementary material).

Table 1. Mean and amplitude (within parentheses) of topsoil (0 - 5 cm) chemical properties in the study site. Samples were collected in the center of each sampled plot.

pH	P	K	Ca	Mg	H+Al	CEC^1	V^2
(CaCl2)	(mg/dm^3)	----------------------(mmolc/dm^3)--------------------				-	(%)
3.1	23.4	1.8	12.1	7.3	357.6	378.7	7.1
(2.7 - 4.4)	(6 - 38)	(0.6 - 3.2)	(3 - 31)	(1 - 14)	(22 - 726)	(27.9 - 755)	(2 - 38)

[1] cation exchange capacity; [2] base saturation.

In order to compare our results with those in the literature, the forest structure and composition data are shown separately for three groups with distinct inclusion criteria: i) DBH ≥1 cm from 16 plots; ii) DBH ≥4.8 cm from 40 plots; iii) DBH ≥10 cm from 40 plots. For each inclusion criteria, the number of individuals per hectare, relative density, basal area, relative dominance, the percentages of multi-stemmed individuals and singletons (species with one individual in the total sample), and the Shannon diversity index (H'; Magurran 1988) were calculated using FITOPAC 2.0 (Shepherd 2009). In order to obtain a better comparison of the values among the three DBH inclusion criteria, the number of species in the DBH ≥4.8 cm and DBH ≥10 cm inclusion criteria were rarefied by sample (20×20-m plots), and the mean species richness values for 0.64 ha and 1 ha, and their respective confidence intervals, were calculated. Sample-based rarefaction curves were calculated using the *vegan* package (Oksanen et al. 2016) in R 3.4.4 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 4222 individuals from 44 families and 141 species were sampled (Table 2). The results varied with the DBH inclusion criteria under consideration (Tables 3 and 4; see also Supplementary Material tables S1 and S2). The smaller the DBH, the greater the tree density, species richness, and Shannon diversity index. The estimated species richness varied between 93 and 114 species per hectare for DBH ≥4.8 cm, and between 73 and 89 species per hectare for DBH ≥10 cm (Supplementary Material Figure S1). Sampling smaller individuals (between 1 and 4.8 cm DBH) had only a minor effect (~3% increase) on the estimated basal area. The proportions of multi-stemmed individuals were similar among the three DBH inclusion criteria (Table 3), and 76 out of 141 species (53.9%) had at least one multi-stemmed individual. Canopy height varied between 14 and 20 m, with emergent trees reaching up to 25 m.

The richest family in all of the DBH inclusion criteria was Myrtaceae, which had nearly twice the number of species than the second-richest (Lauraceae) (Supplementary Material Table S2). The peak in Myrtaceae, Rubiaceae, and Melastomataceae richness was found in the DBH ≥1 cm group, as some species in these families do not have large diameters. The most abundant family in all of the DBH inclusion criteria was Ebenaceae, which was represented by only one species (*Diospyros brasiliensis*), and Myrtaceae was the second-most abundant family for all DBH inclusion criteria because of the abundance of many species (Supplementary Material Table S2). Annonaceae, Arecaceae, and Lauraceae were the most abundant families in the two lowest DBH inclusion criteria, being replaced by Euphorbiaceae and Bignoniaceae, in addition to Lauraceae, when DBH ≥10 cm.

Table 2. Species list and voucher numbers in the herbarium of the Universidade de São Paulo - Campus Piracicaba (ESA) of a Restinga forest in Caraguatatuba - SP, Brazil.

Family/Species	Voucher
Anacardiaceae	
Tapirira guianensis Aubl.	ESA134411
Annonaceae	
Anaxagorea dolichocarpa Sprague & Sandwith	ESA134412
Guatteria australis A.St.-Hil.	ESA134413
Xylopia langsdorfiana A.St.-Hil. & Tul.	ESA134414
Aquifoliaceae	
Ilex dumosa Reissek	ESA134415
Ilex pseudobuxus Reissek	ESA134416
Ilex theezans Mart. ex Reissek	ESA134417
Araliaceae	
Dendropanax monogynus (Vell.) Seem.	ESA134418
Oreopanax capitatus (Jacq.) Decne. & Planch.	
Schefflera angustissima (Marchal) Frodin	ESA134419
Arecaceae	
Astrocaryum aculeatissimum (Schott) Burret	
Attalea dubia (Mart.) Burret	
Bactris setosa Mart.	
Euterpe edulis Mart.	
Geonoma schottiana Mart.	
Bignoniaceae	
Handroanthus albus (Cham.) Mattos	ESA134420
Handroanthus umbellatus (Sond.) Mattos	ESA134421
Jacaranda puberula Cham.	ESA134422
Tabebuia cassinoides (Lam.) DC.	ESA134423
Tabebuia sp. Gomes ex DC.	
Boraginaceae	
Cordia sellowiana Cham.	
Calophyllaceae	
Calophyllum brasiliense Cambess.	ESA134424
Celastraceae	
Maytenus gonoclada Mart.	ESA134425
Clethraceae	
Clethra scabra Pers.	ESA134426
Clusiaceae	
Garcinia gardneriana (Planch. & Triana) Zappi	ESA134427
Cyatheaceae	
Cyathea atrovirens (Langsd. & Fisch.) Domin	
Ebenaceae	
Diospyros brasiliensis Mart. ex Miq.	ESA134428
Elaeocarpaceae	
Sloanea guianensis (Aubl.) Benth.	ESA134429
Euphorbiaceae	
Alchornea triplinervia (Spreng.) Müll. Arg.	ESA134430
Aparisthmium cordatum (A.Juss.) Baill.	ESA134431
Mabea piriri Aubl.	
Maprounea guianensis Aubl.	ESA134433
Fabaceae	
Albizia pedicellaris (DC.) L.Rico	
Andira fraxinifolia Benth.	ESA134434
Hymenolobium janeirense Kuhlm.	
Inga edulis Mart.	
Inga sessilis (Vell.) Mart.	ESA134435
Inga striata Benth.	ESA134436
Machaerium nyctitans (Vell.) Benth.	
Ormosia arborea (Vell.) Harms	ESA134437
Pterocarpus rohrii Vahl	ESA134438
Lacistemataceae	
Lacistema lucidum Schnizl.	
Lamiaceae	
Aegiphila integrifolia (Jacq.) Moldenke	
Vitex polygama Cham.	
Lauraceae	
Aiouea saligna Meisn.	ESA134439
Aniba viridis Mez	ESA134440
Cryptocarya mandioccana Meisn.	
Endlicheria paniculata (Spreng.) J.F.Macbr.	ESA134441
Nectandra grandiflora Nees	
Nectandra oppositifolia Nees	ESA134442
Ocotea aciphylla (Nees & Mart.) Mez	ESA134443
Ocotea brachybotrya (Meisn.) Mez	
Ocotea lanata (Nees & Mart.) Mez	ESA134444
Ocotea mosenii Mez	ESA134445
Ocotea pulchella (Nees & Mart.) Mez	ESA134446
Ocotea venulosa (Nees) Baitello	ESA134447
Persea willdenovii Kosterm.	
Malvaceae	
Eriotheca pentaphylla (Vell. & K.Schum.) A.Robyns	ESA134448
Melastomataceae	
Clidemia cf. *urceolata* DC.	
Huberia ovalifolia DC.	ESA134449
Miconia cinnamomifolia (DC.) Naudin	
Miconia dodecandra Cogn.	ESA134450
Miconia prasina (Sw.) DC.	ESA134451
Miconia pusilliflora (DC.) Naudin	ESA134452
Ossaea sp. DC.	
Tibouchina pulchra Cogn.	ESA134454
Tibouchina stenocarpa (Schrank & Mart. ex DC.) Cogn.	ESA134453
Meliaceae	
Cabralea canjerana (Vell.) Mart.	
Guarea macrophylla Vahl	ESA134455
Trichilia pallens C.DC.	
Monimiaceae	
Mollinedia cf. *ovata* Ruiz & Pav.	

Continuation Table 2.

Family/Species	Voucher
Mollinedia schottiana (Spreng.) Perkins	ESA134456
Moraceae	
Ficus enormis Mart. ex Miq.	
Ficus gomelleira Kunth	ESA134458
Ficus insipida Willd.	
Ficus pulchella Schott	ESA134457
Sorocea jureiana Romaniuc	ESA134459
Myrtaceae	
Blepharocalyx salicifolius (Kunth) O.Berg	ESA134460
Calyptranthes concinna DC.	
Calyptranthes lucida Mart. ex DC.	ESA134461
Eugenia astringens Cambess.	ESA134462
Eugenia brasiliensis Lam.	ESA134463
Eugenia cerasiflora Miq.	ESA134464
Eugenia cereja D.Legrand	
Eugenia melanogyna (D.Legrand) Sobral	
Eugenia monosperma Vell.	ESA134465
Eugenia neoglomerata Sobral	ESA134466
Eugenia oblongata O.Berg	ESA134467
Eugenia stigmatosa DC.	ESA134468
Eugenia sulcata Spring ex Mart.	ESA134469
Marlierea clausseniana (O.Berg) Kiaersk.	ESA134470
Marlierea racemosa (Vell.) Kiaersk.	ESA134471
Marlierea tomentosa Cambess.	ESA134472
Myrcia brasiliensis Kiaersk.	ESA134473
Myrcia glabra (O.Berg) D.Legrand	ESA134474
Myrcia hexasticha Kiaersk.	ESA134475
Myrcia multiflora (Lam.) DC.	ESA134476
Myrcia pubipetala Miq.	ESA134477
Myrcia racemosa (O.Berg) Kiaersk.	ESA134478
Myrcia spectabilis DC.	ESA134479
Myrcia splendens (Sw.) DC.	ESA134480
Psidium cattleianum Sabine	ESA134481
Syzygium jambos (L.) Alston *	ESA134482
Nyctaginaceae	
Guapira hirsuta (Choisy) Lundell	ESA134483
Guapira opposita (Vell.) Reitz	ESA134484
Pisonia sp. L.	ESA134485
Ochnaceae	
Ouratea multiflora (Pohl) Engl.	ESA134486
Ouratea parviflora (A.DC.) Baill.	ESA134487
Olacaceae	
Heisteria silvianii Schwacke	ESA134488
Peraceae	
Pera glabrata (Schott) Poepp. ex Baill.	
Phyllanthaceae	
Hyeronima alchorneoides Allemão	ESA134432
Piperaceae	
Piper gaudichaudianum Kunth	ESA134489
Podocarpaceae	
Podocarpus sellowii Klotzsch ex Endl.	ESA134490
Primulaceae	
Myrsine coriacea (Sw.) R.Br. ex Roem. & Schult.	ESA134491
Myrsine guianensis (Aubl.) Kuntze	
Myrsine venosa A.DC.	ESA134492
Stylogyne lhotzkyana (A.DC.) Mez	ESA134493
Rosaceae	
Prunus myrtifolia (L.) Urb.	ESA134494
Rubiaceae	
Amaioua intermedia Mart. ex Schult. & Schult.f.	ESA134495
Cordiera cf. *myrciifolia* (K.Schum.) C.H.Perss. & Delprete	ESA134496
Ladenbergia hexandra (Pohl) Klotzsch	
Posoqueria latifolia (Rudge) Schult.	ESA134497
Psychotria hastisepala M. Arg.	
Psychotria hoffmannseggiana (Willd. ex Schult.) M. Arg.	ESA134498
Psychotria mapourioides DC.	
Rudgea coriacea (Spreng.) K.Schum.	ESA134499
Rutaceae	
Zanthoxylum rhoifolium Lam.	
Sapindaceae	
Matayba intermedia Radlk.	ESA134500
Sapotaceae	
Ecclinusa ramiflora Mart.	ESA134501
Manilkara subsericea (Mart.) Dubard	ESA134502
Pouteria beaurepairei (Glaz. & Raunk.) Baehni	ESA134503
Pouteria psammophila (Mart.) Radlk.	ESA134504
Siparunaceae	
Siparuna brasiliensis (Spreng.) A.DC.	ESA134505
Styracaceae	
Styrax glabratus Schott	ESA134506
Symplocaceae	
Symplocos estrellensis Casar.	
Theaceae	
Laplacea fructicosa (Schrad.) Kobuski	
Thymelaeaceae	
Daphnopsis racemosa Griseb.	ESA134507
Daphnopsis schwackeana Taub.	
Urticaceae	
Cecropia glaziovii Snethl.	ESA134508
Cecropia pachystachya Trécul	
Coussapoa microcarpa (Schott) Rizzini	ESA134509

* Exotic species.

Table 3. Community-level attributes for three diameter at breast height (DBH) inclusion criteria of a Restinga forest fragment located on coastal plain of Caraguatatuba, Brazil.

Atribute	DBH inclusion criteria		
	≥ 1 (0.64 ha)	≥ 4.8 (1.6 ha)	≥ 10 (1.6 ha)
Total of individuals	2659	2587	1268
Individuals/ha[#]	4154 ± 1127	1616 ± 393	792 ± 263
Basal area (m^2/ha)[#]	31.76 ± 13.3	30.9 ± 10.9	27.08 ± 11.1
Multi-stemmed (%)	14.7	15.7	13.4
Total of species	125	119	95
Species/ha*	--	104 (93-114)	81 (73-89)
Species/0.64ha*	125	90 (77-103)	69 (59-79)
Shannon	3.64	3.61	3.41
Singletons (%)	24.8	24.4	26.3
Families	38	42	37

[#] These values represent the mean ± standard deviation. * These values were estimated by sample-based rarefaction methods. See Supplementary Material for complete rarefaction curves.

Table 4. Relative densities (%) and ranking order (within parentheses) of 15 most abundant species for DBH ≥ 1 cm and their values for larger diameter at breast height (DBH) inclusion criteria sampled in a Restinga forest fragment located on coastal plain of Caraguatatuba, Brazil.

Species	DBH inclusion criteria		
	DBH ≥ 1 (0.64 ha)	DBH ≥ 4.8 (1.6 ha)	DBH ≥ 10 (1.6 ha)
Diospyros brasiliensis	13.8 (1)	19.1 (1)	23.8 (1)
Anaxagorea dolichocarpa	11.5 (2)	6.3 (2)	2.4 (12)
Euterpe edulis	5.7 (3)	6.3 (3)	0.2 (50)
Tabebuia cassinoides	5.1 (4)	3.5 (7)	4.5 (5)
Guarea macrophylla	4.2 (5)	2.6 (10)	0.6 (32)
Nectandra oppositifolia	3.8 (6)	3.1 (8)	4.5 (4)
Eugenia monosperma	3.3 (7)	5.1(4)	3.5 (8)
Sloanea guianensis	3.3 (8)	4.2 (5)	4.7 (3)
Aniba viridis	2.8 (9)	0.9 (28)	0.2 (57)
Bactris setosa	2.7 (10)	0.4 (43)	-
Guapira opposita	2.4 (11)	1.4 (17)	1.5 (15)
Schefflera angustissima	2.4 (12)	2.8 (9)	3.4 (9)
Alchornea triplinervia	2.3 (13)	3.5 (6)	5.7 (2)
Stylogyne lhotzkyana	2.3 (14)	0.6 (36)	0.2 (58)
Myrcia racemosa	1.9 (15)	1.4 (19)	0.5 (36)

A strong dominance by one species, in terms of the number of individuals, was observed in all of the DBH inclusion criteria considered. *Diospyros brasiliensis* had the highest abundance, basal area, and frequency values in all of the DBH inclusion criteria (Table 4 and Supplementary Material Table S1). *Anaxagorea dolichocarpa*, *Euterpe edulis*, and *Alchornea triplinervia* were the second-most abundant species in DBH ≥1 cm, DBH ≥4.8 cm, and DBH ≥10 cm, respectively (Table 4 and Supplementary Material Table S1). For DBH ≥1 cm, the threatened species *E. edulis* and *Tabebuia cassinoides* were also well represented as regenerating individuals (Table 4). Only one exotic species (*Syzygium jambos*) was sampled, with three individuals. *Nectandra oppositifolia* had relatively high basal area values because it can grow to a great size (DBH ≥60 cm). *Tapirira guianensis*, *Calophyllum brasiliense*, and *Manilkara subsericea* had the highest DBH and height values. Only around 10 species were present in more than a half of the plots, and almost a quarter of the species were only found in one plot, irrespective of DBH.

Discussion

Tree density in the study area (1616 ind./ha for DBH ≥4.8 cm) was lower than the average density estimated from 14 Restinga forests in São Paulo state (1442–2756 ind./ha, average 1907 ind./ha; see Lima et al. 2015 and references therein). In addition, the tree density for DBH ≥1 cm (4154 ind./ha) had similar values to the only study of our knowledge that has used this inclusion criteria (3533–4256 ind./ha, average 3895 ind./ha in Oliveira et al. 2014). Differences in tree density could be caused by abiotic factors such as flooding period (Menezes et al. 2010, Marques et al. 2015). Restinga forests that are susceptible to flooding seem to have a greater stem density than those that are not (Guedes et al. 2006, Magnago et al. 2012). However, some studies have found the opposite pattern (e.g., Marques et al. 2009) or no significant differences in stem density between flooded and unflooded Restinga forests (Oliveira et al. 2014). This suggests that other ecological factors could also be relevant, such as successional stage (Chazdon 2003).

In general, forests in early successional stages tend to have greater stem density and lower biomass than old-growth forests (Chazdon et al. 2009). Thus, the low density found in our study site when compared with other Restinga forests could be indicative of the relatively well-conserved status of this site, besides the five recognized secondary plots (see Supplementary Material Table S3). However, the lower density observed could also have been a consequence of harvesting *Euterpe edulis* (101 ind./ha), which probably had long-lasting effects that continue today. In addition, the selective extraction of *Tabebuia cassinoides* individuals (56 ind./ha), which is not currently evident but probably occurred in the past, could also have contributed to the low stem density values obtained. Despite their high relative abundances in this study, these species are usually found at much higher densities in Restinga forests that are susceptible to flooding (for example, 639 ind./ha of *E. edulis* in Rotta et al. 1997, 365 ind./ha in Vanini 1999, and 293.8 ind./ha in Oliveira et al. 2014). However, the *E. edulis* density found for DBH ≥1 cm (237 ind./ha) may indicate that its population is increasing, and could achieve similar values of density as those reported in the above-mentioned studies. Of course, this will only occur if selective extraction ceases or is drastically reduced.

Basal area values (30.9 m²/ha for individuals with DBH ≥4.8 cm) were slightly above the average of other Restinga forests in Brazil (27.3 ± 11.1 m²/ha; Lima et al. 2015 and references therein). The relationship between flooding and basal area that we observed is not supported by the literature, as flooded and periodically flooded sites usually have forests with lower basal areas than unflooded sites (Sztutman & Rodrigues 2002, Guedes et al. 2006, but see Marques et al. 2009). Restinga soils subjected to flooding usually have higher nutrient availability than dry, well-drained sandy soils (Magnago et al. 2012). In a study that compared flooded and unflooded Restinga forests, the relative growth rate was higher on poorly drained soils than on well-drained soils (Marques et al. 2009). It is possible that species that are able to deal with flooding benefit from the higher levels of nutrients available in these sites and accumulate biomass, so have larger basal areas. Large basal areas may also be related to a relatively advanced successional stage (Chazdon 2003), which is the case in our study site (but see Supplementary Material Table S3).

Although multi-stemmed individuals are considered a common feature of Restinga forests (Silva 1990), the average proportion of multi-stemmed individuals in this study was only 14.6%, which is similar to that found by Lima et al. (2011) in a Restinga forest with a mixture of periodically flooded and unflooded plots in Ilha do Cardoso State Park. The proportion of species with multi-stemmed individuals (53.9%) was within the range of other Atlantic Forest types (44–68%; Lima et al. 2011), and lower than the proportion (74%) observed by Lima et al. (2011) in a Restinga forest in southern São Paulo state. The development of multi-stemmed trees has been related to increased light interception, and resprouting and survival after physical damage (Martini et al. 2008). In our study site, the growth of multi-stemmed trees could be related to both of the above-mentioned factors, as well as the open canopies of Restinga forests (Araújo & Lacerda 1987, Sugiyama 1998) and soil instability, which is a common feature of flooded sites and can make species more susceptible to uprooting during storm events (Fraser 1962, Kurtz et al. 2013). The consistency in the proportion of multi-stemmed individuals among the three DBH inclusion criteria sampled in our study site indicates that this feature generally appears in trees at early life stages.

The overall species richness (119 species) was relatively high in comparison with the results obtained by previous studies on other Restinga forests with similar sampling efforts. Only two Restinga forest sites in Brazil have had higher richness values reported for trees with a DBH ≥4.8 cm (Espírito Santo, 159 species and 2 ha sampled, Leite 2010; and Santa Catarina, 128 species and 1 ha sampled, Negrelle 1995). Local environmental heterogeneity may contribute to the establishment of a large number of species (Lundholm 2009). In our study site, variations in environmental features could be mainly related to different levels of waterlogging (Pansonato M.P., pers. obs.) and soil properties. When richness values were rarefied to a common sampling effort (0.64 ha), a richness increase was observed when small individuals were included, probably because of the inclusion of shrub and treelet species in the Myrtaceae, Rubiaceae, and Melastomataceae families.

The Shannon diversity index value was relatively high (H' = 3.61) in comparison to other flooded and unflooded Restinga forests (Menezes et al. 2010 and references therein; Assis et al. 2011 and references therein), particularly considering the high relative abundance of *Diospyros brasiliensis* (ca. 20%), which tends to reduce Shannon values due to reduced equability. Restinga forests with lower species richness than our study site have higher values of diversity (e.g., 71 species and 0.5 ha sampled, H' = 3.703, Fabris & Cesar 1996; 84 species and 0.4 ha sampled, H' = 3.897, Urbanetz et al. 2010; 94 species and H' = 3.637 per hectare, Lima et al. 2011), probably because of higher equability among species. The proximity of our study site to continuous areas of rainforest on clayey soils may have facilitated constant colonization by propagules that originated in the continuous forest and this would tend to increase species richness. Furthermore, if local factors in our study site favored the establishment of certain species, they would also have had their density greatly increased by having a source of propagules in nearby rainforests.

As expected, Myrtaceae was the richest family in our study area. This is a major floristic pattern in the Atlantic Forest (Mori et al. 1983, Scudeller et al. 2001). Lauraceae, Fabaceae, Rubiaceae, and Melastomataceae were also very rich families, as previously found in other studies on Restinga forests (Guedes et al. 2006, Lopes 2007, Martins et al. 2008, Assis et al. 2011, Magnano et al. 2012). Our study site was dominated by *Diospyros brasiliensis* (Ebenaceae), which was the most representative species in terms of density, frequency, and basal area. This species occurs in the coastal plain forests of Rio de Janeiro, São Paulo, and Paraná (Ivanauskas 1997, Santos & Sano 2007, Martins et al. 2008), as well as in the lowlands and riverine forests. It has also been recorded in semi-deciduous forests in São Paulo and Pernambuco states (Soares et al. 2005, Holanda et al. 2010), although this should be viewed with caution, as the misidentification of this species is common (Santos & Sano 2007). Despite species identification, the highest density recorded for this species until now was 59 ind./ha in a seasonal semi-deciduous forest in Pernambuco state (Holanda et al. 2010).

Additionally, this species was found to be dominant in the subcanopy layer of a Restinga forest in a floristic inventory conducted in the nearby municipality of Bertioga, although species abundances were not quantified in this study (Martins et al. 2008). The high abundance of *Diospyros brasiliensis* in our study site (309 ind./ha or 19.1% of all individuals for DBH ≥4.8 cm) is, therefore, very peculiar. The high abundance of *Anaxagorea dolichocarpa* (Annonaceae) (102 ind./ha or 6.3% of all individuals for DBH ≥4.8 cm) is also unusual in Restinga forests.

Sandy coastal plains are geologically recent, and are considered as a subset of species that were originally present in the surrounding Atlantic Forest (Rizzini 1979, Araújo 2000). However, when the densities of some species are compared between coastal plains and adjacent slope forests, they usually differ widely (Scarano 2002, Assis et al. 2011). Some indicator species in coastal plain forests, such as *Ilex theezans* (Marques et al. 2011), confirm this hypothesis. *Calophyllum brasiliense*, *Andira fraxinifolia*, *Myrcia multiflora*, and *Ocotea pulchella* are more abundant in Restinga forests than in slope forests along the Brazilian coast (Lima et al. 2015 and references therein). Low floristic similarities between Restinga forests and nearby rainforests on clayey soils may be caused by soil characteristics (Araújo 2000, Assis et al. 2011). However, the unusual abundance of *Diospyros brasiliensis* and *Anaxagorea dolichocarpa* in our study site should not be solely attributed to general soil characteristics, but colonization processes such as founder effects (Grime 1998) and mass effects (Shmida & Ellner 1984) should also be considered. Further studies on species similarity that take into account species abundance are needed, as they would improve our understanding of differences in species distributions between rainforests on clayey soils and Restinga forests, as well among Restinga forests.

Our study site is located in a region under strong anthropogenic pressure with only a few forest fragments remaining. The recent construction of a road across the Caraguatatuba coastal plain has altered the drainage pattern in a small proportion of the southern part of our study site (Pansonato M.P., pers. obs.). This has also occurred in several areas along the BR-101 highway, and has reduced vegetation cover on the coastal plain (Souza 2006). Therefore, the long-term monitoring of the structure and composition of our study site would improve our understanding of the effects of road construction on coastal plain forests in São Paulo. Furthermore, recent ongoing projects related to transport and seaport infrastructure are generating a high demand for compensatory forest restoration on the northern coast of São Paulo. Knowledge of the local vegetation may provide guidelines for the restoration initiatives, and providing information on local species composition could encourage the use of native local species in restoration initiatives. Forest structure data could help to establish restoration strategies that accelerate the recovery of the main attributes of vegetation.

Our study site is important for both biodiversity conservation and ecosystem services. Its location is strategic in connecting slope forests and the coastal plain, and it probably acts as a stepping stone that facilitates the movement of animals (e.g., seed dispersers) across the landscape. In addition, as our study site is one of the largest Restinga forest remnants in the region, it may function as a source of propagules to recolonize nearby smaller fragments, and areas under secondary succession. Because of the high frequency of heavy rain, many areas on the Caraguatatuba coastal plain are frequently flooded (Boulomytis et al. 2015), so the conservation of this Restinga forest remnant is important because it could act as a water sink that mitigates overflow events (Neary et al. 2009). As overflow events are capable of transporting sediments, Restinga forest remnants probably contribute to sediment retention by preventing sediments being deposited in watercourses or urban areas (Carter 1988). Finally, the Restinga forest studied is only partly protected by ecological-economic zoning, which recommends a soil occupation rate of 20% (Estado de São Paulo, 2017). Given the importance of our study site to the landscape and its proximity to urban areas, we recommend the creation of a Private Reserve of Natural Heritage for its long-term conservation.

Supplementary material

The following online material is available for this article:

Figure S1 - Rarefaction sample-based curves and their respective confidence intervals. A) DBH ≥4.8 cm. B) DBH ≥10 cm.

Table S1 - Parameters of the species sampled for three diameter inclusion criteria of a Restinga forest fragment located on coastal plain of Caraguatatuba, Brazil. N: Number of individuals, Ind./ha: Individuals per hectare, RD: Relative density, BA: Basal area and RDo: Relative dominance. Data ordered by their N for DBH ≥4.8 cm.

Table S2 - Parameters of the families sampled for three diameter inclusion criteria of a Restinga forest fragment located on coastal plain of Caraguatatuba, Brazil. N: Number of individuals, Ind./ha: Individuals per hectare, RD: Relative density, BA: Basal area, RDo: Relative dominance and Nsp: Number of species. Data ordered by their N for DBH ≥4.8 cm.

Table S3 - Parameters of the 72 species sampled in five secondary plots of a Restinga forest fragment located on coastal plain of Caraguatatuba, Brazil. N: Number of individuals, RD: Relative density and Ind./ha: Individuals per hectare. Data ordered by their N for DBH ≥4.8 cm.

Author Contributions

Marcelo Petratti Pansonato: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Renato Augusto Ferreira de Lima: Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Alexandre Adalardo de Oliveira: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Ricardo Bertoncello: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Adriana Maria Zanforlin Martini: 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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Article

Leaf architecture of Rubiaceae Juss. from caatinga vegetation in Brazil

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Abstract: The study and characterization of leaf venation plays a key role in the recognition of taxonomic groups that have been identified mainly based on reproductive traits. This study aimed at characterizing the pattern of leaf venation of Rubiaceae, recognizing individual characters among the taxa of this group and testing the use of leaf architecture as a taxonomic tool capable of providing vegetative and diagnostically characteristics between species. Leaves of 14 species distributed in eight genera from Rubiaceae were diaphanized and classified. The study area is located in Área de Proteção Ambiental Serra Branca/Raso da Catarina, Bahia State, Brazil. The characterization of secondary veins, including type, spacing and number of pairs, third veining patterns, fourth and fifth order, and the conformation of the areola were useful to delimit taxa when used together, providing subsidies to more comprehensive studies.

Keywords: *Diaphanization, Leaf veining, Raso da Catarina, Semiarid.*

Arquitetura foliar de espécies de Rubiaceae Juss. da caatinga brasileira

Resumo: O estudo e a caracterização da venação foliar representam uma possibilidade no reconhecimento de grupos taxonômicos, identificados predominantemente com base em caracteres reprodutivos. O presente estudo objetivou caracterizar o padrão de nervação foliar de Rubiaceae, reconhecer caracteres particulares dentre os táxons deste grupo e testar a utilização da arquitetura foliar como ferramenta taxonômica capaz de fornecer características diagnósticas vegetativas entre as espécies. Foram diafanizadas e classificadas as folhas de 14 espécies distribuídas em 8 gêneros de Rubiaceae na APA Serra Branca/Raso da Catarina. A caracterização das nervuras secundárias, incluindo tipo, espaçamento e número de pares, padrões de nervuras de terceira, quarta e quinta ordem, bem como a conformação das aréolas foram úteis para delimitar os táxons quando utilizados em conjunto, fornecendo subsidío para estudos mais abrangentes.

Palavras-chave: *Diafanização, Nervação foliar, Raso da Catarina, Semiárido.*

Introducion

Rubiaceae Juss. is the fourth largest family in number of species of angiosperms (Robbrecht 1988). In Brazil, the family is represented by 126 genera and 1,412 species, of these 84 genera and 406 species occur in the Northeast, with the greatest diversity in the state of Bahia, where it was recorded 78 genera and 364 species (Barbosa et al. 2018).

Rubiaceae is a monophyletic group of easy circumscription. However, it still faces problems regarding intrafamily classification (Verdcourt 1958, Bremekamp 1966, Bremer & Jansen 1991). The use of vegetative characteristics as diagnostic elements for plant specimens have been considered important in the identification process, in particular those related to the shape of the constituent parts of the plant. Although the shape is accepted as an important element in the taxonomic study, its application has not been explored to its full potential due to the inherent difficulty of description and comparison (Niklas 1994).

Acording to Stace (1989) states that leaf morphology has always played an important role in plant systematics as a whole, particularly to characterize and identify taxa where variation in floral structures is not informative. The leaves present characteristics that can also be used in evolutionary studies, being considered as useful as the floral characteristics, pollen morphology and most of the anatomical characteristics traditionally used in the systematics (Cabral et al. 2016, Ellis et al. 2009, Judd et al. 2009, Hickey & Taylor 1991).

The study of foliar architecture, which was created to assist in the identification of fossils (Hickey 1973), has developed considerably over the years, constituting an important tool for classifying and differentiating problematic groups (Ellis et. al. 2009, Judd et al. 2009, Leaf Architecture 1999).

According to Hickey (1973) the term "leaf architecture" indicates the position and shape of the elements that constitute the external expression of the leave structure including venation pattern, marginal configuration, leaf shape and glandular position, this term is appropriate because the elements of the leaves are arranged in certain defined structural standards that can be described.

Therefore, this study aimed at characterizing the pattern of Rubiaceae leaf venation in the Área de Proteção Ambiental Serra Branca/Raso da Catarina, located in Jeremoabo, Bahia State, recognizing individual characters among the taxa of this group and testing the use of leaf architecture as taxonomic tool capable of providing vegetative diagnostic characteristics among species.

Material and Methods

The Área de Proteção Ambiental Serra Branca/Raso da Catarina (APASB) is located in the municipality of Jeremoabo, northeast of Bahia, between the coordinates 09º53'15.5" to 09º44'34.6"S and 38º49'36,1" to 38º52'20,4"W. The area have 67,237 hectares, bordering to the north with the Estação Ecológica Raso da Catarina (ESEC) and to the south with the Vaza-Barris river, which is the main affluent of the São Francisco river in the region. The area is flat with sandstone formations (Szabo et al., 2007).

There were a total of six collection of the botanical material in different days, from May/2014 to June/2015, covering both periods: rainy and hot/dry. The samples obtained in the field were herborized, according to the methodology of Fosberg & Sachet (1965) and Mori et al. (1989), and are deposited in the herbarium of the Universidade do Estado da Bahia (HUNEB - Paulo Afonso Collection). Fully expanded sun leaves of the third and fourth knot were selected for the study. The fresh materials were fixed in 70% FAA according to the methodology described by Johansen (1940), remaining for 72 hours in the solution. Thereafter, the leaves were transferred to 70% ethanol solution (v/v), where they remained preserved until the technical diaphanization procedure.

For each species, 3-4 leaves were selected. The leaves were mostly intact and uninjured. The leaves were diaphanized in order to not occur any distortion of venation or leaf shape as a whole, resulting from the process of pieces of assembly.

The diaphanization technique used in this work was a modification of the method proposed by Shobe & Lersten (1967). Posteriorly, semipermanent slides with 50% glycerin solution (v/v), and slides with Canada balsam were prepared, and were photographed using a camera (AxioCam ERc5s) attached to the microscope (Zeiss Primo Star). Hickey (1973), Leaf Architecture (1999) and Ellis et al. (2009) were used as references for the description and classification of the veining patterns.

Results

There were a total of 14 species distributed in eight different genera of Rubiaceae of APASB, and described below.

1. *Borreria spinosa* (L.) Cham. & Schltdl.

Symmetrical slide, elliptic to lanceolate, attenuate base, acute apex, entire margin. Pinnate venation, eucamptodromous-brochidodromous. Secondary vein decreasing to the base four-five pairs/side. Intersecondary moderately developed veins, strongly printed. Tertiary veining with alternating percorrent, branched (Figure 1A). Fourth and fifth order reticulated veining in regular polygons. Areola moderately developed, forming three-five sides. Final veins forming one-two branches with no extended vascular endings. Terminal venation of the margin without teeth.

2. *Borreria verticillata* (L.) G. Mey.

Symmetrical and elliptical slide, truncated base, acute apex, serrated margin. Pinnate venation, eucamptodromous. Secondary veins growing to base with three-four pairs/side. Absent intersecondary veins. Randomly reticulated tertiary veins, sinuous, moderately branched. Dichotomized veins of fourth and fifth order (Figure 1B). Undeveloped areolas with three-five sides (Figure 1C). Free final veins forming a branch with enlarged vascular endings (Figure 1D). Terminal venation of the margin forming incomplete ties without teeth.

3. *Cordiera rigida* (K. Schum.) Kuntze

Symmetrical slide elliptic to obovate, acute base, obtuse to subacute apex, entire margin. Pinnate venation, brochidodromous. Secondary veins decreasing to base (Figure 1E) with five-seven pairs/side. Developed intersecondaries veins, heavily printed (Figure 1F). Tertiary opposite percorrent veins, winding. Fourth and fifth order reticulated veins in regular polygons. Developed areolas with four-five sides (Figure 1G). Free final veins forming one-three branches with no extended vascular endings.

4. *Eumachia depauperata* (Müll. Arg.) M.R. Barbosa & M.S. Pereira.

Symmetrical slide, elliptical, convex base, acute apex, entire and revolute margin. Pinnate venation, weak brochidodromous. Irregular secondary veins, with 10 pairs/side. Well developed intersecondaries veins, strongly printed. Tertiary veins randomly reticulated, sinuous, branching. Fourth and fifth order reticulated vein in regular polygons. Areola moderately developed (Figure 1H), with three-five sides. Free final veins forming one-two branches (Figure 1I) with no extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

5. *Hexasepalum apiculatum* (Willd.) Delprete & J. H. Kirkbr.

Symmetrical slide oblong to lanceolate, acute apex, attenuate base, entire margin. Pinnate venation, eucamptodromous-brochidodromous. Secondary veins decreasing to the base with three pairs/side. Absent intersecondaries veins. Tertiary percorrent alternating veins, winding to ramified. Quaternary reticulated in regular polygons veins. Dichotomized fifth order veins. Undeveloped areolas with four-five sides. Free final veins forming one branch with no extended vascular endings (Figure 1J). Venation margin of terminal forming ties incomplete without teeth.

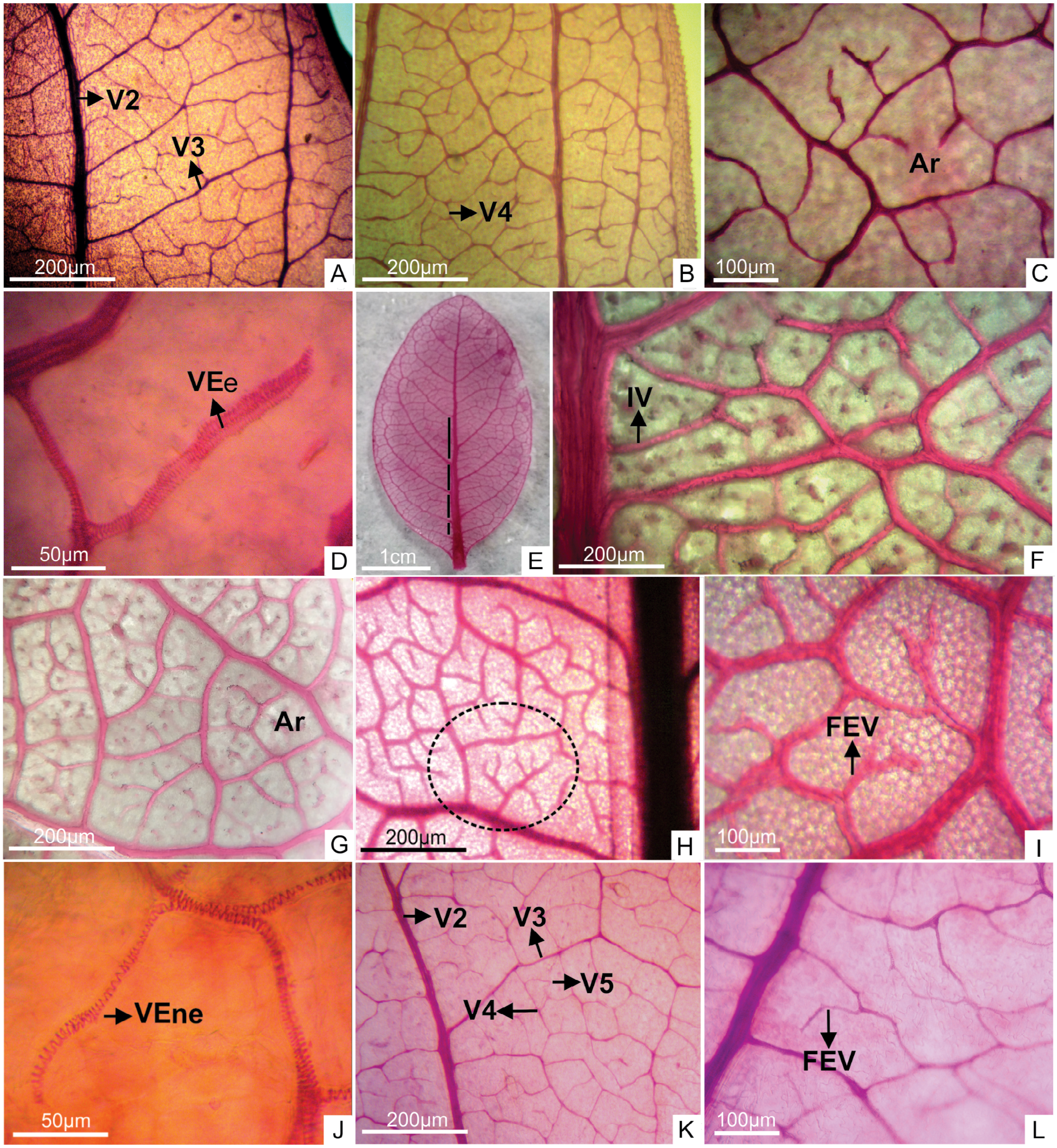

Figure 1. Diaphanized leaves of Rubiaceae of Área de Proteção Ambiental Serra Branca/Raso da Catarina, Bahia. **(A)** *Borreria spinosa*, detail of branched tertiary veins; **(B-D)** *Borreria verticillata*, **B-** with the fourth and fifth order dichotomized veins, **C-** undeveloped areolas and **D-** extended vascular endings; **(E-G)** *Cordiera rigida*, **E-** showing secondary veins decreasing to the base, **F-** intersecondary veins and **G-** developed areolas. **(H)** *Hexasepalum apiculatum*, detail of the final free vein with extensive vascular endings; **(I,J)** *Eumachia depauperata*, **I-** detail of moderately developed areolas and **J-** free end with two branches; **(K,L)** *Mitracarpus salzmannianus*, **K-** detail of the fourth and fifth order reticulated veins in regular polygons and **L-** free end veins with one branch.

6. *Hexasepalum radulum* (Willd.) Delprete & J. H. Kirkbr.

Elliptical slide with asymmetrical base, acute apex, entire margin. Pinnate venation, eucamptodromous. Secondary veins decreasing to base with four-five pairs/side. Absent intersecondaries veins. Mixed opposite/alternating tertiary veins, winding. Quaternary reticulated veins in regular polygons. Areolas developed with three-five sides. Free final veins without branches or forming a (two-three) branch with extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

7. *Hexasepalum teres* (Walter) J. H. Kirkbr.

Symmetrical slide, lanceolate, attenuate base, acute to acuminate apex, entire margin. Pinnate venation, eucamptodromous-brochidodromous. Growing secondary veins to the base, with 4 pairs/side. Absent intersecondaries veins. Tertiary percorrent opposite veins, winding. Quaternary reticulated veins in regular polygons. Dichotomized fifth order veins. Areolas developed with three-five sides. Free final veins without branches or forming a (one-two) branch with extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

8. *Mitracarpus baturitensis* Sucre

Symmetrical slide, linear-elliptic, cuneate base, acute apex, entire margin. Pinnate venation, eucamptodromous. Secondary veins decreasing to base with four-five pairs/side. Little evident intersecondaries veins, weak printed. Tertiary opposite percorrent veins, winding. Fourth order reticulated veins in regular polygons. Dichotomized fifth order. Areola moderately developed with four-five sides. Free final veins forming a branch with broad vascular endings. Venation margin of terminal forming ties incomplete without teeth.

9. *Mitracarpus longicalyx* E.B. Souza & M.F. Sales

Symmetrical slide, elliptical, attenuate base, acute-mucronate apex, entire margin. Pinnate venation, eucamptodromous. Secondary veins decreasing to base with five-six pairs/side. Little evident intersecondary veins, weak printed. Opposite/alternating mixed veins, tertiary winding. Fourth and fifth order reticulated veins in regular polygons. Areola moderately developed with four-five sides. Free final one-two veins forming branches with no extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

10. *Mitracarpus robustus* E.B. Souza & E.L. Cabral

Symmetrical slide, elliptical, attenuate base, acute apex, entire margin. Pinnate venation, eucamptodromous. Secondary veins decreasing to base with five-seven pairs/side. Underdeveloped intersecondary veins, poorly printed. Alternated percorrent tertiary veins, branched. Fourth and fifth order reticulated veins in regular polygons. Undeveloped areolas with three-five sides. Free final one-two veins forming branches with no extended vascular termination. Venation margin of terminal forming ties incomplete without teeth.

11. *Mitracarpus salzmannianus* DC.

Symmetrical slide, elliptical, attenuate base, acute-mucronate apex, entire margin. Pinnate venation, eucamptodromous-brochidodromous. Secondary veins decreasing to the base, with five pairs/side. Intersecondary underdeveloped veins, poorly printed. Percorrent alternate tertiary veins, branched. Fourth and fifth order reticulated veins in regular polygons (Figure 1K). Areola moderately developed with four-five sides. Final ending veins forming an enlarged vascular termination branch (Figure 1L). Venation margin of terminal forming ties incomplete without teeth.

12. *Richardia grandiflora* (Cham. & Schltdl.) Steud.

Symmetrical slide, elliptical, attenuate base, acute apex, entire margin. Pinnate venation, eucamptodromous. Secondary veins growing to the base, with five pairs/side. Absent intersecondary veins. Alternated percorrent tertiary veins, straight, little branched. Dichotomized fourth and fifth order veins. Areola moderately developed with four-five sides. Free final one-two veins forming branches with no extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

13. *Staelia galioides* DC.

Symmetrical slide, linear-lanceolate, attenuate base, acute apex, entire margin. Pinnate venation, brochidodromous. Secondary veins growing to the base, with four-five pairs/side. Underdeveloped intersecondary veins, poorly printed. Percorrent alternate tertiary veins, branched. Fourth and fifth order reticulated veins in regular polygons. Areola moderately developed, with three-four sides. Free final one-two veins forming branches with no extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

14. *Tocoyena formosa* (Cham. & Schltdl.) K. Schum

Symmetrical slide, oval to elliptical, cuneate base, acute apex, entire margin. Pinnate venation, brochidodromous. Uniform secondary veins, with nine-twelve pairs/side. Intersecondary, developed veins, heavily printed. Alternated percorrent tertiary veins, straight. Fourth and fifth order reticulated veins in regular polygons. Moderately developed areolas with four-five sides. Free final veins forming one-two branches with no extended vascular endings. Venation margin of terminal forming ties incomplete without teeth.

Key for Rubiaceae species from APA Serra Branca, Bahia, based on standard characters of leaf venation

1. Eucamptodromous venation; terminal venation margin in incomplete ties.
  2. Secondary veining with decreasing spacing to the base.
    3. Vein network forming up to the fourth order; free final veins with up to three branches .... *Hexasepalum radulum*
    3'. Vein network forming up to the fifth order; free final veins with up to two branches.
      4. Mixed opposite/alternating tertiary veins .... *Mitracarpus longicalyx*
      4'. Tertiary opposite percorrent or alternate percorrent veins.
        5. Intersecondary veins absent ...... *Hexasepalum apiculatum*
        5'. Intersecondary veins present.
          6. Secondary veins with four-five pairs; free final veins with extended vascular endings .... *Mitracarpus baturitensis*
          6'. Secondary veins with five-seven pairs; free final veins with not extended vascular endings .... *Mitracarpus robustus*

2'. Secondary veins with spacing growing to the base.
  7. Secondary veins with three-four pairs; undeveloped areolas; free final veins with extended vascular endings ..... *Borreria verticillata*
  7'. Secondary veins with five pairs; moderately developed areola; free final veins with non-extended vascular endings .... *Richardia grandiflora*

1'. Broquidodromous venation, eucamptodromous-brochidodromous or weak brochidodromous; terminal venation of margin in ties.
  8. Tertiary randomly reticulated veins .... *Eumachia depauperata*
  8'. Tertiary percorrent or alternate percorrent veins.
    9. Strong intersecondary veins.
      10. Secondary veining with decreasing spacing to the base with five-seven pair; tertiary winding veins .... *Cordiera rigida*
      10'. Secondary veins evenly spaced, with nine-twelve pairs; tertiary straight veins .... *Tocoyena formosa*
    9'. Weak intersecondary veins, or absent.
      11. Undeveloped areolas; free final veins with enlarged vascular endings .... *Hexasepalum teres*
      11'. Moderately developed areola; free final veins with no extended vascular endings.
        12. Secondary veining with decreasing spacing to the base; free final veins forming a branch ..... *Mitracarpus salzmannianus*
        12'. Secondary spaced veins growing to the base; free end veins forming up to two branches.
          13. Areolas with up to five sides .... *Borreria spinosa*
          13'. Areolas with up to four sides .... *Staelia galioides*

Discussion

The most used vegetative morphological characters for taxa delimitation are the forms, especially the leaves, because it is the growing body of better access. In this study, these elements were not relevant since there was no significant variation between species, characterized primarily by elliptical type, which may be related to genetic issues. This statement agrees with Parkhurst & Loucks (1972) who state that the size and shape of the leaves are controlled by heredity, as demonstrated in their studies, by high variation of types that occur between different species coexisting in a particular environment. Still, it should be noted that the origin of leaf form occurs for a brief morphogenesis initial period, being more influenced by the formation of secondary veins, since the veins of smaller caliber arise only during expansion of the sheet (Dengler & Kang 2001).

The primary standards of leaf architecture of the studied species correspond to the pinned type, where a main vein of larger caliber diverges from the base to the tip of the slide. In general, the primary and secondary veins are the backbones of the leaf, and tertiary veins are on the smaller-caliber veins, which form a kind of mesh or lattice. The primary vein has the highest caliber and generally extends along the leaf, starting from the base or near the base of the leaf and moving toward the margin or apex (Obermüller et al. 2011).

The secondary veins ranged from broquidodromous to eucamptodromous, although this first type is a common standard for Rubiaceae (Mattos 2011, Mantovani et al. 1995, Da Cunha & Vieira 1997), the latter was well represented in this study, constituting 50% of the species studied, Varjão et al. (2013) observed the same parameters. The species *Borreria spinosa*, *Cordiera rigida*, *Hexasepalum teres* e *Mitracarpus salzmannianus* showed a variation between these two types, described as eucamptodromous-brochidodromous, where secondary veins are strongly curved, joining lightly to each other. In addition, *Eumachia depauperata* showed the standard semibroquidodromous, where secondary veins grow towards the margins and unite forming slightly undeveloped arches. The secondary veins are the next set to be measured after the primaries, they often also have a long haul, and that usually goes from the base of the leaf or a primary vein toward the margin or following in arcs towards the apex (Obermüller et al. 2011).

The spacing between the secondary veins varied between species, allowing the delimitation of taxa. *Borreria spinosa* presented secondary veins decreasing to base with four-five pairs/side, whereas in *B. verticillata* they were found growing to the base with three-four pairs/side. The species of the genus *Hexasepalum* showed decreasing spacing to the base with three pairs/side in *H. apiculatum* and four-five pairs/side *H. radulum*. On the other hand, *H. teres* secondary veins were found to grow for four base pairs. All species of the genus *Mitracarpus* presented spacing of secondary veins decreasing to the base, with four-five pairs/side in *M. baturitensis*, five-six pairs/side in *M. longicalyx*, five-seven pairs/side in *M. robustus* and five pairs/side in *M. salzmannianus*.

The other genera, which are represented in this study by one species each, also presented different spacings. In *Cordiera rigida*, secondary veins were falling to the base with five-seven pairs, in *Eumachia depauperata*, the spacing is irregular with ten pairs. *Richardia grandiflora* and *Staelia galioides* showed growing spaces for the base, with five pairs in the representative of the genus *Richardia* and four-five pairs for the kind of *Staelia*. *Tocoyena* presented secondary veins with uniform spacing.

Tertiary veins and smaller orders veins are less visible and keep a similar caliber, forming reticles. Tertiary veins generally have a narrower size than the set of secondary veins and often connect with the primary and secondary veins throughout the sheet (Obermüller et al. 2011). The importance of the tertiary venation pattern is emphasized by Hickey (1973) and Dilcher (1974). The pattern is a dense, well-structured network that supports webs of smaller calibers. The percorrent alternate type was the most representative of the species, which agress to Saha et al. (2014). Tertiary patterns found in this study were essential to distinguish species.

The highest standards of crosslinking are formed until the fifth order of veins for most species herein studied. Only *Hexasepalum radulum* presented veins just until the fourth order. This ratio of the number of cores orders in one species is not linked to the size of the leaf, since relatively large *Mitracarpus* leaf has the same number of cores orders compared to the *Borreria* species, which have tiny slides. These observations are supported by the work of Saha et al. (2014) for Rubiaceae in India. Most species were fourth and fifth reticulated order veins in regular polygons. However, *H. apiculatum*, *H. teres*, *M. robustus* e *M. baturitensis* differed with dichotomized fifth order veins, and *B. verticillata* and *Richardia grandiflora* with both dichotomized orders. It is noteworthy that the higher the density of the veins, more channels per unit area are available for driving, thus helping transpiration and therefore the maintenance of the leaves for water supply (Fonsêca et al. 2006), and sustention (Souza et al. 2013).

Smaller areas formed by tertiary veins and lower orders of veins originate the so-called areolas, which can provide development, arrangement and different form and may or may not present free end veins inside, which may or may not contain branches. They are considered developed when they have mesh size and regular shape; moderately developed, when the meshes vary in size and have an irregular manner; or undeveloped, incomplete or without limiting the sides of the mesh. In this work, the last two types were observed. The areolas are considered diagnosed vegetative characters consistent in a taxon, being very relevant to the taxonomy (Metcalf & Chalk 1950, Solereder 1908). According to Saha et al. (2014), the nature of the final formation of the areola and the endings free veins are of less importance to the family as a whole, including the tribal level classification system. However, in this study, it was observed that the arrangement and shape of the areola were different and unique for each taxon. These characters were important to differentiate taxa, corroborating to the work from Oliveira et al. (2011), where these characters were used to differentiate Myrtaceae species.

The free end veins (FEVs) or free venules are commonly composed by two-three branches. They are thin and can fill the areal space according to their length. The length is not uniform for a species and is randomly oriented (Saha et al. 2014). The species showed mostly FEVs forming one-two branches usually with ramification. However, *Borreria verticillata*, *Hexasepalum apiculatum*, *Eumachia depauperata*, *Mitracarpus baturitensis* e *M. robustus* presented FEVs with only one branch, and *Cordiera rigida* and *H. radulum* with one-three branches.

The free venules have dual function: carry water and dissolved solutes in the transpiration stream and absorb and translocate the products of photosynthesis to other parts of the plant (Menezes et al. 2012). They can also differentiate for presenting or not extended vascular endings and are used in the delimitation of taxa, as stated by Alvarez et al. (2006) and Mattos (2011). In this study the presence of extensive vascular endings were essential to differentiate *Mitracarpus baturitensis* from *M. robustus*, since they present non-extended vascular endings.

The results showed that the standard features of venation when used together serve as a good taxonomic tool for the separation of genera and species within the family Rubiaceae.

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Author Contributions

Mirella Priscila de Souza Lima: Contribuição substancial no conceito e design do estudo, coleta, análise e interpretação de dados, preparação do manuscrito e revisão crítica.

Adriana Soares: Contribuição na coleta, análise e interpretação de dados, e preparação do manuscrito.

José Lucas Ribeiro de Sousa: Contribuição na análise e interpretação de dados.

Márcia Santos Carvalho: Contribuição na coleta, análise e interpretação de dados.

Jorge Marcelo Padovani Porto: Contribuição para revisão crítica, adicionando conteúdo intelectual.

Francyane Tavares Braga: Contribuição no conceito e design do estudo, coleta e análise de dados, e revisão crítica.

Conflicts of interest

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

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Additional information on reproductive behavior of the Red-breasted Toucan, *Ramphastos dicolorus* (Aves: Piciformes: Ramphastidae)

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Abstract: Ramphastidae is Family of secondary cavity-nesting birds that is endemic to the Neotropical region. Here we provide a comprehensive literature review of nest measurements, and new information on characteristics of the nest cavity, parental nest attendance, incubation and nestling period of the Red-breasted Toucan, *Ramphastos dicolorus*. We also add information on the origin of the seeds found inside toucan nests and propose a potential function for them. Eight nests were studied in three cavities during six breeding seasons at Parque Estadual das Fontes do Ipiranga, São Paulo, and at a private allotment in Ibiúna, southeastern Brazil. The reproductive activities of the birds were observed from September to February. In one cavity, we found the first record of a Meliponini bee hive (*Scaptotrigona bipunctata*) co-existing with a toucan nest. At the beginning of the nesting season, the parents regurgitate seeds on the floor of the cavity, covering it with them. This preparation plays a role in nest sanitation, once the seeds act as a substrate for the feces facilitating the process of carrying them off the nest by the female. The incubation and nestling periods lasted 17.2 ± 1.2 and 43 ± 5.4 days, respectively, and both parents shared egg incubation, nestling, brooding and provisioning, but only the female performed nest sanitation. This differs from published observations on other species of the group, suggesting that the participation of each sex in nest attendance may not be the same for all ramphastids. Additionally, our observations suggest that selecting anthropic locations for nesting can negatively influence toucan nest success.
Keywords: *Breeding biology, cavity-nester, hive coexistence, incubation and nestling periods, regurgitated seeds.*

Informações adicionais sobre o comportamento reprodutivo do tucano-de-bico-verde, *Ramphastos dicolorus* (Aves: Piciformes: Ramphastidae)

Resumo: Ramphastidae compreende uma Família de aves que nidificam em cavidades, são endêmicas da região Neotropical, mas possuem os aspectos relacionados à sua biologia reprodutiva ainda pouco conhecidos. No presente estudo, nós fornecemos uma revisão detalhada da literatura acerca de medidas de ninhos, características das cavidades, cuidado parental e períodos de incubação e permanência do tucano-de-bico-verde *Ramphastos dicolorus*. Nós também descrevemos e propomos uma possível função para as sementes encontradas dentro dos ninhos dessa espécie. Foram estudados oito ninhos, em três diferentes cavidades durante seis temporadas reprodutivas no Parque Estadual das Fontes do Ipiranga, São Paulo, e em um loteamento particular em Ibiúna, ambos no sudeste do Brasil. As atividades reprodutivas foram observadas de Setembro a Fevereiro. Em uma das cavidades foi registrada a coexistência entre uma colmeia de abelhas Meliponini *Scaptotrigona bipunctata* e um ninho de tucanos, sendo este o primeiro registro desse tipo de interação para Ramphastidae. No início do período de nidificação os parentais regurgitavam sementes no fundo da cavidade, cobrindo-o, e nossas observações mostram que essa preparação desempenha o papel de facilitar na limpeza do ninho, uma vez que as sementes servem como substrato para as fezes e possibilitam que a fêmea possa carrega-las para fora. Os períodos de incubação e permanência dos filhotes no ninho duraram 17,2 ± 1,2 e 43 ± 5,4 dias, respectivamente, e ambos os parentais dividiram a incubação dos ovos, aquecimento e alimentação dos ninhegos, mas somente a fêmea realizou a limpeza do ninho. Entretanto, tais comportamentos observados para o tucano-de-bico-verde são diferentes do que se conhece para outras espécies do grupo, sugerindo que o investimento de cada membro do casal no atendimento ao ninho pode não ser o mesmo para todos os ramphastídeos. As observações realizadas também mostram que a seleção de locais antropizados para nidificar poderia influenciar negativamente o sucesso reprodutivo dos tucanos.
Palavras-chave: *Biologia reprodutiva, coexistência com uma colmeia, nidificação em cavidade, períodos de incubação e permanência, sementes regurgitadas.*

Introduction

Ramphastidae, including toucanets and toucans, is a family of thirty-four medium to large birds that are endemic to the Neotropical region (Short & Horne 2002, Remsen et al. 2017). These birds are remarkable for their colorful plumage and very large bills, which play several ecological and behavioral functions (Sick 1997, Tattersall et al. 2009). Ramphastid diversity is greatest in forested environments, from the lowlands of the subtropical Atlantic Forest to the montane forests of the Andes (Short & Horne 2002). Toucans are primarily frugivorous and consume many fruits of a wide range of plants, playing an important role in seed dispersal (Galetti et al. 2000, Pizo et al. 2008). Furthermore, their substantial participation in the predation of eggs and nestlings of other birds has been reported (Santos & Ragusa-Netto 2013, Cockle et al. 2016), highlighting their ecological role as nest predators, while they can also be the prey of larger carnivorous birds (Rangel-Salazar & Enriquez-Rocha 1993, Arévalo & Araya-Salas 2013).

Toucans are obligatory cavity nesters. This means that to be able to nest they need to find vacant cavities or usurp occupied ones (Sick 1997, Short & Horne 2002). Nests are selected mostly from tree cavities made by woodpeckers, or produced by wood decay (Van Tyne 1929, Skutch 1958). Although the basic aspects of toucan nests are well documented, more detailed information on the breeding biology of particular species is scarce. The largest species and subspecies of ramphastids are found in the genus *Ramphastos*. Generally, the following is known about the nesting habits of species in this genus: the nests of seven species have been described; the eggs and nestling characteristics of six species have been reported; and incubation or nestling periods have been described for five species (Short & Horne 2001, 2002 and therein references, Filadelfo 2008, Jesus et al. 2012). In the case of the Red-breasted Toucan *Ramphastos dicolorus* Linnaeus, 1766, only two nest cavities have been measured, with nestling characteristics provided in one contribution (Lange 1967, Jesus et al. 2012). Information on eggs is provided for at least three clutches, one provided by Lange (1967) and two deposited in the Ornithological Collection of the Museu de Zoologia da Universidade de São Paulo – MZUSP from 1905 and 1907. The incubation period is only known for individuals in captivity (Brehm 1969) and parental participation in nestling provisioning has been quantified, without discriminating between female and male contributions (Jesus et al. 2012).

The Red-breasted Toucan is a large bird (42 to 48 cm), with black back and tail, red breast and uppertail coverts, and a yellow throat with a central orange area. The bill is mainly green, with a black basal line and some red above the tomial tooth (Short & Horne 2002). This toucan is found mainly in the Atlantic Forest, from the states of Espírito Santo, Minas Gerais and Goiás to Rio Grande do Sul - Brazil, extending to eastern Paraguay and northeastern Argentina (Sick 1997). Here, we provide new information on the reproduction of the Red-breasted Toucan associated with a literature review. The specific objectives are to 1) present information on the breeding phenology, parental nest attendance, incubation and nestling periods; 2) provide averaged measurements of the cavities used for nesting considering data from the field and literature; and 3) describe the seeds regurgitated by toucans in the nest chamber and hypothesize their potential function.

Materials and Methods

1. Study area

Observations were conducted at Parque Estadual das Fontes do Ipiranga in the city of São Paulo (PEFI, 23º 39’ 05” S and 46º 37’ 24” W, 526.38 ha, 810 m a.s.l.) and at a private allotment in the city of Ibiúna (23º 38’ 47” S and 47º 19’ 17” W, 151 ha, 860 m a.s.l.), both in the state of São Paulo, southeastern Brazil.

The allotment in Ibiúna has private houses with extensive lawns, gardens and exotic trees, but also some remnants of native vegetation in the surroundings, which are classified as Lower Montane Rain Forest (Oliveira-Filho & Fontes 2000). The climate is temperate without a dry season and with hot summers, classified as Cfa according to Koppen-Geiger (Peel et al. 2007). The annual rainfall varies from 40 to 214.9 mm and the temperature averages from 15.3 to 22.3ºC (Cepagri 2017).

In PEFI the climate is temperate with dry winters and hot summers (Cwa according to Koppen-Geiger classification, Peel et al. 2007). Rainfall ranges from 34.1 to 238.2 mm and the average temperature varies from 17 to 24ºC (Cepagri 2017). This Park consists of a preserved fragment of Lower Montane Rainforest, is isolated from other remnants of native vegetation and is surrounded by the urban area of São Paulo (Oliveira-Filho & Fontes 2000, Fernandes et al. 2002).

2. Field procedures

Data were collected for six years, from September to February of 2012/2013, 2013/2014, 2014/2015, 2015/2016, 2016/2017 and 2017/2018. At PEFI one nest was monitored in 2012, and at Ibiúna seven nests were monitored from 2013 to 2017. The nest at PEFI was found while we were walking along a road and observed a toucan getting out of a cavity. The locations of the other nests were reported by local workers. After they were located, the nests were checked once every week. When important events such as egg laying, hatching and fledging were near, we monitored the nests every day after 10:00 a.m. to ensure higher precision in the determination of incubation and nestling periods.

The incubation period was delimited from the first day of incubation to the day before hatching, and the nestling period from the day of hatching to the day before fledging (Winkler 2004). Each period of egg or nestling was considered separately, and when hatching or fledging was asynchronous, it was assumed that the laying order predicted the order of hatching or fledging. Nestlings were never touched to avoid interfering with the duration of nestling period (Skutch 1945). When eggs or nestlings disappeared before the estimated time for hatching or fledging, we assumed that they had been predated (Francisco 2006).

To evaluate participation of the parents in nest attendance during incubation and nestling stages, one-hour sessions of observation were conducted using 8 X 42 mm binoculars behind a blind (Van Tyne 1929, Skutch 1971), or video between 6:00 and 10:00 a.m. Observations were held in three breeding events of the same cavity in Ibiúna, in which one parent was identifiable due to a large and evident scar on the bill. We considered the scar-billed toucan as the female because its bill was shorter, and this represents a sexual dimorphism feature for *R. dicolorus* (Castro et al. 2003).

All nests were located inside tree cavities and were measured after the breeding period. The cavities were measured with a metal caliper of 0.1 mm precision (horizontal and vertical lengths of the chamber entrance) or a measuring tape (for other dimensions). The following measurements were made: height of the cavity (from the lower lip of the entrance to the ground), diameter of the tree at breast height, vertical and horizontal diameters of the entrance (taken in the larger diameter of each one), depth of the cavity (from the lower lip of the entrance to the cavity bottom), and the diameter of the cavity, measured from the lower internal lip of the entrance to the back wall of the cavity (Lange 1967). It was not possible to take the eggs out to measure, but they were classified according to their shapes, as proposed by Winkler (2004). To describe the material deposited inside the nest cavity, the contents of the bottom of one nest (from PEFI) were collected after the fledgling left the nest. The content was washed only with water, and then dried and identified.

3. *Statistical analyses*

Parental investment during incubation, nestling provisioning and nest sanitation were averaged, and ranges were given for the number or duration of events per hour of observation. Differences in the participation of male and female for each activity were analyzed using the Student T-test in software R 2.15.3 (R Development Core Team, 2013). The average and the standard deviations of the measurements of cavities were calculated considering also the nests observed by Lange (1967) and Jesus et al. (2012), and were presented as means ± SD. The amount of seeds found in the content of cavity was presented as percentages for each species in relation to the total amount of seeds.

Results

Eight nesting attempts of Red-breasted Toucans were recorded in three cavities during six breeding seasons; all in anthropic habitats near forest remains (Table 1). The reproductive activity, considering all seasons, occurred from early October to early February (Figure 1). The earliest reproductive activity was observed on 02 September 2015 during nest preparation for laying, and the latest nestlings were observed on early February 2017.

1. *Nest site and seeds used inside cavity*

The nest sites selected were cavities produced by wood decay (n = 1 nest) or excavated by woodpeckers (n = 2). One of the trees excavated by woodpeckers (unidentified species, Figure 2) and the tree with wood decay cavity (*Persea americana* Mill.) were alive and apparently healthy. The third tree (excavated by woodpecker) was a dead trunk of an unidentified palm. The trees were near the edges of a lake (n = 1) or a dam (n = 2), and the diameter of the trunks were 81.3 cm ± 46.7 (range = 42 -133 cm; n = 3 cavities). In the tree found in PEFI, produced by wood decay, there was an active hive of Meliponini bees *Scaptotrigona bipunctata* (Holmberg), apparently positioned directly under the floor of the toucan nest. The entrance of the hive was located directly below the entrance of the toucan nest, but no bee attacks on toucans were observed.

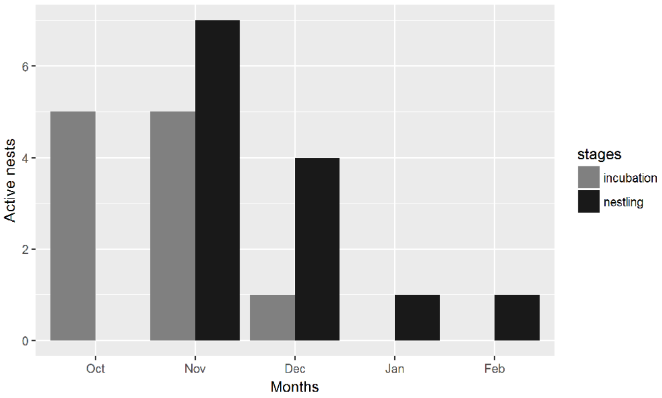

Figure 1. Cumulative number of active nests during incubation and nestling stages from 2012/2013 to 2017/2018 breeding seasons.

The floors of all cavities were covered with several seeds, from at least 10 species of plants. The most highlighted seed in the entire content was from the exotic *Archontophoenix* sp and the most abundant was from the native *Cecropia glaziovii* Snethl. (Table 2). The nest contents also included heads and legs of Coleoptera insects *Macraspis* sp (n = 4 individuals) and old bones of a young toucan, probably from previous nesting events.

During four breeding seasons we were able to record the adults preparing the nests for egg-laying in the same tree cavity at Ibiúna. During a 3-hour observation at this stage, male and female visited the cavity 1 - 4 times/ h and remained inside the nest for an average of 9 min/ h ± 6 min (range = 2 - 16 min per visit), sometimes together. Although we were not directly observing toucans bringing and depositing the seeds (because they regurgitate it), the daily monitoring showed evident changes inside the nests: 1) before the toucans began to use the cavity, the floor was dirty, with dark material, and sometimes it was possible to see sprouted seedlings (Figure 3 a). 2) When toucans started visiting,

Table 1 - Cavities measurements considering nests monitored in the present study and reported in literature.

Nest	Tree condition	Cavity measurements (cm)					
		Cavity height	Tree Diameter	Entrance horizontal diameter	Entrance vertical diameter	Cavity depth	Cavity diameter
Lange 1967	-	600	-	8.5	6.5	15	13
Jesus et al. 2012	Alive	90	-	4.0	9.0	62	15
		70		3.5	11		
PEFI	Alive	284	42	6.0	9.0	151	12
Ibiúna 1	Alive	121	133	7.5	7.0	56	12
Ibiúna 2	Dead	580	69	9.0	10.5	40	7.5
Total average	-	290	81.3	6.4	8.8	64.8	11.9

Table 2 - Seeds found in one nest of *Ramphastos dicolorus*.

Taxa	Scientific name	Status	Units
Arecaceae	*Archontophoenix* sp	Exotic	542 (18%)
	Euterpe edulis Mart.	Native	08 (0.3%)
Caricaceae	*Carica papaya* L.	Exotic	369 (12%)
Cecropiaceae	*Cecropia glaziovii* Snethl.	Native	1900 (62%)
Lauraceae	*Ocotea odorífera* (Vell.) Rohwer	Native	70 (2.3%)
Myrsinaceae	*Rapanea* sp	Native	11 (0.4%)
Myrtaceae	*Myrcia tomentosa* (Aubl.) DC.	Native	92 (3%)
Verbenaceae	*Cytharexyllum myrianthum* Cham.	Native	36 (1%)
-	Not identified1	-	09 (0.3%)
-	Not identified 2	-	22 (0.7%)

Figure 2. Woodpecker excavated cavity used by *Ramphastos dicolorus* and an adult provisioning late staged nestling.

some seeds were observed inside the nests and their number increased daily until the floor was completely covered (Figures 3b and c). We believe that these seeds were regurgitated because they were scarified and totally clean. 3) Thus, toucans were laying the eggs on a "bed" of seeds (Figure 3d). 4) Later, when the nestlings hatched they messed up the "bed" while developing (Figure 3e). 5) After fledging, the cavity floor was dirty again, and remained so until the next nesting activity (Figure 3f).

The walls of the cavities were scratched, and some pieces of wood were observed in one nest during seed deposition (Figure 3b), suggesting that toucans lightly tear the walls of cavities before laying. The height of the cavities ranged from 1.21 to 5.8 m (3.3 m ± 2.3; n = 3) above the ground, cavity depth averaged 82.3 cm ± 60 (range = 40 - 151 cm, n = 3), inside diameter 10.5 cm ± 2.6 (range = 7.5 - 12 cm; n = 3), and cavity entrance averaged 7.5 cm ± 1.5 (horizontal length, range = 6 - 9 cm; n = 3) per 8.8 ± 1.7 (vertical length, range = 7 - 10.5; n = 3).

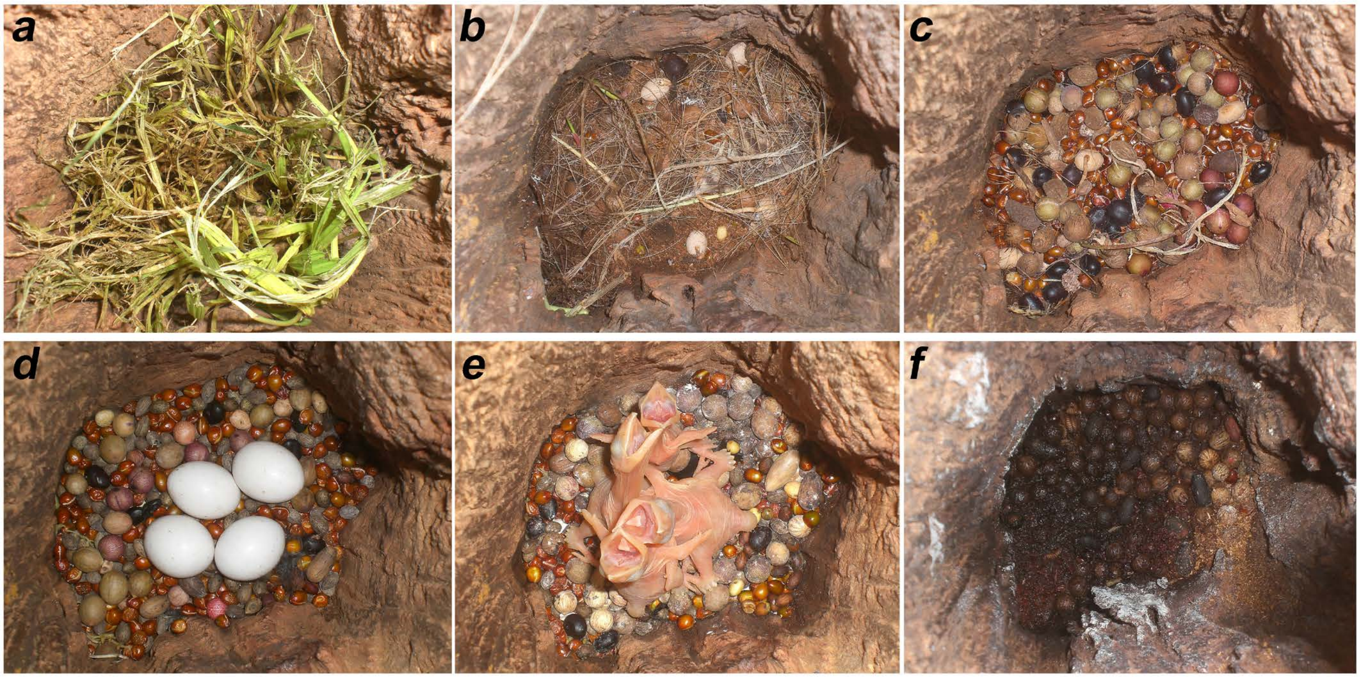

Figure 3. Seeds on the floor during different nesting stages: a – cavity floor dirty with sprouted seedlings; b – initiation of nest cleaning and seed depositing; c – nest floor with many seeds before laying; d – Eggs on the "bed" of seeds; e – Three days old nestling and seeds becoming dirty; f – seeds dirty in the nest after fledging.

The average of cavities' measurements, including the two nests reported in the literature, are presented in Table 1.

2. *Eggs, nestlings, and periods*

Clutch size varied between three (n = 2 nests) or four eggs (n = 5 nests). Eggs were white, and short oval (Figure 3d), but became dirty during incubation. In three of the eight breeding events monitored, the incubation period was recorded with precision. In two nests, eggs were laid on consecutive days, and in one nest with tree eggs, the first two were laid on consecutive days, but the third was laid two days later. The incubation began in the morning that the females laid the first eggs in three nests. The incubation periods were 16 (4 eggs), 17 (2 eggs), 18 (2 eggs), or 19 days (2 eggs) averaging 17.2 ± 1.2 days (10 eggs from three nests).

Hatching was synchronous in one nest (with three eggs, only two of which hatched) and asynchronous in other two nests (with four eggs, three of which hatched in the same day and one hatched one day later). The nestling periods of six young from two nests were 39 (n = 2), 40 (n = 2), and 50 days (n = 2), averaging 43 ± 5.4 days. Hatchings were devoid of a down, were blind, had pinkish skin and displayed pronounced heel pads (n = 4 nests). Their bills and the interior of their mouths were short and pinkish (Figure 3e). They did not have swallow flanges but the tip and borders of their maxillae and mandible were bright white. In two nests we were able to observe the entire development of nestlings, on the second week the nestlings were considerably larger, and their tracts were greyish and visible. On the third week, their eyes were open, sheaths were present, and their bills became greenish. On the fourth week the sheaths were open, and their bills were apparently solid and green. On the fifth week the nestlings were well feathered, and on the sixth week they would put their heads out of the entrance of the cavity to wait for food from the parents (Figure 2). Fledgling are very similar to adults in plumage, but their bills are considerably shorter and paler.

3. *Nest attendance*

In over 16 hours of observations of three nests during the incubation stage, eggs were attended by both parents on average 94% of the time. The female incubated the eggs for 445 min while the male spent 454 min in this activity, resulting in no difference in the average time spent for incubation between sexes (female 27.5 ± 22.4 min/ h, male 28.2 ± 20.5 min/ h, t = - 0.07, P = 0.94). During incubation, parents put their heads out of the cavity to keep a watch, for about 1, 2 or 4 times per hour during 0.1 - 17 minutes for female (2.1 ± 4.5 min/ h) and 0.15 - 6.8 minutes for male (1.5 ± 2.5 min/ h).

In 20 hours of observation at three nests during the nestling stage (all nests with four nestling each one), the young were provisioned 1 - 6 times per hour by both parents (female 2.6 ± 1.1 times/ h, male 1.8 ± 1.2 times/ h), but the proportion of visits by the female was significantly higher (t = 2.2, P = 0.03). During these observations, parents were recorded brooding young six times (female 32.5 ± 10.5 min/ h, and male 19.2 ± 9.2 min/ h), only in the early nestling stage. Nest sanitation was performed only by the female, who carried away dirty seeds with feces in her bill 0 - 4 times per hour (1.5 times/ h ± 1.4). Nestlings were provisioned mainly with entire fruits carried by parents in their bills. During the visits where the content of the bill could be identified, 98% were fruits and 2% were bird eggs. A bird leg was observed being carried out once. When nestlings became larger, the parents began to deliver food from outside of the cavity's entrance, and episodes were recorded in which toucans also carried fruits in their gizzards to regurgitate and to offer to the nestlings (Figure 2).

4. *Nest loss and predation*

From the eight breeding events that were monitored, two nests were predated, one was abandoned and five were successful. Predation occurred during incubation (12.5%) and the nestling stage (12.5%).

One nest was lost due to human disturbance during the nestling stage (12.5%), and five others were successful raising their young to the fledging stage (62.5%). On one instance we saw a Southern Caracara *Caracara plancus* (Miller, 1777) predating upon one fledgling that was caught while perching on an exposed scrub on the lawn around the nest tree. We also saw the fledglings of another nest being attacked by a Black-and-white Teju *Salvator merianae* (Duméril & Bibron, 1839) when they went down to the ground. During the attacks, the parents remained on the trees above for a long time, restless and vocalizing frequently.

Abandonment due to human disturbance occurred after the pruning of a tree near the nest. The parents ceased provisioning the nestlings probably as a result of the loud sounds and intense movement around it. The hungry young left the nests earlier and flightless. Although the young were returned to the nest by our team, they disappeared the next day.

5. *Cavity reuse*

The cavity in PEFI and one of the cavities in Ibiúna were monitored only during one breeding season, but the third one could be monitored for longer and was used by toucans in all seasons from 2012 to 2017. Although the toucans were not marked, we believe that at least one member of the couple used the cavity for reproduction in all events. We could recognize it based on a scar on its bill. In 2016, the young from this cavity's nest were predated upon during the nestling stage around 15 November, and was reused in early December, resulting in four fledging in early February 2017. This same cavity was also reused in September 2017.

Discussion

The height of the cavities used by Red-breasted Toucan measured by Jesus et al. (2012) and Lange (1967) had discrepant values, ranging from 70 to 600 cm above ground, respectively. In the present study, the nests were 121 and 580 cm above ground. Only one nest in our observations was recorded on a dead tree. It has been documented that nest success can be higher in living trees than in dead ones, because the walls of the holes of living trees are stronger and thicker (Wesołowski 2002, Cockle et al. 2015). Height above ground, in contrast, does not seem to be significant in nest survival for large cavity-nesting birds as toucans (Cockle et al. 2015). Small nest entrances also seem to be an important feature in the cavities used by the Red-breasted Toucan, but although species of smaller birds have higher nest survival rates when selecting cavities with smaller entrances (Wesołowski & Rowiński 2004, 2012, Zhu et al. 2012), large birds apparently do not (Cockle et al. 2015). Small entrances might help to avoid large nest predators as coatis and birds of prey (Van Tyne 1929, López-Lanús 2000, Pizo et al. 2008).

Although nests in cavities have been considered more efficient to ensure offspring survival (Auer et al. 2007), many species of predators are able to access the contents of these nests (Berkunsky et al. 2011, Cockle et al. 2016). The construction of nests in association with insect colonies has been reported as a protection strategy used by several species of birds (see Hansell 2000 for review), since ants, wasps and bees can be aggressive and attack other animals that are approaching their nests (Myers 1935, Moreau 1942). To our knowledge, this is the first record of a toucan nesting in association with a social insect, and we believe that this interaction was beneficial for *R. dicolorus* because no attack to the toucans was observed during field work, while the bees always tried to attack people who got close to the nest for inspection. Meliponini bees are stingless, but their aggressive behavior against intruders is common in some species (Knoll et al. 1993). The absence of attacks against the toucans was probably related to the disjunction between the toucan cavity and the hive cavity, besides the fact that the birds tend to enter and exit the nest very rapidly.

Nest attendance between the sexes has been recorded for several species of Ramphastids as *Aulacorhynchus prasinus* (Gould, 1833), *A. caeruleogularis* Gould, 1854, *Ramphastos sulphuratus* Lesson, 1830, *R. vitellinus* Lichtenstein, 1823 and even *R. dicolorus* (Van Tyne 1929, Skutch 1944, Lill 1968, Riley 1986, Jesus et al. 2012), but the proportion of the contribution during incubation, provisioning and nest sanitation by each sex has remained poorly understood. The *R. dicolorus* couple monitored by us shared incubation and nestling provisioning, but only the female was involved in nest sanitation. Females of the Emerald Toucanet, *Aulacorhynchus prasinus* (Riley 1986), tend to be more engaged in nest attendance, while in the case of the Pale-billed Araçari *Pteroglossus erythropygius* (Gould, 1843) both sexes seem to be equally involved (Berg 2001). However, for these two species, the male participated in nest sanitation, suggesting that the nest attendance activities may not be the same for all Ramphastidae species or individuals.

Seeds found inside the nest can reflect a portion of the diet of the adults in the study area (Riley 1986, Van Tyne 1929). About 62% of the seeds that were identified in our study are consumed by *R. dicolorus* (Galetti et al. 2000, Guix et al. 2001, Alvarenga et al. 2004, Fonseca & Antunes 2007). Nesting in cavities that have seeds inside is common among toucans and toucanets (Van Tyne 1929, Skutch 1958, Lill 1968, Jesus et al. 2012), and the presence of these seeds in nests have been considered accidental (Van Tyne 1929, Lill 1968). However, we believe that the deposition of seeds in cavities is part of the nest preparation process and plays an essential role in nest sanitation. Since toucans do not produce fecal sacs, the seeds act as a substrate for the feces, facilitating the process of carrying them off the nest by the female bird (Filadelfo 2008, Jesus et al. 2012).

Toucans and toucanets are mostly frugivorous and important seeds dispersers in the habitats where they live (Galetti et al. 2000, Ragusa-Netto 2008). However, consumption of animals such as arthropods, eggs and nestlings has been recorded for many species (Skutch 1958, Riley 1986, Cockle et al. 2016), but in lower rates than fruits (Remsen et al. 1993, Ragusa-Netto 2008). During our observations of nestling attendance, a few instances of animal matter consumption were recorded. Yet, this might be underestimated because the toucans brought unseen food in the craw. Toucans in general seem to be representative predators of eggs and nestlings (see Menezes & Marini 2017 for review), which would be related to the timing of the toucans' and passerines' breeding season, since young Ramphastids usually eat protein sources during their development even when their diet is mostly frugivorous (Brehm 1969, Remsen et al. 1993).

Red-breasted Toucan nests were observed in environments disturbed by humans, and although toucans can find fruits from exotic tree species for nestling provisioning even in these landscapes (Guix et al. 2001, Fonseca & Antunes 2007) and to be well succeeded in some reproduction attempts in anthropic habitats (Jesus et al. 2012, this work), in two situations the fledglings were exposed to predation by the absence of an understory structure for perching and hiding. Moreover, in a third situation, direct human disturbance resulted in premature fledging. Thus, we believe that the selection of anthropic locations by toucans for nesting negatively influenced nest success in the reported cases.

In conclusion, our contribution provides information about parental attendance, incubation and nestling period in *R. dicolorus*, and our literature review subsumes all available data on nest measurements for this species, providing a better understanding of its breeding biology. Our observations also suggest a different interpretation how these birds use regurgitated seeds on the floor, as nest material deposited purposely for facilitating nest sanitation.

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Author Contributions

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 manuscript preparation; Contribution to critical revision, adding intelectual content. Fernanda Junqueira Vaz Guida: Substantial contribution in the concept and design of the study; Contribution to data collection; 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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Article

Genetic diversity in Mexican wild populations of the Great Curassow (*Crax rubra*)

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Abstract: The Great Curassow (*Crax rubra*) is a Neotropical bird with a wide distribution; it is classified under different threat categories and is listed as a vulnerable species by the IUCN. The Official Mexican Standard, the NOM-059-SEMARNAT-2010, indicates that the Great Curassow is a threatened species, and the subspecies *Crax rubra griscomi*, which is restricted to the island of Cozumel, is classified as critically endangered. Habitat loss and fragmentation, hunting, overexploitation, and illegal trade are among the main factors that have placed the bird at an endangered status. The objective of the present study was to determine the genetic structure and variation of the species within the Mexican populations of *Crax rubra* by using three mitochondrial markers, and one nuclear marker (COI, ND2, Cyt b, and MUSK). We used 47 samples obtained by noninvasive collection (feathers) including the two different color phases of the female plumage: dark brown and barred (rare in Mexico). Gene flow between the remaining populations is recent and extensive, even between the continental and the island population (*C. r. griscomi*). The results indicate that the subspecies *C. r. rubra* and *C. r. griscomi* do not present a marked genetic differentiation because the second exhibits an exclusive haplotype and a shared haplotype. With this study, we provide the first genetic-geographic approximation of the curassow in Mexico, where a gradual geographic differentiation is observed between the western and eastern populations of the Isthmus of Tehuantepec, and we provide a baseline for future studies. Finally, the information obtained indicates that important genetic diversity persists in the Mexican populations of the Great Curassow and that sufficient conservation within the ecosystems of these subspecies can be obtained by protecting them from overexploitation and by conserving and restoring their habitat.
Keywords: *Cracids, noninvasive collection, genetic variability, mitochondrial genes, nuclear gene, gene pool, conservation.*

Diversidad genética en poblaciones silvestres mexicanas del hocofaisán (*Crax rubra*)

Resumen: El hocofaisán (*Crax rubra*) es un ave de la región Neotropical con amplia distribución, que se encuentra en diferentes categorías de riesgo, por la IUCN está catalogada como una especie Vulnerable. A nivel nacional, dentro de la NOM-059-SEMARNAT-2010 está considerada como una especie amenazada, y la subespecie *Crax rubra griscomi* restringida a la isla de Cozumel, está categorizada como en peligro de extinción. Entre los factores principales por los que se encuentra en grave riesgo, destacan la pérdida y fragmentación del hábitat, la cacería, la sobreexplotación, la extracción y el comercio ilegal. El objetivo del presente estudio es conocer la estructura y variación genética de la especie dentro de las poblaciones silvestres mexicanas de *Crax rubra*, mediante el uso de tres marcadores mitocondriales y uno nuclear (COI, ND2, Cyt b y MUSK). A partir de 47 muestras obtenidas mediante colecta no invasiva (plumas) que incluyen las dos fases de plumaje de la hembra: café oscura y barrada (rara en México). Se observó que el flujo génico entre las poblaciones remanentes es reciente y extenso, incluso entre las poblaciones continentales y la isleña (*C. r. griscomi*). Los resultados indican que las subespecies *C. r. rubra* y *C. r. griscomi* no presentan una marcada diferenciación genética dado que la segunda presentó un haplotipo exclusivo y uno compartido. Con el presente estudio brindamos la primera aproximación genético-geográfica del hocofaisán en México y una línea de base para futuros estudios, en el que se observa una diferenciación geográfica gradual entre las poblaciones del oeste y del este del Istmo de Tehuantepec. Finalmente, la información obtenida indica que en las poblaciones mexicanas del hocofaisán persiste una diversidad genética importante y que su conservación en los ecosistemas puede ser suficiente mediante la protección a la sobreexplotación, la conservación y restauración de su hábitat.
Palabras clave: *Crácidos, colecta no invasiva, variabilidad genética, genes mitocondriales, gen nuclear, acervo genético, conservación.*

Introduction

The Cracidae is a bird family native to the Neotropical region, and these birds constitute one of the most vulnerable groups found on the American continent (Delacour & Amadon, 2004; International Ornithological Union, 2018). In Mexico, eight species of Cracids are present, of which five are listed by the International Union for the Conservation of Nature (IUCN) under some endangered category (Pinilla-Buitrago et al., 2014), with the Great Curassow *Crax rubra* (Linnaeus, 1758) being considered vulnerable (BirdLife International, 2016). Additionally, according to the Official Mexican Standard NOM-059-SEMARNAT-2010, the nominal species is considered threatened, and the endemic subspecies *C. r. griscomi* (Nelson, 1926) is considered endangered. *C. r. rubra* is distributed from southern Tamaulipas to the Yucatan Peninsula through the central plateau of the Isthmus of Tehuantepec and continues through Central America, with its range culminating west of the Andes in Colombia and Ecuador (del Hoyo, 1994), whereas *C. r. griscomi* is found exclusively on the Island of Cozumel. Unlike other cracids, *C. rubra* presents a marked sexual dimorphism (Delacour & Amadon, 2004), and the plumage of females have three different color patterns, which are described as the dark brown phase (the most common in Mexico), the barred phase (rare to observe, but present in the country), and the red phase (absent in Mexico). The Cozumel Curassow is distinguished by its reduced body size in comparison to that of the continental curassow (Nelson, 1926; Martínez-Morales, 1996).

The Great Curassow plays a key role in the regeneration of ecosystems due to its highly complex dynamics in seed dispersal and predation (Sedaghatkish, 1996; Muñoz & Kattan, 2007). Given its high sensitivity to the destruction of its habitat (Silva & Strahl, 1997), the species is considered an indicator of habitat quality. Due to its large body size, the Great Curassow is very important within the trophic chains as prey for mammals and raptors (Aranda & Sánchez-Cordero, 1996; Chinchilla, 1997; Delacour & Amadon, 2004). Additionally, in several indigenous communities, it is an important source of food, since it has the largest biomass of birds killed by hunters (Begazo, 1997; Pérez-Irineo & Santos-Moreno, 2017).

Estimates of population densities of *C. r. rubra* indicate that 6,700 to 40,000 individuals are found within the Great Curassow distribution, with a decreasing trend (BirdLife, 2016), whereas, in 2005, *C. r. griscomi* had an estimated population size of approximately 372 individuals, with its conservation status currently unknown (Martínez-Morales et al., 2009). Among the main threat factors for *C. rubra* are habitat loss, fragmentation, or deterioration, hunting, overexploitation, illegal trade, and climate change (Begazo, 1997; Silva & Strahl, 1997; Brooks & Strahl, 2000; Baur, 2008; Kattan et al., 2015; Peterson et al., 2015). Because it is located on an island, *C. r. griscomi* is more susceptible to the introduction of exotic species, hurricanes, urbanization, and the increase in tourism infrastructure (Martínez-Morales, 1999; Cuarón et al., 2009).

Regarding genetic diversity, habitat reduction in cracids may result in a decrease in genetic diversity, as suggested for *C. fasciolata* (Pereira & Wanjtal, 2001; Goncalves et al., 2010). In Mexico, the main risk factors could be overexploitation and habitat loss and reduction. Recent estimates indicate that the country has lost 82% of its original forest cover and that this cover continues to decline (Koleff et al., 2012; Hansen et al., 2013). Given that the distribution of the species is linked to primary forests, *C. rubra* populations in the country are already isolated and extirpated in most of their historical distribution areas. Therefore, to propose a solid foundation for its conservation within the country, the present work aims to assess the genetic diversity and differentiation among the main wild populations throughout its distribution in Mexico.

Material and Methods

1. *Samples*

The sampling strategy was carried out as follows: first, the potential distribution of *Crax rubra* was determined from exclusive records from Mexico obtained from the Database of Global Occurrences of Cracids (Pinilla-Buitrago et al., 2014). Additionally, more recent records that extend the distribution of *C. rubra* (Hernández-Pérez et al. 2014) were included. Once the potential distribution map was obtained, the best-conserved areas were selected for fieldwork, which included the following natural protected areas: El Cielo Biosphere Reserve (Tamaulipas), Sierra Gorda Biosphere Reserve (Querétaro), Los Tuxtlas Biosphere Reserve (Veracruz), Reserva Campesina de Los Chimalapas (Oaxaca), Calakmul Biosphere Reserve (Campeche), Montes Azules Biosphere Reserve (Chiapas), El Triunfo Biosphere Reserve (Chiapas), and the Palenque National Park (Chiapas) (Figure 1, STable 1).

Table 1. Genetic heterogeneity statistics (Kxy= average number of nucleotide differences between populations. Measure of genetic differentiation= Fs, Gst,Nst Δst, γst, and Da= nucleotide substitutions per site between populations) at the three mitochondrial genes of the 10 Mexican populations of the curassow (*Crax rubra*). Populations groups based on the network of their geographic correlation and similarity of habitat.

Population 1	Population 2	Kxy	Gst	Δst	γst	Nst	Fst	Da
Cozumel Island	Escarcega-Conhuas	4.6	0.0708	0.00006	0.039	0.03293	0.03259	0.00005
Cozumel Island	Veracruz_Oaxaca	5.5	0.0423	0.00022	0.136	0.19022	0.18990	0.00037
Cozumel Island	Queretaro_Tamaulipas	6.0	0.0427	0.00072	0.781	0.66688	0.66667	0.00140
Cozumel Island	Chiapas_Belice_Qroo	3.4	-0.0059	0.00016	0.166	0.02806	0.02778	0.00003
Escarcega-Conhuas	Veracruz_Oaxaca	5.7	0.0183	0.00017	0.095	0.14037	0.14051	0.00028
Escarcega-Conhuas	Queretaro_Tamaulipas	5.9	0.2155	0.00037	0.218	0.58355	0.58422	0.00121
Escarcega-Conhuas	Chiapas_Belice_Qroo	3.8	-0.0003	0.00004	0.029	0.01539	0.01535	0.00002
Veracruz_Oaxaca	Queretaro_Tamaulipas	3.5	0.2324	0.00020	0.162	0.29825	0.29841	0.00037
Veracruz_Oaxaca	Chiapas_Belice_Qroo	4.3	0.0014	0.00017	0.120	0.13045	0.13042	0.00020
Queretaro_Tamaulipas	Chiapas_Belice_Qroo	4.2	0.2953	0.00054	0.533	0.68898	0.68889	0.00104

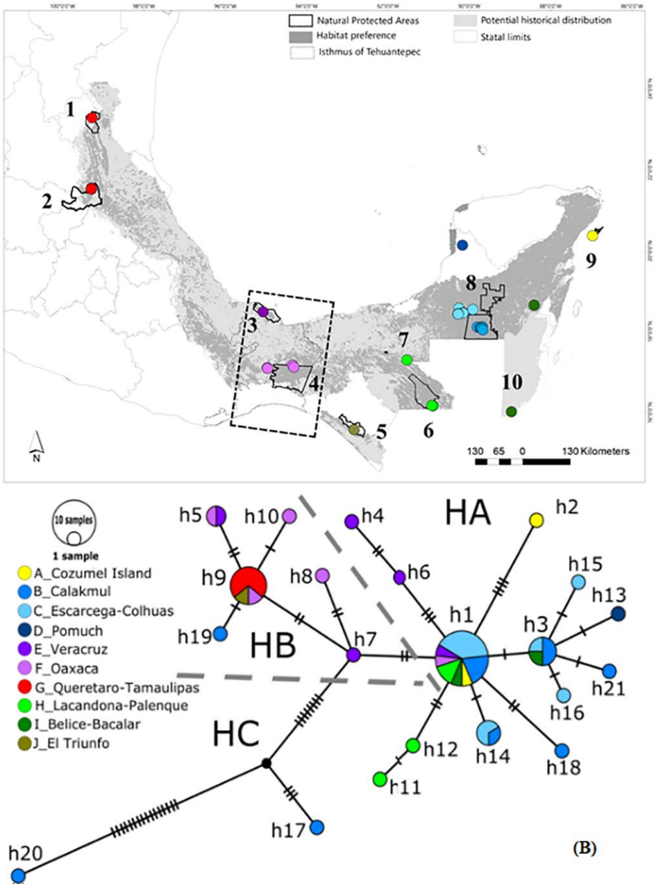

Figure 1. (A) Collection sites throughout the *Crax rubra* distribution range within Mexico. The circles represent the records of the individuals collected. Potential historical distribution of *Crax rubra* (light gray) and current preferred habitat (dark gray). The polygons show the boundaries of the protected areas. (1) El Cielo Biosphere Reserve (Tamaulipas), (2) Sierra Gorda Biosphere Reserve (Querétaro), (3) Los Tuxtlas Biosphere Reserve (Veracruz), (4) Campesina Reserve of Los Chimalapas (Oaxaca), (5) El Triunfo Biosphere Reserve (Chiapas), (6) Montes Azules Biosphere Reserve (Chiapas), (7) Palenque National Park (Chiapas), (8) Calakmul Biosphere Reserve (Campeche), (9) Cozumel Island Biosphere Reserve (Quintana Roo), and (10) Belize. (B) Haplotype network for Mexican populations of the Great Curassow (*Crax rubra*). Barrs indicate numbers of individual mutation, and the size of the circle represents the frequency of each haplotype. The dotted gray line represents the HA and HB haplogroups relatively separated by the Isthmus of Tehuantepec, and the HC haplogroup present in Calakmul.

Because the Great Curassow is an evasive species, the acquisition of direct samples is difficult. A noninvasive sampling method using biological material (feathers) was undertaken for the purpose of not disturbing or stressing individuals. An intensive survey was carried out for collecting samples from perching, or scratching sites being located through vocalizations. To distinguish among individuals, feathers were collected at a minimum distance of 5 km from other samples and sorted in male (black feathers) and female (dark brown phase and barred phase). Additionally, nine samples were included, from scientific collections: Bird Collection of the Museum of Mayan Biodiversity, UACAM (two feathers), and Museum of Zoology, Faculty of Sciences, UNAM (three tissues); from Oaxaca (blood preserved on FTA Whatman cards samples from two adults and a chick), a donation from Belize (one feather), and two samples (feathers) from Cozumel Island (*C. r. griscomi*).

2. *Sequences*

DNA extraction was performed with a DNeasy Blood & Tissue extraction kit (Qiagen); in particular, the feathers were processed using the method used by Morales-Contreras et al. (2016). To reduce the probability of obtaining pseudogenes (Pereira & Baker, 2004a), three mitochondrial regions larger than 1000 bp were amplified using the polymerase chain reaction (PCR) of Cytochrome Oxidase I (COI), NADH subunit 2 (ND2), and Cytochrome b (Cyt b) and the noncoding nuclear gene MUSK (muscle skeletal receptor tyrosine kinase). A platinum Taq PCR kit (Invitrogen) was used, and the reaction volume of the PCR sample was 12 µl: DNA Taq polymerase (1 unit), 1X PCR buffer, 1.5 mM MgCl2, 10% Trehalose, 0.2 mM dNTP mix, 0.2 µM of forward and reverse primers, and 2 to 3 µL of DNA. We used the primers Ltyr (Tavares & Baker, 2008), H8205 (Pereira & Baker, 2004b), COIBird F1 (Hebert et al., 2004), and internal primers AwCintF4 and COIBird R2 (Kerr et al., 2009) to amplify COI. For Cyt b, L14841 (Kocher et al., 1989) and H4a (Harshman, 1996) primers were used. L5215 (Hackett, 1996) and H6313 (Sorenson et al., 1999) primers to ampify ND2. MUSK F and MUSK R (Barker, 2004) primers were used for MUSK gene. The amplification conditions for the three genes were: a denaturation of 94°C for 1 min, followed by 40 cycles at 94°C for 45 seg, 58°C for 1 min, and 72°C for 2 min; and a final extension of 72°C for 10 min. Internal primers of COI were amplified with the conditions suggested by Lijtmaer et al. (2012). MUSK was amplified with a denaturation of 94°C for 3 min, followed by 35 cycles of 94°C for 35 sec, 48°C for 40 sec, and 72°C for 1 min and an extension of 72°C for 10 min. The products were visualized on 1% agarose gels and subsequently sent to the Molecular Biology Laboratory of Biodiversity and Health of the Institute of Biology (UNAM) for sequencing.

3. *Phylogenetic analysis and genetic diversity*

The sequences were edited with CodonCode Aligner V5 (CodonCode Corporation). Using MEGA v6 (Tamura et al., 2013), we verified the absence of stop codons for the translation to amino acids, as well as the multiple sequence alignment for each gene, using ClustalW (Thompson et al., 2002). In MESQUITE (Madisson & Madisson, 2011), the three mitochondrial genes and the MUSK nuclear gene were concatenated. Using DNASP 5.0 (Librado & Rozas, 2009), number of haplotypes (H), haplotypic diversity (H_d), nucleotide diversity (π), genetic diversity (θ), and mismatch distribution analysis were calculated. Genetic differentiation was tested through calculation of genetic heterogeneity statistics (Kxy, Gst, Δst, γst, Nst, Fst and Da) again in DNASP 5.0, with the three mitochondrial genes for mexican populations of *Crax rubra*, grouped with their geographic correlation and similarity of habitat network. In PopART, (Leigh & Bryant, 2015), the haplotype network was constructed using the median-joining algorithm (Bandelt et al., 1999), which is related to the coalescence of the common ancestor. To evaluate the degree of genetic differentiation between and within populations, a molecular variance analysis (AMOVA) was performed. Finally, the Nei genetic distances were obtained for populations according to the geographical distribution of haplotypes.

The phylogenetic reconstruction was done by maximum likelihood (ML) analysis and Bayesian Inference (Irisarri and Zardoya, 2013), with the evolution model of nucleotide substitution TrN with invariant sites I (Reeves, 1992; Tamura and Nei, 1993) obtained under the Akaike information criterion (AIC) with jMODELTEST version 0.1.1 (Reeves, 1992; Posada, 2008). *Crax daubentoni* (KJ914544.129) and *Pauxi pauxi* (AF 165473, AF 1654971, KX356255) were included as external groups. The ML analysis was performed with 2000 bootstrap replicates to evaluate branches support using PhyML 3.0 (Guindon et al., 2010). The Bayesian analysis (BY) was performed with Mr. Bayes version 3.2 (Ronquist et al., 2011), with 10 million generations, discarding 25% of the trees in the burning phase to get the optimal topology. In both analyzes, the evolutionary model of nucleotide substitution previously obtained was used.

Results

Fifty-nine feathers were collected, of which only three corresponded to the barred phase (C_Escarcega_Conhuas_02, C_Escarcega_Conhuas_03 and B_Calakmul_02). In total, genomic DNA was obtained from 41 feathers and six blood samples, which were also incorporated into the analyses. The MUSK gene was not informative because no nucleotide substitutions were founded in 11 of the sequences obtained, and for that reason it was not included in the phylogenetic analyses. The final data matrix consists of 141 sequences belonging to 47 individuals, of which 1034 bp were from ND2 (position 5140 - 6109 of the mitochondrial genome), 1440 bp were from COI (position 6597 - 8037 of the *mt* genome), and 1029 bp were from Cyt b (position 14897 – 15933 of the *mt genome*), and none of the genes exhibited stop codons.

The concatenated sequence data matrix has a total of 3503 bp, with 66 (1.8%) variable sites and 26 (0.7%) parsimoniously informative sites. Regarding the diversity indices, the haplotypic diversity (Hd) was high at 0.874, whereas the nucleotide diversity per site (π) was 0.00169. The average number of nucleotide differences (k) was 4.81, and the genetic diversity (θ) per sequence was 12.68.

In total, 21 different haplotypes were detected sorted in a network containing three main haplogroups. This arrangement showed some geographical congruence with a gradient of frequencies from north to east (Figure 1). Haplogroup HA was formed by the most common haplotype (H1), which includes individuals from most of the populations of southern Mexico and the Yucatan peninsula (green, blue and purple). The second haplogroup (HB) included populations from the North (Querétaro and Tamaulipas, red), with the haplotype of the second-greatest frequency (H9). An individual from the southeastern population (moss green; from El Triunfo, Chiapas), was also included in haplotype H9, which is geographically more isolated within the Mexican distribution. The individuals of Veracruz and Oaxaca (lilac and purple) were represented in the two main haplogroups (HA and HB). The third haplogroup (HC) joins the network through the absence of a haplotype (black color) and included two samples from southern Mexico (Calakmul, Campeche), and these two samples were excluded by eight mutational steps, in addition to three and 16 more steps, respectively. About of the two Cozumel Island samples (*C. r. griscomi*), one of the individuals presented an exclusive haplotype (H2, yellow) with four mutational steps from H1, whereas the second sample was included in the haplotype of greatest frequency (H1).

The genetic distances within and between populations were almost all extremely low (Supplementary Figure 1), even between *C. r. griscomi* and *C. r. rubra*, whose values range from 0 to 0.01%. However, the greatest genetic distance observed was between individuals from Calakmul (B_Calakmul_10) and an individual from Veracruz (E_Veracruz_01), and between the same individual from Calakmul (B_Calakmul_10) and an individual from Cozumel Island (A_ Cozumel_Island_02), both with a distance of 1.0 %. The analysis of paired distributions (Mismatch) resulted in a bimodal pattern, suggesting an equilibrium and not a population expansion (Figure 2A). In addition, the Tajima's D test revealed negative population changes (-2.17342), which confirm that the population shows stability and not expansion. For the other statistics, 10 populations were considered, which correspond to the geographical distribution (Supplementary Table 1). The N_{ST} and F_{ST} values showed that the most distant populations are the most differentiated (Table 1); the Belize-Bacalar representatives compared to the Querétaro-Tamaulipas population had the highest genetic differentiation (Fst=0.888). According to the analysis of molecular variance (AMOVA), all variation is distributed within the populations with a variance component of 17.762, which represents 100% of the genetic variation, whereas among populations, the values are null, which indicates that the gene flow is high and genetic differentiation is low in the species.

The results of the phylogenetic analyses using ML and BY (Figure 2) indicate that *C. rubra* forms a monophyletic group using *C. daubentoni* and *Pauxi pauxi* as outgroups, in which the two samples of haplotype HC (Calakmul) separate from the rest of the samples at the base of the clade (>60%). Within this second clade, two main groups are formed and correspond to the other two haplogroups obtained (HA and HB), with bootstrap support >89% and a posterior probability equal to 1.0. The separation of the populations in the Isthmus of Tehuantepec is notable but incomplete. On the one hand, the Querétaro and Tamaulipas populations are grouped (HB), but the group also includes some samples from Oaxaca and southern Chiapas (El Triunfo). On the other hand, the second group (HA) is formed by haplotypes from Veracruz, northern Chiapas, and from the Yucatán Peninsula, and Cozumel Island. In each of the groups, other internal clades with the same support that is greater than 80% are observed (pp=0.9).

Discussion

The potential historical distribution indicates an extensive distribution of *C. rubra* associated with lowland and highland Neotropical forests in Mexico. However, the current potential distribution reveals low connectivity and isolated populations because of the loss and modification of tropical forests and, consequently, the recent decline of the wild populations of The Great Curassow in Mexico. In disturbed places, no samples or sightings of *C. rubra* were obtained, although inhabitants of the different localities confirmed the presence of the curassow in recent years (10-15 years). On the other hand, in well-conserved areas (core zones of protected natural areas), most of the time, some indication of the presence of the species in the area was found. For example, in the southern part of their distribution, where the continuity of the primary vegetation is wide, the largest number of samples was collected, and the greatest genetic diversity was observed. In the northern part of their distribution, the number of populations was low, and it is where the greatest forest loss has occurred. Additional factors that directly affect the presence of the Great Curassow were confirmed and identified, as subsistence hunting (Chiapas and Oaxaca), or hunting as a recreational activity (some parts of Campeche); the construction of roads throughout the species distribution, land use change, illegal trafficking, and the use of specimens as pets (military camps in Chiapas). Therefore, the conservation of the original vegetation remnants, as well as their connection and protection, is crucial.

The diversification of the Cracidae, which is of Mesoamerican origin, occurred in South America approximately 3 million years ago. *Crax rubra* is the ancestral species within the genus and recolonized Mesoamerica in recent times, possibly in the Pliocene (Hosner et al., 2016). The recent expansion of cracids may explain the low genetic differentiation observed within Mexican populations of *C. rubra* and the low genetic structure. The mismatch analyses revealed that the species has maintained a stable population size and shows no recent expansion, which may imply that wild populations are currently dividing or experiencing a reduction due to fragmentation. We noticed that a gradual geographical differentiation exists from northern to southern and eastern Mexico. With regard to plumage phases of females, they did not show any significant genetic difference, nor exclusive morphological characters of a lineage. The lack of genetic congruence with phases of coloration in the plumage has already been recorded previously (Zink et al., 2005).

In this study, we did not find indications of isolation or reduction of the historical population, possibly due to the prevalence of populations with a moderate size in protected natural areas. However, if the habitat continues to contract and the exploitation pressure does not cease, bottlenecks and loss of genetic diversity could occur as a result of genetic drift.

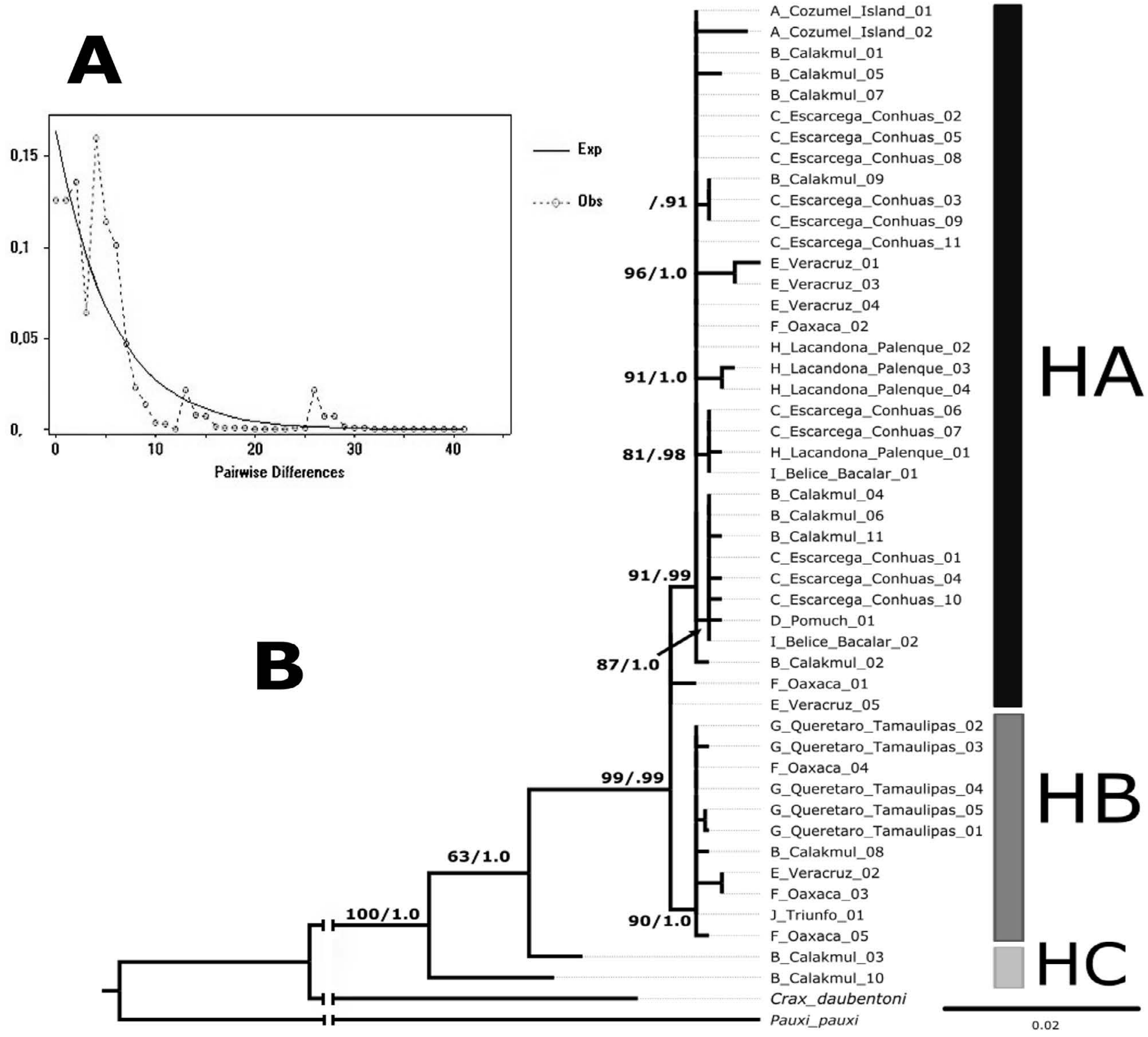

Figure 2. (A) Mismatch distribution of three mitochondrial genes concatenated in the Mexican populations of the Great Curassow (*Crax rubra*). (B) ML tree with 2000 bootstrap replicates for individuals of wild Mexican populations of Great Curassow (*Crax rubra*). Bootstrap values and posterior probabilities of the Bayesian analysis are found in the branches of the tree. The dotted gray bar separates the northern haplogroup (HA) from the eastern haplogroup (HB).

On the other hand, in the southern portion of the distribution range, genetic flow has been high between regions; however, these areas are no longer connected.

Populations of curassows had high levels of gene flow, and a low genetic structure: This may also be due to a slow evolution rate because the species is a large long-lived bird (Nunn & Stanley, 1998). On the one hand, *C. rubra* possibly presents a polygamous reproduction system (Alvarez-Prada & Ruiz-García 2015) and thus is able to maintain levels of considerable genetic diversity. Coupled with its limited exclusivity for a given habitat, the species can maintain gene flow and therefore a low genetic structure. The Great Curassow can inhabit different types of tropical vegetation (e.g., deciduous forests; sub-deciduous, evergreen, and sub-evergreen forests; montane tropical forests; natural pastures; and mangroves), provided that they are conserved, a key feature for maintaining a high gene flow. High levels of genetic diversity were also found in *Crax globulosa* in three Amazonian islands (Ruiz García et al., 2007).

Although the habitat is degraded, no genetic evidence exists yet of habitat fragmentation. The low genetic differentiation among the included populations, even among the most distant, seems to indicate that the time elapsed since the fragmentation is very recent, so the populations have not yet differentiated clearly at the genetic level. On the other hand, the low rates of mitochondrial evolution in recent lineages in cracids has resulted in a low population genetic structure (van Tuinen & Dike, 2004; Pereira et al., 2004). Fragmentation and loss of habitat are very recent processes in the evolutionary history of a species; therefore, the use of more variable markers such as microsatellites or SNPs is advisable to identify greater variability in the parameters (Allendorf et al. 2010; Guichoux et al., 2011), or in the markers subject to selection (i.e., Hemmer-Hansen et al., 2007).

The Cozumel Curassow maintains a low genetic differentiation from *C. r. rubra.* The two included samples with different haplotypes: one island exclusive and the other shared with mainland samples does not support differentiation. In contrast, other island species such as the Pygmy Raccoon (*Procyon pygmaeus*) and the Dwarf Coati (*Nasua nelsoni*) have haplotypes exclusive to Cozumel and not shared with the continent populations (McFadden et al., 2008). The presence of an exclusive Cozumel haplotype (yellow) in the Cozumel Curassow may still signal an incipient genetic differentiation with the continental population, as has been observed in other endemic island birds (Rojas-Soto et al., 2010). While adaptation to the ecological conditions of the island may be reflected in the small size of the Cozumel Curassow, this particular adaptation may be the result of different selection pressures (Mayr, 1963). A more complete sampling is essential to corroborate whether this haplotype is exclusive to the island.

The Great Curassow does not fly long distances, so the origin of its presence in Cozumel is unknown. On the one hand, Cozumel is an oceanic island that originated between the Oligocene and the Pliocene, and it has not been connected to the continent, which was submerged when the sea level increased 121,000 ± 6,000 years ago (Spaw, 1978; Weidie, 1985). Its distribution may be the result of an intentional introduction by the Maya people like it has occurred with other species of large game birds such as *Meleagris gallopavo* and *M. ocellata* (Hamblin, 1980).

Both endemics species, the coati and the raccoon inhabited Cozumel Island for several thousand years, as evidenced by fossils (Hamblin, 1984). However, in recent excavations, bones of *Crax rubra* and of domestic birds such as *Meleagris gallopavo* and other species, were found on the island, which demonstrate a long-distance trade route. Species important to human diets were also confirmed, as were animals of other possible cultural, religious, or ceremonial use. (Hamblin, 1980). These deposits date back to the Late Postclassic period, that is, 800 years ago. In Cozumel, *C. rubra* has been recorded in six archaeological sites. However, it was apparently used only as food, and not as offerings (Hamblin, 1980).

With respect to the two haplotypes found in the population of Calakmul-Escárcega, which are highly differentiated from the other individuals (0.9% and 0.5% in the three included genes, 24 and 11 steps away from the other predominant haplogroups, Figure 1), this result corresponds to other reports of high intrapopulation genetic diversity that have been found in Neotropical birds, such as in *Amazilia tzacatl* (Miller et al., 2011), *Henicorhina leucosticta* (Aguilar et al., 2016) or in *Arremon aurantiirostris* (López et al., 2016). This haplotypic diversity suggests, in the first instance, the possibility of cryptic species, but a closer analysis of the phenotypic and ecological differentiation indicates that these diversity patterns may have been generated by modifications in the distribution of habitats due to climatic changes. These changes could have varied the configuration of barriers, allowing populations to expand and have secondary contacts, becoming sympatric, and sharing haplotypes that had previously differentiated. This seems to be exactly what occurred in the Mayan jungle in the populations of *Ara macao* (Schmidt, 2013). Other examples of secondary contacts in neotropical birds of lowland habitats are being found (e.g., Brumfield, 2005; Miller et al., 2008, 2010; Vázquez-Miranda, 2009). These findings show a pattern of geographic isolations followed by secondary contacts, without leading to the formation of biological species and which are also not congruent with the nominal subspecies.

Analyzing the role of barriers in the diversification of taxa from the lowlands (Weir, 2006) and the mountains (Weir, 2009) it has been shown that mountain taxa have a wide range of divergence times. These findings support a history of extensive dispersion after the formation of the barrier, detected for species such as the Azure-crowned Hummingbird (*Amazilia cyanocephala*) (Rodríguez-Gómez & Ornelas, 2013) or *Lepidocolaptes affinis* (Arbelaez-Cortés et al., 2010), which found in the Isthmus of Tehuantepec a permeable barrier, or the Common Bush-Tanager (*Chlorospingus ophthalmicus*) where a very different marked population differentiation was found between the different mountain ranges (García-Moreno et al., 2004). This finding is also mentioned by Barber and Klicka (2010), who found two pulses of diversification across the Isthmus of Tehuantepec in 10 mountain taxa, both within the Pleistocene or late Pleistocene.

However, for lowland taxa, a tendency exists for the rate of diversification to decrease (Weir, 2006), suggesting that habitat alterations due to global climate were not sufficient to trigger an increase in the diversification rate of lowland faunas. This finding agrees with our work on *Crax rubra,* where we found that the differentiation across the Isthmus of Tehuantepec is not complete and suggests only an incipient differentiation by distance.

Noninvasive sample collection was an effective tool for the analysis of the genetic diversity of an elusive and endangered species such as *C. rubra*. Despite the loss of habitat and overexploitation to which the Great Curassow has been subjected, wild populations persist, with significant genetic variability, in the natural protected areas visited. The results indicate that the low genetic population differentiation is a consequence of historical gene flow, and to maintain genetic flow, connectivity must be maintained among populations. Since the only barriers that create isolated populations are anthropogenic, it is important to design conservation plans that highlight the need to maintain populations of considerable sizes, with these populations ultimately being connected to avoid isolation and subsequent loss of genetic diversity. Implementation of a constant monitoring system is needed in places where the populations of Great Curassow have been drastically reduced to prevent hunting, illegal trafficking, and nest poaching, because the overexploitation in some places was very evident. Additionally, planning for education campaigns that are inclusive in nature and address the current distribution of the curassow is important.

Although *C. r. griscomi* does not present the same level of differentiation as other Cozumel endemics such as the raccoon and coati, maintaining the distinction of this subspecies as an evolutionary unit while genetic studies are improved is important. More studies are needed on the genes subject to selection that have been detected using SNP markers. Such studies will help clarify whether the morphological differentiation found has developed with an evolutionary adaptive base since the species arrival on the island, and whether the subspecies evolved in isolation from major exchange events with its congeners on the continent. In this way, the conservation priority of the subspecies is maintained while better information is produced.

Supplementary material

The following online material is available for this article:

Table S1- Samples of wild Mexican populations of the Great Curassow *(Crax rubra)* used in the present study, indicating the taxonomic level, key for the assigned geographical group, type of sample (feather, blood, or tissue), collection site, geographic coordinates, and access numbers in GenBank. The female individuals of barred phase were assigned (*).

Figure S1- Genetic distance matrix between individuals of the Mexican populations of the Great Curassow *(Crax rubra)*, grouped according to their populations. The color ranges from lighter blue (0%) (0) to navy blue (1.0%).

Author Contributions

Jonathan Morales Contreras, contributed in data collection, data analysis and interpretation, and in manuscript preparation

Patricia Escalante Pliego contributed with data analysis and interpretation, manuscript preparation, and critical revision

Noemí Matías-Ferrer contributed in data collection, data analysis and interpretation, manuscript preparation, and critical revision

Conflicts of interest

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

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Termite assemblages (Blattaria, Isoptera) in two montane forest (Brejo de Altitude) areas in northeastern Brazil

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Abstract: Wet enclaves of montane forests in Caatinga domain, locally known as "Brejos de Altitude", are associated with plateau at altitudes greater than 500 m a.s.l. and to orographic rainfall. Termite assemblage structures were studied in two areas of montane forest (Brejo de Altitude) located in the municipalities of Bezerros and São Vicente Ferrer, Pernambuco State, Brazil. Sampling was performed in 65 x 2 m transects, totaling 300 m^2/per area; the densities of nests in each area were estimated using six 1300 m^2 plots. A total of 35 morphospecies were recorded that belonged to 21 genera and three families, with 133 encounters. The soil-feeders feeding group predominated, followed by wood-feeders species. A total of five species constructing conspicuous nest were recorded in the two areas. The mean density of active conspicuous nests was 2.6 ± 6.3 nests/ha (mean ± sd) in Bezerros, and 21.8 ± 21.4 nests/ha in São Vicente Ferrer. Termite richness in the study areas were within the amplitude ranges recorded in other montane forests. Thus, the results presented here, combined with data from literature, reinforce need additional studies of the termite fauna in montane forest areas, once the "Brejos" are currently under high ecological pressure and their preservation is urgent.

Keywords: *Biodiversity, feeding groups, nest density, Acorhinotermes, Neotropical region.*

Taxocenoses de térmitas (Blattaria, Isoptera) em duas florestas de altitude (Brejo de Altitude) no nordeste do Brasil

Resumo: Enclaves de floresta úmida de altitude nos domínios da Caatinga, localmente conhecidos como "Brejos de Altitude", estão associados a áreas com mais de 500 m de altitude e às chuvas orográficas. O presente estudo teve como objetivo caracterizar a estrutura da taxocenose de térmitas em duas áreas de Brejo de Altitude localizadas nos municípios de Bezerros e São Vicente Ferrer, Estado do Pernambuco, Brasil. A amostragem foi realizada através de transectos de 65 m x 2 m, totalizando 300 m^2/por área. Em cada área, a densidade de ninhos foi estimada em seis parcelas de 1300m^2. Trinta e cinco morfoespécies foram registradas nas duas áreas, pertencentes a 21 gêneros e três famílias, com 133 encontros. O grupo alimentar dos humívoros foi predominante, seguido pelos xilófagos. Um total de cinco espécies construtoras de ninhos conspícuos foram registradas nas duas áreas. A densidade média de ninhos conspícuos ativos foi de 2,6 ± 6,3 ninhos/ha (média ± dp) em Bezerros, e de 21,8 ± 21,4 ninhos/ha em São Vicente Ferrer. A riqueza de térmitas das áreas estudadas ficou dentro da amplitude já registrada para áreas de Brejo de Altitude. Assim, os resultados aqui apresentados, combinados com dados da literatura, reforçam a necessidade de estudos adicionais da fauna de térmitas em áreas de floresta montana, uma vez que os Brejos estão atualmente sob alta pressão ecológica e sua preservação é urgente.

Palavras-chave: *Biodiversidade, grupos alimentares, densidade de ninhos, Acorhinotermes, Região Neotropical.*

Introduction

The Caatinga domain is a mosaic of spiny shrubs and seasonally dry tropical forests covering approximately 735,000 km^2 in northeastern Brazil (Leal et al. 2005). The Caatinga domain is bordered by the Atlantic Forest to the east, the Amazon Forest to the west, and Cerrado (neotropical savanna) to the south (Leal et al. 2005, Prado 2003), and it harbors enclaves of humid montane forests locally known "Brejos de Altitude" or "Brejos Nordestinos" (Andrade-Lima, 1982). The origin of these mountains has been associated with climatic shifts during the Pleistocene, with the current forest enclaves representing the results of expansion and retraction cycles of more humid forests within the dryer Caatinga matrix (Andrade-Lima 1982, Santos et al. 2007).

Montane forests are associated with plateaus at altitudes greater than 500 m a.s.l. and orographic rainfall with precipitation levels above 1200 mm/year (Andrade-Lima 1960, Veloso et al. 1991, Tabarelli & Santos 2004), with perennial, sub-perennial, and sub-deciduous forests predominating on the highest slopes (Andrade-Lima 1966, Andrade-Lima 2007). When compared to adjacent semiarid regions, those montane forests show milder temperatures and higher humidity levels (Andrade-Lima 1966, Rodal & Sales, 2008). Studies focusing on the phylogeography of anuran amphibians (Carnaval, 2002; Carnaval & Bates, 2007), reptile distributions (Borges-Nojosa & Caramaschi, 2003), arboreal physiognomy and diversity (Tavares et al., 2000; Rodal & Sales, 2008), and parsimony analyses of woody plant endemism (Santos et al., 2007) have all indicated that the areas have a biota with distinct characteristics between them. These forests have high rates of endemism (Andrade-Lima, 1982), biological communities little known (Silva et al. 2018), and there is a strong degradation of the original formation, being considered currently priority areas for the conservation of the biodiversity (Prado 2003; Silva et al. 2018).

Termites are eusocial insects of the infraorder Isoptera (order Blattaria) that have important functions in the dynamic ecological processes of nutrient recycling, soil aeration, and the maintenance of soil fertility (Wood & Sands 1978, Holt & Lepage 2000), and their activities are principally associated with benefits to primary production (Bignell & Eggleton 2000). Termite distribution is predominately tropical, with humid equatorial forests and savannas having the greatest species richness, abundance, and biomass; diversity declines with increasing latitude and altitude (Eggleton 2000, Jones & Eggleton 2011, Cancello et al. 2014; Nunes et al., 2017).

Termite fauna of montane forests have been investigated in six different areas, generating species lists (Vasconcellos & Moura 2014, Couto et al. 2015) and studies of the influence of anthropogenic disturbances on termite assemblages (Bandeira & Vasconcellos 2002, Bandeira et al. 2003, Bandeira & Vasconcellos 2004) and their vertical stratification (Araújo et al. 2015). The present study sought to characterize the structures of termite assemblages in two montane forest areas in northeastern Brazil in terms of their species richness, relative abundance, feeding groups, and nest densities.

Materials and Methods

1. Study area

The present study was undertaken in two areas of montane forest in northeastern Brazil: i) Mata Vertentes (BEZ) in the municipality of Bezerros, Pernambuco State, Brazil (8º11'12"S x 35º47'29"W); ii) the Mata do Estado Municipal Reserve (SVF) in the municipality of São Vicente Ferrer, Pernambuco State, Brazil (7º37'07"S x 35º30'15"W) (Figure 1). The Mata Vertentes comprises an area of 129 ha at approximately 960 m a.s.l. The climate there is tropical rainy, usually with dry summers (KÖppen 1936, Alvares et al. 2013); the mean annual rainfall is 442.5 mm (APAC 2017), with a mean annual temperature of 22.9 ºC (DCA 2016). The Mata do Estado Municipal Reserve comprises an area of 600 ha, at approximately 570 m a.s.l., with a rainy tropical climate with dry summers (KÖppen 1936, Alvares et al. 2013), a mean annual rainfall rate of 928.7 mm (APAC 2017), and mean annual temperatures between 22º C and 26º C (SEMAS 2014). The vegetation of the studied areas is perennial, formed by fragments of montane rain forests. Due to anthropogenic disturbances, vegetation varies from primary forests to highly impacted secondary forests (Veloso & Góes Filho 1982; Andrade-Lima 2007).

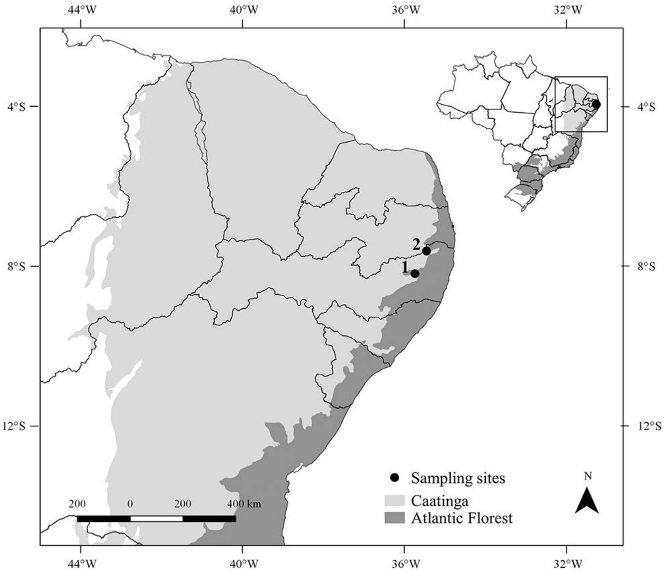

Figure 1. Locations of the study areas in northeastern Brazil. (1) Mata Vertentes, municipality of Bezerros, Pernambuco State, Brazil (2) Mata do Estado Municipal Reserve, municipality of São Vicente Ferrer, Pernambuco State, Brazil.

2. Sampling protocols

The termite assemblages were sampled between April and July/2017 using protocols based on transect sampling (modified from De Souza & Brown [1994] and Jones & Eggleton [2000]) similar to those described by Cancello et al. (2014). Six 65 m x 2 m transects (separated one from the other by at least 200 m) were established in each locality. Each transect was subdivided into five 5 x 2 m plots every 10 m, totaling 30 plots (300 m^2) per locality. The sampling effort in each plot was 1 hour-person. We searched for termites in active and abandoned nests (up to 2 m above the ground), in tunnels, in the trunks of live and dead trees, in the leaf litter, inside the fallen tree boughs, beneath the ground (up to 15 cm below the surface), under stones, and within dead roots. The specimens collected were stored in flasks containing 80% alcohol and subsequently deposited in the Isoptera Collection at the Federal University of Paraíba.

3. *Species determination*

The genera were determined using taxonomic keys present in Constantino (2002) and Bourguignon et al. (2016). At the species level were used the descriptions listed in Constantino (1998; 2017). All the material was compared with the species housed in the Entomological Collection of the Termitology Laboratory/Lab*Termes* of the Federal University of Paraíba, Brazil.

4. *Feeding groups*

Feeding group assignments were based on *in situ* observations and information available in the specialized literature concerning neotropical termites (De Souza & Brown 1994, Constantino 1999, Bandeira et al. 2003, Sena et al. 2003, Vasconcellos et al. 2005, Cancello et al. 2014). The species were classified as belonging to the following feeding groups (FG1): (i) wood-feeders, those that consume the wood of living trees, or of dead trees in various stages of decomposition; (ii) soil-feeders, those that feed on humus and generally live in the soil; (iii) wood/soil-feeders, those that consume humus, as well as wood in different stages of decomposition and generally transport soil to within the wood they are consuming; and (iv) wood/leaf-feeders, those that consume wood in advanced stages of decomposition, as well as leaves gathered on the forest floor. The termites were also categorized following the proposals of Donovan et al. (2001) (see Table 1, FG2).

5. *Nest densities*

In each area, the densities of conspicuous nests were estimated within six 65 x 20 m plots established parallel to the transects used in the faunal inventory. All nests with volumes ≥ 2.0 L in those plots were identified, counted, and characterized as: (i) arboreal, nests constructed on the trunks and branches of living or dead trees; (ii) epigeal, nests generally initiated below ground level, but eventually growing and emerging with an above-ground portion.

6. *Analyses*

The number of plots in which a given species was present (encounters) was used as an indirect measure of relative abundance (Jones 2000). Species accumulation curves were constructed using the Mao Tau method, considering 95% confidence intervals for 1000 randomizations without replacement of the original data. Chao 2 non-parametric estimator was used to evaluate species richness in each area. Those analyses were performed using EstimateS 9.1.0 software (Colwell 2017).

Results

A total of 35 termite species were recorded in the two study areas, of which 19 (54%) were identified and 16 (46%) were morphotyped; they belonged to 21 genera and three families, with 133 encounters (Table 1). The family Termitidae comprised the greatest numbers of species (80.0%) and encounters (93.2%), Rhinotermitidae comprised 11.4% of the species and 3.0% of the encounters, while Kalotermitidae comprised 8.6% of the species and 3.8% of the encounters.

Fifteen termite morphospecies were encountered in Bezerros, belonging to 12 genera and three families, with 54 encounters. São Vicente Ferrer had a richness of 24 morphospecies belonging to 14 genera and three families, with 79 encounters. The most abundant species in Bezerros was *Nasutitermes corniger* (Motschulsky, 1855) (14 encounters), and the two most abundant species in São Vicente Ferrer were Apicotermitinae sp.4 and *Diversitermes diversimiles* (Silvestri, 1901) (12 and 9 encounters respectively) (Table 1).

In relation to the subfamilies of Termitidae, the subfamily Apicotermitinae had the greatest species richness (6 species; 50.0%) and numbers of encounters (26 encounters; 53.1%) in Bezerros, while the subfamily Nasutitermitinae had the greatest number of species (9; 45%) and number of encounters (38; 50.1%) in São Vicente Ferrer (Figure 2).

The estimated species richness in Bezerros was 16.16 ± 0.32 (mean ± standard error) (Chao2), and the estimated species richness in São Vicente Ferrer was 50.58 ± 4.03 (Chao2). The species accumulation curve in Bezerros had a slight tendency towards attaining its asymptote; the accumulation curve in São Vicente Ferrer had an ascending tendency. According to the confidence intervals, there were no significant differences between the species accumulation curves of the two areas (Figure 3).

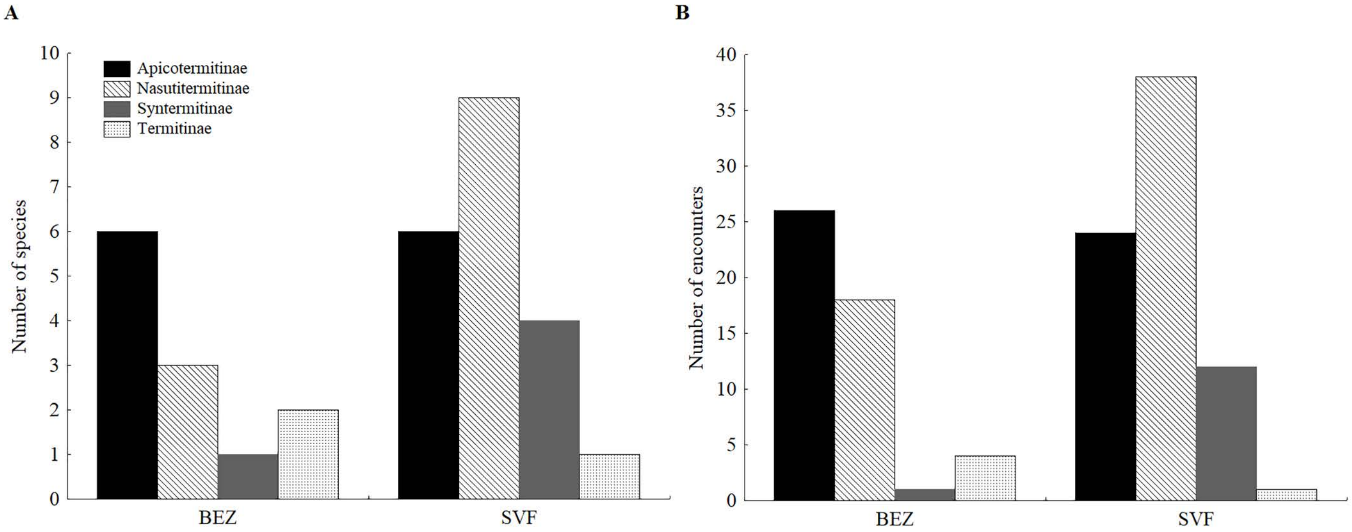

Figure 2. Species richness (a) and numbers of encounters (b) of termites per subfamily of Termitidae in the municipalities of Bezerros (BEZ) and São Vicente Ferrer (SVF), Pernambuco State, Brazil.

Table 1. Species richness, relative abundances (numbers of encounters), feeding group, and micro-habitats of termites in the study areas in the municipalities of Bezerros (BEZ) and São Vicente Ferrer (SVF), Pernambuco State, Brazil. Micro-habitat: Li, leaf litter; Wo, wood; Ne, nest; So, soil. FG: feeding groups. FG1: W, wood-feeders; S, soil-feeders; W/L, wood/leaf-feeders; W/S, wood/soil-feeders. FG2: following the categories of Donovan et al. (2001). * Conspicuous nest.

Family/subfamily/species	**Sites**		**Micro-habitat**	**Feeding groups**	
	BEZ	**SVF**		**FG1**	**FG2**
KALOTERMITIDAE					
Glyptotermes sp.	1		Wo	W	I
Neotermes sp.		1	Wo	W	I
Rugitermes sp.	3		Wo	W	I
RHINOTERMITIDAE					
Acorhinotermes sp.		1	Wo	W	I
Heterotermes longiceps (Snyder, 1924)		1	Wo	W	I
Heterotermes tenuis (Hagen, 1858)	1		Wo	W	I
Rhinotermes hispidus Emerson, 1925		1	Wo	W	I
TERMITIDAE					
Apicotermitinae					
Anoplotermes sp.1		1	So	S	IV
Anoplotermes sp.2	5	1	So	S	IV
Apicotermitinae sp.1		1	So	S	IV
Apicotermitinae sp.2		6	So	S	IV
Apicotermitinae sp.3		3	So	S	IV
Apicotermitinae sp.4		12	So	S	IV
Apicotermitinae sp.5	9		So	S	IV
Apicotermitinae sp.6	4		So	S	IV
Apicotermitinae sp.7	3		So	S	IV
Grigiotermes sp.	1		So	S	IV
Ruptitermes reconditus (Silvestri, 1901)	4		Li/So	W/L	III
Nasutiterminae					
Diversitermes diversimiles (Silvestri, 1901)		9	Li/Wo/So	W/L	II
Nasutitermes calimorphus Mathews, 1977		3	Li/Wo	W	II
Nasutitermes corniger (Motschulsky, 1855)	14*	5*	Wo/Ne	W	II
Nasutitermes ephratae (Holmgren, 1910)		2*	Wo/Ne	W	II
Nasutitermes gaigei Emerson, 1925		5	Wo	W	II
Nasutitermes jaraguae (Holmgren, 1910)	2	7	Wo	W	II
Subulitermes microssoma (Silvestri, 1903)		1	So	S	III
Subulitermes sp.	2	3	Wo/So	S	III
Velocitermes velox (Holmgren, 1906)		3	Li/Wo/So	W/L	II
Syntermitinae					
Embiratermes neotenicus (Holmgren, 1906)		5*	So/Ne	W/S	III
Embiratermes parvirostris Constantino, 1993		1	So	S	III
Ibitermes inflatus Vasconcellos, 2002	1		So	S	III
Labiotermes labralis (Holmgren, 1906)		1*	Ne	S	IV
Silvestritermes holmgreni (Snyder, 1926)		5*	Li/Wo/Ne	W/S	III
Termitinae					
Dihoplotermes inusitatus Araujo, 1961	2		So	S	III
Microcerotermes strunckii (Sörensen, 1884)		1	Wo	W	II
Neocapritermes sp.	2		So	W/S	III
Encounters	**54**	**79**			
Morphospecies	**15**	**24**			

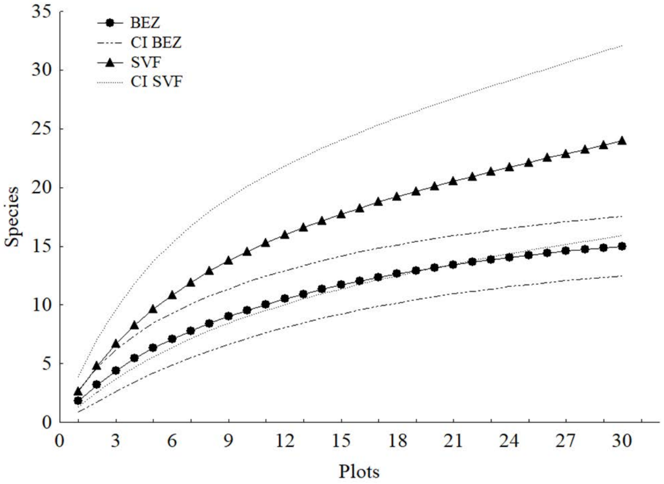

Figure 3. Accumulation curves of termite species (Mao Tau), and 95% confidence intervals (CI).

The soil-feeders (FG1) were predominant in Bezerros, representing 53.3% of the species and 50.0% of the encounters; the second most predominant feeding group was wood-feeders. There were equal numbers of soil-feeders and wood-feeders termite species (41.7% each) in São Vicente Ferrer, with the soil-feeders demonstrating the greatest numbers of encounters (38.0%) (Figure 4). According to the classification of Donovan et al. (2001), feeding groups III and IV demonstrated the greatest numbers of species in Bezerros (33.3% each), while group II (33.3%) and group IV (29.2%) had the greatest numbers of species in São Vicente Ferrer (Table 1, FG2).

The mean nest density in Bezerros was 2.6 ± 6.3 (mean ± standard deviation) active nests/ha, with *Nasutitermes corniger* being the only termite that built conspicuous nests, while the mean nest density in São Vicente Ferrer was 21.8 ± 21.4 active nests/ha. Four arboreal nest building species were encountered in the latter area: *Labiotermes labralis* (Holmgren, 1906) (2.6 ± 6.3 active nests/ha), *Nasutitermes corniger* (3.8 ± 6.4), *Nasutitermes ephratae* (Holmgren, 1910) (5.1 ± 6.3), and *Silvestritermes holmgreni* (Snyder, 1926) (2.6 ± 4.0). *Embiratermes neotenicus* (Holmgren, 1906) was the only species recorded in the study that constructed epigeal nests, with a density of 7.7 ± 6.9 active nests/ha in São Vicente Ferrer.

Discussion

A total 35 species were found, of which 19 (54%) were identified and 16 (46%) were morphotyped. The species *Rhinotermes hispidus, Nasutitermes gaigei*, and *Velocitermes velox* represent new records for montane forests. Including those three new records, approximately 48 species have now been identified to the species level in eight inventoried montane forests (Bandeira & Vasconcellos 2002, Bandeira et al. 2003, Bandeira & Vasconcellos 2004, Vasconcellos & Moura 2014, Araújo et al. 2015, Couto et al. 2015). Studies focusing on termite diversity in montane forests have usually been unable to identify between 24 and 53% of the morphospecies encountered, indicating the existence of new species and/or taxonomic difficulties (principally related to the subfamily Apicotermitinae). The report here of the genus *Acorhinotermes* sp. in São Vicente Ferrer is notable, as it is currently considered monospecific (*Acorhinotermes subfusciceps*) and no records of encounters have yet been published for Brazil. Its current distribution is mainly related to the Amazonian, occurring in Guiana and French Guiana (Emerson 1925, Davies 2002, Davies et al. 2003), and little is known about its biology or ecology. Only one colony of *Acorhinotermes* sp. was encountered in the decomposing bark of a live tree in the present research.

The species richness of termites in the study areas (15 and 24 spp.) were within the amplitude ranges reported for other montane forest areas sampled by several protocols and sampling efforts (between 17 and 29 spp.) (Bandeira & Vasconcellos 2002, Bandeira et al. 2003, Bandeira & Vasconcellos 2004, Vasconcellos & Moura 2014, Couto et al. 2015). In relation to the areas sampled with the same protocol employed in the present study, the numbers of morphospecies reported here were within the amplitude ranges for the studies in the Montane Forests (16 to 29 spp.) (Vasconcellos & Moura 2014, Couto et al. 2015), in the Atlantic Forest (4 to 34 spp.) (Souza et al. 2012, Cancello et al. 2014); and in the Caatinga domain (7 to 36 spp.) (Mélo & Bandeira 2004, Vasconcellos et al. 2010, Alves et al. 2011, Vasconcellos & Moura, 2014).

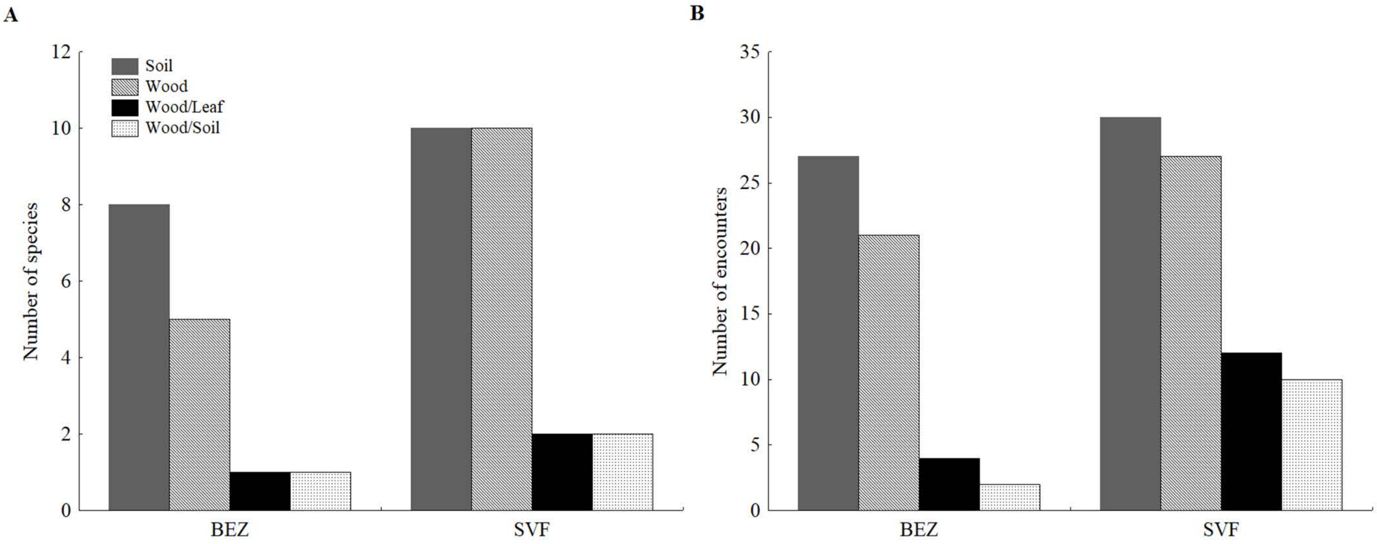

Figure 4. Species richness (a) and numbers of termite encounters (b) per feeding group (category FG1).

The observed and estimated species richness at Bezerros were relatively similar, indicating sufficient sampling efforts for that site. The richness estimates for São Vicente Ferrer (especially that suggested by the Chao2 estimator) were larger than the observed richness. This result may be related to the fact that 45.8% of the species recorded for that area were represented by single sample (*uniques*). Using the same sampling protocol used in the present study, Cancello et al. (2014) reported that Atlantic Forest remnants in northeastern Brazil demonstrated greater numbers of *uniques* in relation to Atlantic Forest remnants at higher latitudes, indicating the necessity of greater sampling efforts for rare species at lower latitudes.

In relation to the species richness and abundances of the subfamilies of Termitidae, we found the Apicotermitinae to be predominant in Bezerros while Nasutitermitinae was predominant in São Vicente Ferrer. Previously published studies concerning the termite fauna in montane forests (Bandeira et al. 2003), Atlantic Forests (Reis & Cancello 2007, Vasconcellos 2010, Souza et al. 2012), and Caatinga sites (Vasconcellos et al. 2010; Viana Junior et al. 2014) have cited the predominance of the subfamily Nasutitermitinae, although Vasconcellos & Moura (2014) noted variations in the predominance of different subfamilies of Termitidae in montane forests.

In general, the present study encountered greater richness of soil-feeders and wood-feeders termites, respectively, corroborating other studies in similar ecosystems (Bandeira & Vasconcellos 2002, Bandeira et al. 2003, Vasconcellos & Moura 2014, Couto et al. 2015). Studies undertaken in humid tropical forests in Brazil (Amazon and Atlantic forests), degraded montane forests, and Caatinga vegetation have reported the predominance of wood-feeders termites (Bandeira et al. 1998, Brandão 1998, Bandeira et al. 2003, Vasconcellos et al. 2005, Reis & Cancello 2007, Vasconcellos et al. 2010, Souza et al. 2012, Alves et al. 2011, Viana Junior et al. 2014). No previous study undertaken in montane forests used the feeding group classification proposed by Donovan et al. (2001), which may be related to the lack of detailed information available concerning the Neotropical termite fauna (Bandeira et al. 2003). At the global level, soil (group IV) and humus (group III) consumers have been reported to represent the greatest fraction of termites in Humid Tropical Forests in Africa (Cameroon), South America (French Guiana), and Southeast Asia (Borneo) (Davies et al. 2003; Jones & Eggleton 2011).

Twelve species of termites constructing conspicuous nests are known to inhabit montane forest areas (varying between one and seven species per site) (Bandeira & Vasconcellos 2002, Vasconcellos & Moura 2014, Couto et al. 2015). Among those 12 species, only one was encountered in Bezerros and five in São Vicente Ferrer. Their nest densities were greater in São Vicente Ferrer (21.8 nests/ha) than in Bezerros (2.6 nests/ha). Those densities are below published records for the Atlantic Forest (24 to 92.8 nests/ha) (Vasconcellos 2010), but within the amplitude for Caatinga vegetation (0.7 to 89 nests/ha) (Martius et al. 1999, Mélo & Bandeira 2004, Vasconcellos et al. 2010, Alves et al. 2011). The nest density recorded here for São Vicente Ferrer is greater than that reported by Bandeira et al. (2003) in another area of montane forest (5 to 10 nests/ha).

In summary, the following findings should be emphasized: (i) as compared to Bezerros, the São Vicente Ferrer site had greater species richness and abundance and higher densities of conspicuous nests, which may be related to factors such as: elevation, climatic variables, levels of anthropogenic disturbances, and/or the sizes of those two areas. The greatest area extension of São Vicente Ferrer can reduce both, the edge effect and the anthropic disturbances on the communities that comprise this ecosystem. Bandeira et al. (2003) consider the effects of environmental degradation and the higher altitude as possible factors resulting in the lower termite diversities observed in montane forests; (ii) was found a greater richness of soil-feeders and wood-feeders termites. Bandeira & Vasconcellos (2002) report that decrease in species richness of termites, mainly by the reduction of humus and intermediate feeders can associated with levels of environmental disturbance; (iii) although some studies underway in Brazil, new records of species such as the *Acorhinotermes* sp. are still little known. Thus, the results presented here, combined with data from literature, reinforce need additional studies of the termite fauna in montane forest areas, once the Brejos are currently under high ecological pressure and their preservation is urgent.

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Author Contributions

Israel Soares da Silva - Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation and critical revision, adding intellectual content.

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

Flávia Maria da Silva Moura - 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, 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

Checklist of marine demersal fishes captured by the pair trawl fisheries in Southern (RJ-SC) Brazil

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Abstract: Demersal fishery resources are abundant on continental shelves, on the tropical and subtropical coasts, making up a significant part of the marine environment. Marine demersal fishery resources are captured by various fishing methods, often unsustainably, which has led to the depletion of their stocks. In order to inventory the marine demersal ichthyofauna on the Southern Brazilian coast, as well as their conservation status and distribution, this study analyzed the composition and frequency of occurrence of fish captured by pair trawling in 117 fishery fleet landings based in the State of São Paulo between 2005 and 2012. The ichthyofauna consisted of 245 species (81 families, 32 orders and 2 classes). Among the species, 50 species were classified as constant, 38 accessory and 157 accidental. Still, 13.47% of the species were listed as endangered; 35.29% of the Chondrichthyes and 11.85% of the Actinopterygii (15.1% of the total species) belong to the endemic fauna of the Biogeographic Province of Argentina. The richness was directly related to the oceanographic features of the study area, which determined the southern boundary of occurrence of several tropical species and the northern boundary of occurrence for temperate species. This is the region with the highest abundance of fishery resources in Brazil.

Keywords: *ichthyofauna, inventory, fishery resources, conservation.*

Lista de peixes demersais marinhos capturados pela pesca de parelha no Sul (RJ-SC) do Brasil

Resumo: Nas costas tropicais e subtropicais, sobre as plataformas continentais, os recursos pesqueiros demersais são abundantes e considerados uma parcela importante do ambiente marinho. Os recursos pesqueiros demersais marinhos são capturados por diversas modalidades de pesca, muitas vezes de forma insustentável, o que vem provocando o esgotamento de seus estoques. Com o objetivo de inventariar a ictiofauna demersal marinha da costa sudeste-sul do Brasil, assim como seu status de conservação e distribuição, foram analisadas a composição e frequência de peixes capturados em 117 cruzeiros de pesca entre 2005 e 2012 pela frota de arrasto de parelha sediada no Estado de São Paulo. A ictiofauna foi composta por 245 espécies (81 famílias, 32 ordens e 02 classes), sendo 50 consideradas como constantes, 38 acessórias e 157 acidentais; 13.47% apresentam algum grau de ameaça; 35.29% dos Chondrichthyes e 11.85% dos Actinopterygii (15.1% do total) pertencem à fauna endêmica da Província Zoogeográfica da Argentina. A riqueza observada está diretamente vinculada às características oceanográficas da área de estudo, sendo estas responsáveis pelo limite meridional de ocorrência de várias espécies tropicais e o limite setentrional de espécies de regiões temperadas. Esta é a região com maior abundância de recursos pesqueiros do Brasil.

Palavras-chave: *ictiofauna, inventário, recursos pesqueiros, conservação.*

Introduction

Demersal fishery resources are abundant on continental shelves, on the tropical and subtropical coasts, making up a significant part of the marine environment. Oceanographic conditions influence the quality and potential of such resources, as well as regionally limit the use of certain types of vessels and fishing gear (Yáñez-Arancibia & Sánches-Gil 1988, Dias-Neto & Ximenes 1998).

The capture of demersal species has been carried out by artisanal and industrial fishing for several decades and it is one of the most important activities for the fishing industry in Brazil (Castro et al. 2003). Small-scale fishing consists of beach seining, gillnets, long-lines, stern trawlers or double rig trawlers along estuarine and coastal and regions. Industrial fishing is carried out by medium and large-sized vessels using pair trawls and otter trawlnets, stern trawlers or double rig trawlers, gillnets and long-lines, as well as fishing with traps, coves and purse seines (Sudepe 1985, Valentini et al. 1991, Ibama 1993, 1995, Haimovici 1997).

The industrial pair trawling stands out among various modalities and categories of fishing aimed to demersal fish. Species belonging to the family Sciaenidae (Weakfishes), such as *Micropogonias furnieri* (Whitemouth croaker), *Macrodon atricauda* (Southern King Weakfish) and *Cynoscion jamaicensis* (Jamaica weakfish); in addition to species of the family Balistidae, such as *Balistes capriscus* (Grey triggerfish) and family Ariidae, such as *Genidens barbus* (White sea catfish) are the most targeted along with elasmobranchs (Valentini et al. 1991, Ibama 1993, 1995, Haimovici 1997, Castro et al. 2003, 2007, Castro & Tutui 2007).

According to Caddy & Sharp (1986), it is necessary to bear in mind that exploited species are linked to others that may be essential for the economic and biological productivity of the ecosystem. This is the only way to minimize the adverse effects of fishing and not to compromise the system's ability to sustain productive human activity. Therefore, the proper management of exploited resources and aquaculture requires knowledge about the dynamics of communities/populations, their ecological interactions, the integration with physicochemical and biological processes at spatial and temporal scales, as well as changes in fishery dynamics (Yáñes-Arancibia et al. 1985, Davis & Anderson 1989, Aryuthaka & Thumbthimsang 1992, Castelo et al. 2007, Castro & Tutui 2007).

This study provides a checklist of marine demersal ichthyofauna of the southern coasts of Brazil captured by the fishery fleet pair trawling based in the state of São Paulo and comments upon the composition, frequency of occurrence, conservation status and geographical distribution of the recorded species.

Material and Methods

The pair trawling fleet operation area had its limits under the coordinates 23º04'S / 42º20'W near Cabo Frio - RJ and 29º19'S / 49º41'W in Torres - RS. Prior to SMA-SP Resolution No. 69 of 2009, activities were concentrated on the south coast of São Paulo under the coordinates 24º31'S / 46º39'W near Peruíbe - SP and 28º37'S / 48º37'W near Cabo de Santa Marta - SC (Figure 1).

The fleet operated between a depth of 14 and 55 meters (m ± SD = 29.2 ± 9.7m). For each year, the following means ± standard deviation and amplitudes were observed: 30.4 ± 8.6m (22 and 50m) in 2005; 27.9 ± 7.5m (20 and 42m) in 2006; 33.2 ± 13.9m (14 and 52m) in 2007; 25.2 ± 11.9 (15 and 55m) in 2008; 28.5 ± 10.9m (14 and 50m) in 2009; 30.1 ± 9.7m (26 and 52m) in 2010; 30.4 ± 6.3m (26 and 50m) in 2011; and 30.9 ± 6.6m (26 and 44m) in 2012.

The ichthyofauna from 117 fishery fleet landings of the industrial pair trawling fleet based in the State of São Paulo were sampled from March 2005 to May 2012. The sampling order was randomly. Only landings at the ports located in the municipalities of Santos and Guarujá-SP, Brazil were recorded.

The following sampling routine was performed for qualitative purposes: (a) the whole screening operation of the marketable product was followed up by collecting all rejected material among commercial products and also part of this products; (b) samples of the rejected and discarded products that fishermen sampled during the whole fishing operation were collected for further evaluation in the laboratory; c) co-ordinates of the most distant bids, as well as of the main fishing grounds and their respective depths, were annotated with the masters. Samples of rejected and discarded fauna were also obtained through 15 shipments to the studied fleet.

The collected specimens were referred to the laboratory in iceboxes. Samples were identified, counted and had their total length measured (in mm). Subsequently, the specimens were fixed in 10% formaldehyde and preserved in 70% ethyl alcohol. Voucher specimens of the some species collected during the study were deposited in the regional collection of fish from the Atlantic Forest coast of the "Acervo Zoológico da Universidade Santa Cecília" (AZUSC), in the city of Santos-SP, Brazil.

Fish species in the samples were identified according to Figueiredo (1977), Figueiredo & Menezes (1978, 1980, 2000), Fischer (1978), Menezes & Figueiredo (1980, 1985), Compagno (1984, 1988), Cervigón et al. (1992), Carvalho-Filho (1999), Moura et al. (2001), Carpenter (2002), Fischer et al. (2004), Marceniuk (2005), Leis (2006), Moura & Lindeman (2007), Sampaio & Nottingham (2008), Carvalho-Filho et al. (2010), Gomes et al. (2010), McBride et al. (2010), Rosa & Gadig (2010), Tavera et al. (2011, 2012), Ruocco et al. (2012), Frable et al. (2013), Menezes et al. (2015), Knudsen & Clements (2016), Last et al. (2016a, b, c); Marceniuk et al. (2016). The systematics and nomenclature followed Nelson et al. (2016) and Eschmeyer et al. (2017), respectively.

Species were classified from their frequencies of occurrence and according to the Dajoz's (1983) scale as "constant" when present in more than 50% of the samples; "accessory" when frequencies ranged from 25 to 50% and "accidental" when frequencies were below 25%.

The conservation status of each species were based on the International Red List of threatened species (IUCN – International Union for Conservation of Nature 2017) and the Brazilian Federal list of threatened species, Ordinance number 445 of the MMA – Brazilian Environment Ministry, December 17th 2014 (MMA 2014).

Species were classified in the biogeographical categories proposed by Floeter et al. (2008) and Luiz Jr. et al. (2008).

Results

Of the 117 fishery fleet landings, the majority occurred in 2011 (20.52%) and the minority in 2010 (5.98%) (Table 1).

The richness consisted of 245 species (Table 2) distributed over 2 classes, 32 orders, 81 families and 170 genera. Out of the total species analyzed, 86.12% of the species belong to Class Actinopterygii and 13.88% to Class Chondrichthyes (Table 3). Of the 81 families comprising the captured ichthyofauna, six families had the highest species richness (33.5% of the total), namely Carangidae (6), Sciaenidae (19), Paralichthyidae (11), Haemulidae and Serranidae (10) and Engraulidae (8).

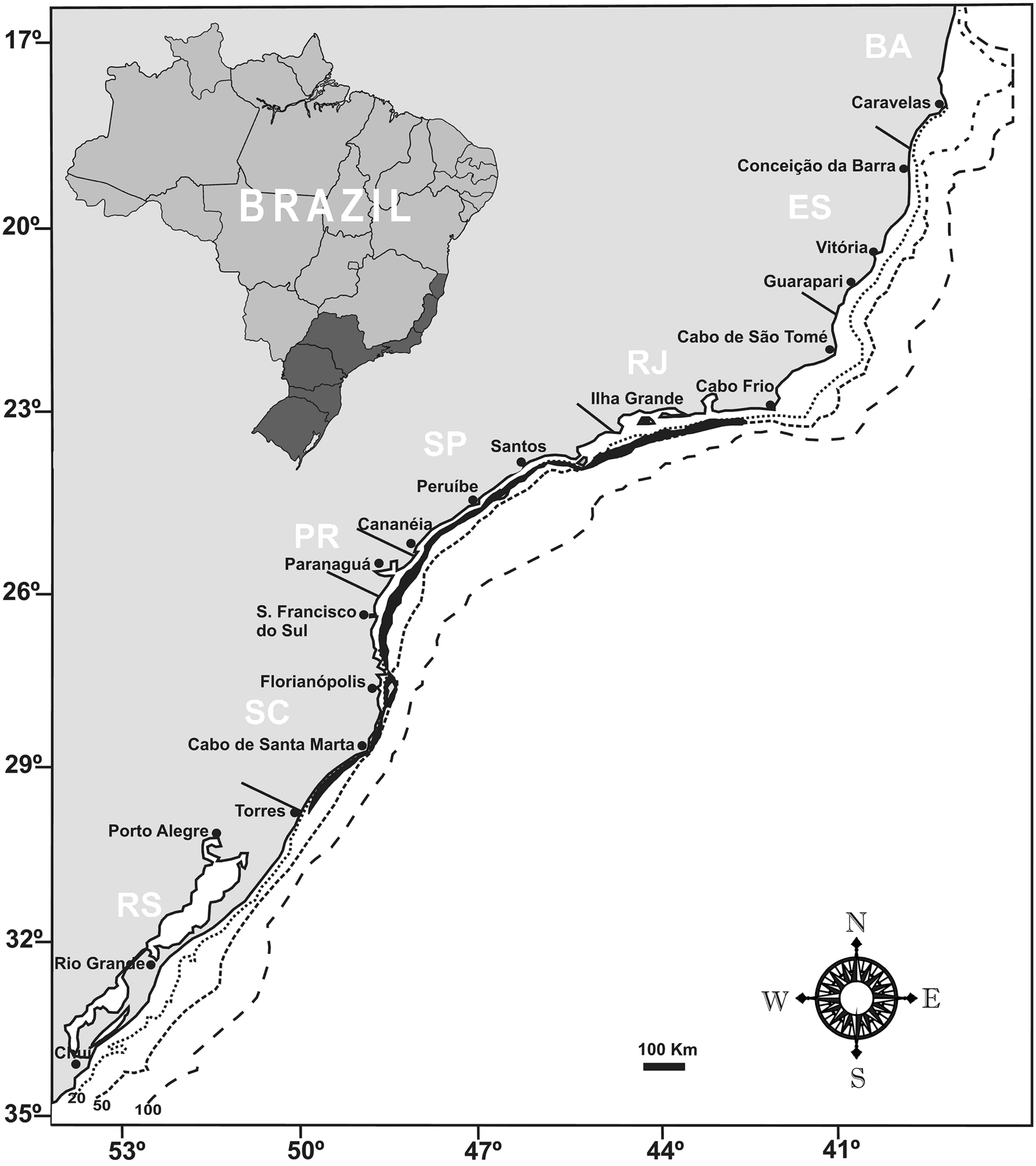

Figure 1. Areas of legal practice of pair trawling fishing in the State of São Paulo (Espírito Santo to Rio Grande do Sul). The study area is highlighted in black.

Table 1. Distribution of the number of fishing operations (N) and percentage (%) in relation to the study period.

	2005	2006	2007	2008	2009	2010	2011	2012	Total
N	10	17	13	18	20	7	24	8	117
%	8.5	14.5	11.1	15.3	17.0	5.9	20.5	6.8	100

Table 2. Ichthyofauna caught by the pair trawling fleet of the state of São Paulo, Dajoz scale (DS): Co = constant, Ac = accessory, Ad = accidental; geographic distribution (GD): CT = circumtropical, TA = Trans-Atlantic (both sides of the Atlantic), WA = Western Atlantic (Northern and Southwestern Atlantic), SWA = Southern West Atlantic (from northern Brazil to Argentina), SSWA = Southern South West Atlantic (species with affinity to temperate areas occurring from Argentina and Uruguay to southern Brazil), Ca = Caribbean (from Florida to Venezuela), Br = Brazilian Province (the area between the Orinoco Delta in Venezuela and Santa Catarina in Brazil) and EP = Eastern Pacific; conservation status in the IUCN Red List (IUCN 2017), in the Brazilian list (MMA 2014): X = Not Evaluated; LC = Least Concern; DD = Data Deficient; VU = Vulnerable; NT = Near-Threatened; END = Endangered, CR = Critically Endangered; vouchers.

Order/ Family/ Species	DS	GD	IUCN	MMA	Voucher
CARCHARHINIFORMES					
Triakidae					
Mustelus schmitti Springer, 1939	Ad	SSWA	END	CR	AZUSC3405
Carcharhinidae					
Carcharhinus brachyurus (Günther, 1870)	Ad	CT	NT		AZUSC3791
Carcharhinus falciformis (Bibron, 1839)	Ad	CT	VU		AZUSC3354
Carcharhinus limbatus (Valenciennes, 1839)	Ad	CT	NT		AZUSC3350
Galeocerdo cuvier (Péron & Lesueur, 1822)	Ad	CT	NT		AZUSC3383
Rhizoprionodon lalandii (Valenciennes, 1839)	Co	WA	DD		AZUSC3293
Rhizoprionodon porosus (Poey, 1861)	Ad	Ca+SWA	LC		AZUSC4811
Sphyrnidae					
Sphyrna lewini (Griffith & Smith, 1834)	Ad	CT	END	CR	AZUSC0893
Sphyrna zygaena (Linnaeus, 1758)	Ad	CT	VU	CR	AZUSC3780
SQUATINIFORMES					
Squatinidae					
Squatina guggenheim Marini, 1936	Ac	SSWA	END	CR	AZUSC3203
TORPEDINIFORMES					
Torpedinidae					
Tetronarce puelcha (Lahille, 1926)	Ad	SSWA	DD	VU	AZUSC3543
Narcinidae					
Narcine brasiliensis (Olfers, 1831)	Co	WA	DD		AZUSC3424
RAJIFORMES					
Rajidae					
Atlantoraja castelnaui (Miranda Ribeiro, 1907)	Ac	SSWA	END	END	AZUSC3147
Atlantoraja cyclophora (Regan, 1903)	Ad	SSWA	VU		AZUSC3346
Atlantoraja platana (Günther, 1880)	Ad	SSWA	VU		AZUSC3349
Psammobatis extenta (Garman, 1913)	Ad	SSWA	LC		AZUSC3446
Psammobatis lentiginosa McEachran, 1983	Ad	SSWA	DD		AZUSC3442
Rioraja agassizii (Müller & Henle, 1841)	Co	SSWA	VU	END	AZUSC3432
Sympterygia bonapartii Müller & Henle, 1841	Ad	SSWA	DD	END	AZUSC3782
PRISTIFORMES					
Rhinobatidae					
Pseudobatos horkelii (Müller & Henle, 1841)	Ac	SSWA	CR	CR	AZUSC3454
Pseudobatos percellens (Walbaum, 1792)	Co	TA	NT		AZUSC3456
Zapteryx brevirostris (Müller & Henle, 1841)	Co	WA	VU	VU	AZUSC3591
MYLIOBATIFORMES					
Dasyatidae					
Hypanus americanus (Hildebrand & Schroeder, 1928)	Ac	WA	DD		AZUSC3355
Dasyatis hypostigma Santos & Carvalho, 2004	Co	SSWA	DD		AZUSC3359
Hypanus guttatus (Bloch & Schneider,1801)	Ad	Ca+Br	DD		AZUSC3358
Pteroplatytrygon violacea (Bonaparte, 1832)	Ad	CT	LC		AZUSC3786

Gymnuridae					
Gymnura altavela (Linnaeus, 1758)	Co	TA	VU	CR	AZUSC3387
Myliobatidae					
Aetobatus narinari (Euphrasen, 1790)	Ad	CT	NT		AZUSC3291
Mobula hypostoma (Bancroft, 1831)	Ad	CT	DD	VU	AZUSC3400
Mobula thurstoni (Lloyd, 1908)	Ad	CT	NT	VU	AZUSC3403
Myliobatis freminvillei Lesueur, 1824	Ad	WA	DD	END	AZUSC3412
Myliobatis goodei Garman, 1885	Ad	WA	DD	CR	AZUSC3418
Rhinoptera bonasus (Mitchill, 1815)	Ad	WA	NT		AZUSC3783
Rhinoptera brasiliensis Müller, 1836	Ad	WA	END	CR	AZUSC0877
ELOPIFORMES					
Elopidae					
Elops smithi McBride, Rocha, Ruiz-Carus & Bowen, 2010	Ad	WA	DD		AZUSC1360
ALBULIFORMES					
Albulidae					
Albula vulpes (Linnaeus, 1758)	Ac	WA	NT		AZUSC1742
ANGUILLIFORMES					
Muraenidae					
Gymnothorax conspersus Poey, 1867	Ad	WA	X		AZUSC3719
Gymnothorax funebris Ranzani, 1839	Ad	WA	LC		AZUSC2108
Gymnothorax moringa (Cuvier, 1829)	Ad	WA	LC		AZUSC2710
Gymnothorax ocellatus Agassiz, 1831	Ac	Ca+SWA	LC		AZUSC2010
Muraena retifera Goode & Bean, 1882	Ad	WA	LC		AZUSC2299
Ophichthidae					
Echiophis intertinctus (Richardson, 1848)	Ad	WA	LC		AZUSC2109
Ophichthus ophis (Linnaeus, 1758)	Ad	TA	LC		AZUSC2256
Ophichthus gomesii (Castelnau, 1855)	Ac	WA	LC		AZUSC3673
Muraenosocidae					
Cynoponticus savanna (Bancroft, 1831)	Ad	Ca+Br	LC		AZUSC3761
Congridae					
Conger orbignianus Valenciennes, 1837	Ad	SSWA	X		AZUSC1120
CLUPEIFORMES					
Pristigasteridae					
Chirocentrodon bleekerianus (Poey, 1867)	Ac	Ca+Br	LC		AZUSC1488
Pellona harroweri (Fowler, 1917)	Co	Ca+Br	LC		AZUSC1477
Engraulidae					
Anchoa filifera (Fowler, 1915)	Ad	Ca+Br	LC		AZUSC1490
Anchoa marinii Hildebrand, 1943	Ad	SSWA	LC		AZUSC4620
Anchoa spinifer (Valenciennes, 1848)	Ad	Ca+Br+EP	LC		AZUSC4621
Anchoa tricolor (Spix & Agassiz, 1829)	Ad	SWA	LC		AZUSC4625
Anchovia clupeoides (Swainson, 1839)	Ad	Ca+Br	LC		AZUSC1476
Anchoviella lepidentostole (Fowler, 1911)	Ad	Br	LC		AZUSC1487
Engraulis anchoita Hubbs & Marini, 1935	Ad	SSWA	X		AZUSC2160
Lycengraulis grossidens (Spix & Agassiz, 1829)	Ad	Br+SSWA	LC		AZUSC4663
Clupeidae					
Brevoortia pectinata (Jenyns, 1842)	Ad	SSWA	LC		AZUSC3897
Harengula clupeola (Cuvier, 1829)	Co	WA	LC		AZUSC1328

Opisthonema oglinum (Lesueur, 1818)	Ad	WA	LC		AZUSC1327
Sardinella brasiliensis (Steindachner, 1879)	Ad	SSWA	X		AZUSC2164
SILURIFORMES					
Ariidae					
Aspistor luniscutis (Valenciennes, 1840)	Co	Br	X		AZUSC1976
Bagre bagre (Linnaeus, 1766)	Ac	Ca+Br	LC		AZUSC1367
Bagre marinus (Mitchill, 1815)	Ad	WA	LC		AZUSC4602
Cathorops spixii (Agassiz, 1829)	Ad	Ca+Br	X		AZUSC1518
Genidens barbus (Lacepède, 1803)	Co	Br+SSWA	X	END	AZUSC1371
Genidens genidens (Cuvier, 1829)	Co	Br+SSWA	LC		AZUSC1756
AULOPIFORMES					
Synodontidae					
Synodus bondi Fowler, 1939	Co	WA	LC		AZUSC2090
Trachinocephalus myops (Forster, 1801)	Ac	CT	LC		AZUSC1470
GADIFORMES					
Merlucciidae					
Merluccius hubbsi Marini, 1933	Ad	SSWA	X		AZUSC1288
Gadidae					
Urophycis brasiliensis (Kaup, 1858)	Ad	SSWA	X		AZUSC2122
HOLOCENTRIFORMES					
Holocentridae					
Holocentrus adscensionis (Osbeck, 1765)	Ad	TA	LC		AZUSC1699
OPHIDIIFORMES					
Ophidiidae					
Genypterus brasiliensis Regan, 1903	Ad	SSWA	X		AZUSC0905
Ophidion holbrookii Putnam, 1874	Ac	WA	LC	CR	AZUSC1352
Raneya brasiliensis (Kaup, 1856)	Ac	SSWA	X		AZUSC1811
BATRACHOIDIFORMES					
Batrachoididae					
Opsanus beta (Goode & Bean, 1880)	Ad	Ca	LC		AZUSC3144
Porichthys porosissimus (Cuvier, 1829)	Co	SSWA	X		AZUSC1146
Thalassophryne montevidensis (Berg, 1893)	Ad	SWA	X		AZUSC3088
MUGILIFORMES					
Mugilidae					
Mugil curema Valenciennes, 1836	Ad	TA+EP	LC		AZUSC1814
Mugil liza Valenciennes, 1836	Ad	WA	DD		AZUSC2698
Polynemidae					
Polydactylus oligodon (Günther, 1860)	Ad	WA	LC		AZUSC2089
Polydactylus virginicus (Linnaeus, 1758)	Ac	WA	LC		AZUSC2011
BELONIFORMES					
Exocoetidae					
Cheilopogon melanurus (Valenciennes, 1847)	Ad	TA	LC		AZUSC2709
Hemiramphidae					
Hemiramphus balao Lesueur, 1821	Ad	TA	LC		AZUSC4008
Hemiramphus brasiliensis (Linnaeus, 1758)	Ad	TA	LC		AZUSC4604
Hyporhamphus unifasciatus (Ranzani, 1841)	Ad	WA+EP	LC		AZUSC4618

Belonidae				
Ablennes hians (Valenciennes, 1846)	Ad	CT	LC	AZUSC2020
Strongylura marina (Walbaum, 1792)	Ad	WA	LC	AZUSC4672
Strongylura timucu (Walbaum, 1792)	Ad	WA	LC	AZUSC4603
Tylosurus acus (Lacepède, 1803)	Ad	TA	LC	AZUSC4619
CARANGIFORMES				
Coryphaenidae				
Coryphaena hippurus Linnaeus, 1758	Ad	CT	LC	AZUSC4601
Rachycentridae				
Rachycentron canadum (Linnaeus, 1766)	Ad	CT	LC	AZUSC2244
Echeneidae				
Echeneis naucrates Linnaeus, 1758	Ad	CT	LC	AZUSC3232
Carangidae				
Alectis ciliaris (Bloch, 1787)	Ad	CT	LC	AZUSC1389
Caranx crysos (Mitchill, 1815)	Ac	TA	LC	AZUSC1319
Caranx hippos (Linnaeus, 1766)	Ac	TA	LC	AZUSC1658
Caranx latus Agassiz, 1831	Ad	TA	LC	AZUSC1322
Chloroscombrus chrysurus (Linnaeus, 1766)	Co	TA	LC	AZUSC1344
Decapterus punctatus (Cuvier, 1829)	Ad	TA	LC	AZUSC1792
Hemicaranx amblyrhynchus (Cuvier, 1833)	Ac	WA	LC	AZUSC1928
Oligoplites palometa (Cuvier, 1832)	Ad	Ca+Br	LC	AZUSC3037
Oligoplites saliens (Bloch, 1793)	Co	Ca+SWA	LC	AZUSC1317
Oligoplites saurus (Bloch & Schneider, 1801)	Ad	WA	LC	AZUSC3771
Parona signata (Jenyns, 1841)	Ad	SSWA	X	AZUSC3604
Pseudocaranx dentex (Bloch & Schneider, 1801)	Ac	CT	LC	AZUSC1329
Selar crumenophthalmus (Bloch, 1793)	Ad	Ca+Br	LC	AZUSC2447
Selene setapinnis (Mitchill, 1815)	Co	WA	LC	AZUSC1323
Selene vomer (Linnaeus, 1758)	Co	WA	LC	AZUSC1388
Seriola dumerili (Risso, 1810)	Ad	CT	LC	AZUSC3668
Seriola fasciata (Bloch, 1793)	Ad	TA	LC	AZUSC3677
Seriola lalandi Valenciennes, 1833	Ad	CT	LC	AZUSC2634
Trachinotus carolinus (Linnaeus, 1766)	Co	WA	LC	AZUSC1336
Trachinotus falcatus (Linnaeus, 1758)	Ad	WA	LC	AZUSC3149
Trachinotus goodei Jordan & Evermann, 1896	Ac	WA	LC	AZUSC2016
Trachinotus marginatus Cuvier, 1832	Ad	SSWA	X	AZUSC2678
Trachurus lathami Nichols, 1920	Ad	WA	LC	AZUSC1122
Uraspis secunda (Poey, 1860)	Ad	CT	LC	AZUSC1963
ISTIOPHORIFORMES				
Sphyraenidae				
Sphyraena guachancho Cuvier, 1829	Co	TA	LC	AZUSC1341
Sphyraena tome Fowler, 1903	Ac	SSWA	X	AZUSC1602
Xiphiidae				
Xiphias gladius Linnaeus, 1758	Ad	CT	LC	AZUSC4598
PLEURONECTIFORMES				
Paralichthyidae				
Citharichthys arenaceus Evermann & Marsh, 1900	Ac	WA	LC	AZUSC4623
Citharichthys macrops Dresel, 1885	Ad	WA	LC	AZUSC1678

Cyclopsetta chittendeni Bean, 1895	Ad	Ca+Br	LC	AZUSC1651
Etropus crossotus Jordan & Gilbert, 1882	Ad	WA+EP	LC	AZUSC2159
Etropus longimanus Norman, 1933	Ad	SSWA	X	AZUSC4622
Paralichthys brasiliensis (Ranzani, 1842)	Ac	SWA	X	AZUSC1521
Paralichthys orbignyanus (Valenciennes, 1839)	Ad	SSWA	X	AZUSC1926
Paralichthys patagonicus Jordan, 1889	Ac	SSWA+EP	X	AZUSC1392
Syacium papillosum (Linnaeus, 1758)	Ad	WA	LC	AZUSC1549
Syacium micrurum Ranzani, 1842	Ad	WA	LC	AZUSC3036
Xystreurys rasile (Jordan, 1891)	Ad	SSWA	X	AZUSC4600
Bothidae				
Bothus ocellatus (Agassiz, 1831)	Ad	WA	LC	AZUSC3086
Bothus robinsi Topp & Hoff, 1972	Ad	WA	LC	AZUSC2088
Achiridae				
Achirus declivis Chabanaud, 1940	Ad	WA	LC	AZUSC2245
Gymnachirus nudus Kaup, 1858	Ac	WA	LC	AZUSC1949
Trinectes paulistanus (Miranda Ribeiro, 1915)	Ad	Ca+Br	LC	AZUSC4664
SYNGNATHIFORMES				
Fistulariidae				
Fistularia petimba Lacepède, 1803	Ad	TA	LC	AZUSC1330
Fistularia tabacaria Linnaeus, 1758	Co	TA	LC	AZUSC2018
Dactylopteridae				
Dactylopterus volitans (Linnaeus, 1758)	Co	TA	LC	AZUSC1147
SCOMBRIFORMES				
Gempylidae				
Thyrsitops lepidopoides (Cuvier, 1832)	Ad	SSWA	X	AZUSC1148
Trichiuridae				
Evoxymetopon taeniatus Gill, 1863	Ad	Ca+SWA+EP	LC	AZUSC2939
Trichiurus lepturus Linnaeus, 1758	Co	CT	LC	AZUSC1391
Scombridae				
Scomber japonicus Houttuyn, 1782	Ad	CT	LC	AZUSC2286
Scomberomorus brasiliensis Collette, Russo & Zavala-Camin, 1978	Co	Ca+Br	LC	AZUSC1369
Scomberomorus cavalla (Cuvier, 1829)	Ad	WA	LC	AZUSC4599
Thunnus alalunga (Bonnaterre, 1788)	Ad	CT	NT	AZUSC1933
Centrolophidae				
Seriolella porosa Guichenot, 1848	Ad	SWA+EP	X	AZUSC2636
Stromateidae				
Peprilus xanthurus (Quoy & Gaimard, 1825)	Co	SWA	X	AZUSC1333
TRACHINIFORMES				
Pinguipedidae				
Pseudopercis semifasciata (Cuvier, 1829)	Ad	SSWA	X	AZUSC2119
Percophidae				
Percophis brasiliensis Quoy & Gaimard, 1825	Ad	SSWA	X	AZUSC1145
Uranoscopidae				
Astroscopus sexspinosus (Steindachner, 1876)	Ad	SSWA	X	AZUSC2113
Astroscopus y-graecum (Cuvier, 1829)	Ad	WA	LC	AZUSC2014
LABRIFORMES				

Labridae					
Xyrichtys novacula (Linnaeus, 1758)	Ad	WA	LC		AZUSC1157
Scaridae					
Nicholsina usta (Valenciennes, 1840)	Ad	WA	LC		AZUSC3644
Sparisoma axillare (Steindachner, 1878)	Ad	Br	DD	VU	AZUSC1828
Sparisoma frondosum (Agassiz, 1831)	Ad	Ca+Br	DD	VU	AZUSC1799
PERCIFORMES					
Centropomidae					
Centropomus parallelus Poey, 1860	Ad	WA	LC		AZUSC1372
Centropomus undecimalis (Bloch, 1792)	Ac	WA	LC		AZUSC1363
Gerreidae					
Diapterus auratus Ranzani, 1842	Ad	WA	LC		AZUSC3898
Diapterus rhombeus (Cuvier, 1829)	Co	Ca+Br	LC		AZUSC1325
Eucinostomus argenteus Baird & Girard, 1855	Ac	WA+EP	LC		AZUSC1326
Eucinostomus gula (Quoy & Gaimard, 1824)	Ad	WA	LC		AZUSC1359
Eucinostomus melanopterus (Bleeker, 1863)	Ad	TA	LC		AZUSC1625
Eugerres brasilianus (Cuvier, 1830)	Ad	WA	LC		AZUSC1875
Polyprionidae					
Polyprion americanus (Bloch & Schneider, 1801)	Ad	CT	DD	CR	AZUSC3656
Mullidae					
Pseudupeneus maculatus (Bloch, 1793)	Ad	WA	LC		AZUSC1661
Upeneus parvus Poey, 1852	Ac	WA	LC		AZUSC1351
Kyphosidae					
Kyphosus vaigiensis (Quoy & Gaimard, 1825)	Ad	TA	LC		AZUSC3867
Kyphosus sectatrix (Linnaeus, 1758)	Ad	TA	LC		AZUSC0372
Serranidae					
Diplectrum formosum (Linnaeus, 1766)	Co	WA	LC		AZUSC1331
Diplectrum radiale (Quoy & Gaimard, 1824)	Ac	WA	LC		AZUSC1355
Dules auriga Cuvier, 1829	Ad	SSWA	X		AZUSC1140
Epinephelus itajara (Lichtenstein, 1822)	Ad	WA	CR	CR	AZUSC1043
Epinephelus marginatus (Lowe, 1834)	Ad	WA+EP	END	VU	AZUSC2377
Epinephelus morio (Valenciennes, 1828)	Ad	WA	NT	VU	AZUSC3646
Hyporthodus niveatus (Valenciennes, 1828)	Ac	WA	VU	VU	AZUSC1600
Mycteroperca acutirostris (Valenciennes, 1828)	Ad	Ca+Br	LC		AZUSC1646
Rypticus randalli Courtenay, 1967	Ad	Ca+Br	LC		AZUSC3721
Serranus atrobranchus (Cuvier, 1829)	Ad	WA	LC		AZUSC3676
Pomatomidae					
Pomatomus saltatrix (Linnaeus, 1766)	Co	CT	VU		AZUSC1630
Priacanthidae					
Priacanthus arenatus Cuvier, 1829	Co	TA	LC		AZUSC1339
Pomacanthidae					
Pomacanthus paru (Bloch, 1787)	Ad	WA	LC		AZUSC1342
Malacanthidae					
Caulolatilus chrysops (Valenciennes, 1833)	Ad	WA	LC		AZUSC1605
Haemulidae					
Anisotremus surinamensis (Bloch, 1791)	Ad	WA	DD		AZUSC1688
Anisotremus virginicus (Linnaeus, 1758)	Ac	WA	LC		AZUSC1343

Boridia grossidens Cuvier, 1830	Ad	SSWA	X		AZUSC1912
Conodon nobilis (Linnaeus, 1758)	Co	WA	LC		AZUSC1320
Genyatremus cavifrons (Cuvier, 1830)	Ad	Ca+Br	DD		AZUSC1968
Haemulon aurolineatum Cuvier, 1830	Ac	WA	LC		AZUSC1398
Haemulon steindachneri (Jordan & Gilbert, 1882)	Ac	Ca+SWA	LC		AZUSC1913
Orthopristis ruber (Cuvier, 1830)	Co	Ca+SWA	LC		AZUSC1138
Haemulopsis corvinaeformis (Steindachner, 1868)	Co	Ca+SWA	LC		AZUSC1345
Pomadasys crocro (Cuvier, 1830)	Ad	WA	DD		AZUSC3099
Lutjanidae					
Lutjanus analis (Cuvier, 1828)	Ad	WA	NT		AZUSC2789
Lutjanus griseus (Linnaeus, 1758)	Ad	WA	LC		AZUSC3100
Lutjanus jocu (Bloch & Schneider, 1801)	Ad	WA	DD		AZUSC4542
Lutjanus synagris (Linnaeus, 1758)	Ad	WA	NT		AZUSC4321
Rhomboplites aurorubens (Cuvier, 1829)	Ad	WA	VU		AZUSC1423
SCORPAENIFORMES					
Scorpaenidae					
Scorpaena brasiliensis Cuvier, 1829	Ac	WA	LC		AZUSC1599
Scorpaena isthmensis Meek & Hildebrand, 1928	Ad	WA	LC		AZUSC3711
Scorpaena plumieri Bloch, 1789	Ac	WA	LC		AZUSC1611
Triglidae					
Prionotus nudigula Ginsburg, 1950	Ad	SSWA	X		AZUSC1609
Prionotus punctatus (Bloch, 1793)	Co	Ca+SWA	LC		AZUSC1141
MORONIFORMES					
Ephippidae					
Chaetodipterus faber (Broussonet, 1782)	Co	WA	LC		AZUSC1334
ACANTHURIFORMES					
Sciaenidae					
Ctenosciaena gracilicirrhus (Metzelaar, 1919)	Co	Ca+Br	LC		AZUSC1142
Cynoscion acoupa (Lacepède, 1801)	Ad	Ca+SWA	LC		AZUSC3202
Cynoscion guatucupa (Cuvier, 1830)	Ac	SSWA	X		AZUSC1143
Cynoscion jamaicensis (Vaillant & Bocourt, 1883)	Co	Ca+SWA	LC		AZUSC1386
Cynoscion leiarchus (Cuvier, 1830)	Co	Ca+Br	LC		AZUSC1966
Cynoscion virescens (Cuvier, 1830)	Co	Ca+Br	LC		AZUSC1358
Isopisthus parvipinnis (Cuvier, 1830)	Co	Ca+Br	LC		AZUSC1390
Larimus breviceps Cuvier, 1830	Co	Ca+Br	LC		AZUSC1318
Macrodon atricauda (Günther, 1880)	Co	Br+SSWA	X		AZUSC1498
Menticirrhus americanus (Linnaeus, 1758)	Co	WA	LC		AZUSC1430
Menticirrhus littoralis (Holbrook, 1847)	Ad	WA	LC		AZUSC4730
Micropogonias furnieri (Desmarest, 1823)	Co	Ca+SWA	LC		AZUSC1144
Nebris microps Cuvier, 1830	Ac	Br	LC		AZUSC1467
Paralonchurus brasiliensis (Steindachner, 1875)	Co	Ca+SWA	LC		AZUSC1425
Pogonias cromis (Linnaeus, 1766)	Ad	WA	LC	END	AZUSC3206
Stellifer brasiliensis (Schultz, 1945)	Ad	Br	X		AZUSC1486
Stellifer rastrifer (Jordan, 1889)	Ad	Br+SSWA	LC		AZUSC1520
Umbrina canosai Berg, 1895	Ad	SSWA	X		AZUSC1123
Umbrina coroides Cuvier, 1830	Ad	WA	LC		AZUSC1365

Acanthuridae				
Acanthurus chirurgus (Bloch, 1787)	Ad	TA	LC	AZUSC2120
SPARIFORMES				
Lobotidae				
Lobotes surinamensis (Bloch, 1790)	Ad	CT	LC	AZUSC2635
Sparidae				
Archosargus aries (Valenciennes, 1830)	Ad	WA	X	AZUSC3239
Archosargus rhomboidalis (Linnaeus, 1758)	Ac	WA	LC	AZUSC1361
Calamus penna (Valenciennes, 1830)	Ad	WA	LC	AZUSC3035
Calamus pennatula Guichenot, 1868	Ad	WA	LC	AZUSC3089
Diplodus argenteus (Valenciennes, 1830)	Co	SWA	LC	AZUSC1366
Pagrus pagrus (Linnaeus, 1758)	Co	TA	LC	AZUSC1426
LOPHIIFORMES				
Lophiidae				
Lophius gastrophysus Miranda Ribeiro, 1915	Ad	WA	LC	AZUSC1608
Antennariidae				
Antennarius striatus (Shaw, 1794)	Ad	WA	LC	AZUSC3143
Ogcocephalidae				
Ogcocephalus vespertilio (Linnaeus, 1758)	Ac	Ca+SWA	X	AZUSC2707
TETRAODONTIFORMES				
Ostraciidae				
Acanthostracion quadricornis (Linnaeus, 1758)	Ad	WA	LC	AZUSC2117
Balistidae				
Balistes capriscus Gmelin, 1789	Co	CT	VU	AZUSC1347
Monacanthidae				
Aluterus monoceros (Linnaeus, 1758)	Ac	CT	LC	AZUSC1362
Aluterus heudelotii Hollard, 1855	Ad	CT	LC	AZUSC4517
Stephanolepis hispidus (Linnaeus, 1766)	Co	TA	LC	AZUSC1332
Molidae				
Mola mola (Linnaeus, 1758)	Ad	CT	VU	AZUSC3950
Tetraodontidae				
Lagocephalus laevigatus (Linnaeus, 1766)	Co	TA	LC	AZUSC1373
Lagocephalus lagocephalus (Linnaeus, 1758)	Ad	CT	LC	AZUSC3234
Sphoeroides greeleyi Gilbert, 1900	Ad	Ca+Br	LC	AZUSC2522
Sphoeroides spengleri (Bloch, 1785)	Ad	TA	LC	AZUSC3330
Sphoeroides testudineus (Linnaeus, 1758)	Ad	WA	LC	AZUSC2163
Diodontidae				
Chilomycterus spinosus (Linnaeus, 1758)	Co	SWA	LC	AZUSC1139

Table 3. Total number of ichthyofaunal taxa captured by the pair trawling fleet based in the State of São Paulo.

Class	**Chondrichthyes**	**Actinopterygii**
Order	6	26
Family	11	70
Genus	22	148
Species	34	211

From the frequency of occurrence (FO) and the classification proposed by Dajoz (1983) the ichthyofauna consisted of 50 species (20.41%) classified as constants; 38 (15.5%) accessory species and 157 (64.08%) accidental species. Seventeen species (6.34%) had 100% frequency throughout the sample period: *Dactylopterus volitans* (Flying gurnard), *Prionotus punctatus* (Bluewing searobin), *Chloroscombrus chrysurus* (Atlantic bumper), *Oligoplites saliens* (Castin leatherjacket), *Selene setapinnis* (Atlantic moonfish), *S. vomer* (Lookdown), *Conodon nobilis* (Barred grunt), *Orthopristis ruber* (Corocoro grunt), *Diplodus argenteus* (South American silver porgy), *Cynoscion jamaicensis* (Jamaica weakfish), *Menticirrhus americanus* (Southern kingcroaker), *Micropogonias furnieri* (Whitemouth croaker), *Chaetodipterus faber* (Atlantic spadefish), *Trichiurus lepturus* (Largehead hairtail), *Balistes capriscus* (Grey triggerfish), *Stephanolepis hispidus* (Planehead filefish) and *Chilomycterus spinosus* (Southern burrfish).

When considering only the Critical, Endangered and Vulnerable categories, both by the IUCN (2017) criteria and by Brazilian federal Legislation (MMA 2014) we observed a total of 33 threatened species (13.47%) in this study. Also, when considering only the threat categories and choosing the most conservative classification, three distinct and worrying scenarios are drawn by correlating the conservation status with the frequency of occurrence (constant, accessory and accidental) (Table 4).

Most of the captured species (35.51%) during this study were distributed in the Atlantic Ocean (WA), followed by the SSWA (15.10%), circumtropical (CT) species (13.06%), Trans-Atlantic (TA) species (11.02%), Ca+Br (9.8%), Ca+SWA (4.9%), SWA (2.45%), Br (2.04%), Br+SSWA (2.04%), and WA+EP (1.63%). One species (0.41% of the total) was found in each of the following areas: TA+EP, SWA+EP, SSWA+EP, Ca+Br+EP, Ca+SWA+EP, and Ca.

Discussion

The richness of demersal organisms is directly related to the continental shelf width, sediment types, oceanographic conditions, the geological events that shaped the continental shelf, as well as its positioning relative to the equator characterizing the environmental temperature (Lowe-McConnell 1987, Longhurst & Pauly 2007).

Although many results of this study can be explained by the oceanographic features of the pair trawling fleet operation area in the state of São Paulo, biotic factors interfere with the species distribution through inter- and intraspecific relations, such as the predator-prey effect and the competitive interactions for food (Sanders 1969, Moyle & Cech 1998, Menge & Olson 1990, Barry et al. 1996, Akin et al. 2003).

Overall, the widest and largest continental shelf is in southern Brazil, consisting of muddy sediment and under the influence of the oscillation of the subtropical convergence between the warm waters

Table 4. Checklist of the threatened species captured according to the International Union for Conservation of Nature (IUCN 2017) and the Brazilian list (MMA 2014) and their frequency of occurrence (Dajoz 1983).

	Accidental	Accessory	Constant
Critically endangered	*Mustelus schmitti* *Sphyrna lewini* *Sphyrna zygaena* *Myliobatis goodei* *Rhinoptera brasiliensis* *Epinephelus itajara* *Polyprion americanus*	*Pseudobatos horkelii* *Squatina guggenheim* *Ophidion holbrooki*	*Gymnura altavela*
Endangered	*Sympterygia bonapartii* *Myliobatis freminvillei* *Epinephelus marginatus* *Pogonias cromis*	*Atlantoraja castelnaui*	*Rioraja agassizii* *Genidens barbus*
Vulnerable	*Carcharhinus falciformis* *Atlantoraja cyclophora* *Atlantoraja platana* *Tetronarce puelcha* *Mobula hypostoma* *Mobula thurstoni* *Sparisoma axillare* *Sparisoma frondosum* *Epinephelus morio* *Rhomboplites aurorubens* *Mola mola*	*Hyporthodus niveatus*	*Zapteryx brevirostris* *Pomatomus saltatrix* *Balistes capriscus*

of the Brazilian current and the cold waters of the Malvinas current. The region has upwelling zones that during the summer and spring cause the South Atlantic Central Water (SACW) to penetrate the shelf reaching the coastal areas with a thermocline of 10 and 15 meters. During the winter, SACW retracts and Tropical Water (TW) fills the space. There is significant water input from continental drainage. Due to such characteristics, the region represents the Southern boundary of occurrence of several tropical species (e.g.: *Hypanus guttatus*, *H. americanus*, *Anchoa tricolor*, *Rypticus randalli*) and the Northern boundary of temperate species (e.g.: *Atlantoraja castelnaui*, *A. cyclophora*, *Sardinella brasiliensis*, *Boridia grossidens*). Moreover, the region has the highest abundance of fishery resources in Brazil (Pires-Vanin et al. 1993, Matsuura 1995, Castro & Menezes 1998, Menezes et al. 2003, Braga & Niencheski 2006, Castro et al. 2006, Castro et al. 2008, Amaral & Nallin 2011, Menezes 2011).

According to Menezes et al. (2003), 1,297 species of marine fish, belonging to 36 orders and 192 families, occur in Brazil. The results of this study showed the Southern demersal ichthyofauna accounted for 18.9% of species recorded in Brazil. The families Carangidae, Sciaenidae, Paralichthyidae, Haemulidae, Serranidae and Engraulidae showed high number of species, with most species being demersal, benthic or benthopelagic (Lowe-McConnell 1987).

According to Dajoz's (1983) scale, we could hypothesize that the common richness of the ichthyofauna by landing is approximately 90 species (constant + accessory). However, this value differs from that observed in landings, which ranged from 49 to 104 species (mean and standard deviation = 68.65 ± 13.20), mainly due to the high number of accidental species.

According to criteria adopted by the International Union for Conservation of Nature (IUCN 2017), species classified as least concern (64.08%) were predominant, followed by not-evaluated species (14.69%), data deficient (8.16%), vulnerable and near-threatened (4.90%), endangered (2.45%) and critically endangered (0.82%). Based on Brazilian federal legislation (MMA 2014), only 10.61% of the species are threatened regarding the conservation status: 4.49% endangered, 3.67% vulnerable and 2.45% endangered. Proportionally, Class Chondrichthyes had higher number of highly endangered species (23.53% critically endangered, 11.76% endangered and 20.59% vulnerable). Based on the three scenarios observed regarding the conservation status of the captured species, the actions of fishery management are priority for the pair trawling fishing operating in the Zoogeographic Province of Argentina. We emphasize this study was carried out in the period before the prohibitions on the capture of several endangered species (MMA 2014); therefore, the continuous monitoring of the composition of this fishing modality is necessary. We also point out there were restrictions of fishing area for pair trawling fishing in the State of São Paulo (SMA 2009). Such restrictions occurred during the last 3 years of this study, which again emphasizes the need for continuous monitoring to verify changes in the composition of the species captured by this type of fishery.

According to Kotas (1991), the pair trawling fishing is characterized as active, multispecies and difficult to manage due to the large number of species captured. The most used management technique in these cases is the restriction of areas and/or periods (High et al. 1969, Caddy 1982), which often causes economic and social problems. There are other ways of ensuring greater selectivity and more sustainability in these fisheries without losing their economic viability, such as adequacy of fishing nets and the use of bycatch reduction devices (BRD). Several BRD models have been developed and tested in different types of environments worldwide, many of which are summarized in the study by Broadhurst (2000). Thus, studies on fishing technology aiming at minimizing the capture of endangered species are essential for biodiversity maintenance.

Caires (2014) listed 120 species (76.67% Actinopterygii and 23.33% Chondrichthyes) as endemic to the zoogeographic province of Argentina. Out of the 92 species of Actinopterygii listed, 24 species (26%) were observed in this study, namely: *Conger orbignianus* (Congridae), *Sardinella brasiliensis* (Clupeidae), *Merluccius hubbsi* (Merlucciidae), *Urophycis brasileinsis* (Gadidae), *Genypterus brasiliensis* and *Raneya brasiliensis* (Ophidiidae), *Porichthys porosissimus* and *Thalassophryne montevidensis* (Batrachoididae), *Parona signata* and *Trachinotus marginatus* (Carangidae), *Sphyraena tome* (Sphyraenidae), *Etropus longimanus*, *Paralichthys orbignyanus*, *P. patagonicus* and *Xystreurys rasile* (Paralichthyidae), *Thyrsitops lepidopoides* (Gempylidae), *Pseudopercis semifasciata* (Pinguipedidae), *Percophis brasiliensis* (Percophidae), *Astroscopus sexspinosus* (Uranoscopidae), *Dules auriga* (Serranidae), *Boridia grossidens* (Haemulidae), *Prionotus nudigula* (Triglidae), *Cynoscion guatucupa* and *Umbrina canosai* (Sciaenidae). Among the 28 species of Chondrichthyes, 11 species (39.3%) were captured in the present study, namely: *Mustelus schmitti* (Triakidae), *Squatina guggenheim* (Squatinidae), *Atlantoraja castelnaui*, *A. cyclophora*, *A. platana*, *Psammobatis extenta*, *P. lentiginosa*, *Rioraja agassizii* and *Sympterygia bonapartii* (Rajidae), *Pseudobatos horkelii* (Rhinobatidae) and *Dasyatis hypostigma* (Dasyatidae). Due to the distribution (Southeast region of Brazil to Argentina), we also consider the following species endemic to the zoogeographic province of Argentina: *Tetronarce puelcha* (Torpedinidae), *Anchoa marinii* and *Engraulis anchoita* (Engraulidae) and *Brevoortia pectinata* (Clupeidae). Thus, 35.29% of Chondrichthyes and 11.85% of Actinopterygii (15.1% of the total) belong to the endemic fauna of zoogeographic province of Argentina.

The importance of the zoogeographic province of Argentina in the diversity of marine and estuarine fish fauna of Southern Brazil was evidenced in two other studies carried out in the Laje de Santos Marine State Park (Luiz Jr et al. 2008) and in the Paranaguá estuarine complex (Passos et al. 2012). The similarity in species composition was low (<50%) due to the different types of environments (rocky reefs, continental shelf and estuary). Totally, 427 species were observed, out of which 46 species (10.77%) are endemic to zoogeographic province of Argentina, being the fourth geographic distribution with the highest number of species, only behind the Western Atlantic with 169 species (39.58%), circumtropical with 52 species (12.18%) and trans-Atlantic with 50 species (11.71%). There was higher rate of endemism in the zoogeographic province of Argentina among marine demersal species (14.29%), followed by estuarine (10.95%) and reef (4.69%) species.

Based on the results of this work, we highlight the need for studies addressing other aspects of exploited communities, such as diversity analysis, spatial and seasonal distribution, as well as on impacts on explored demersal fishes, such as types of fishing, pollution, degradation of coastal areas and the introduction of exotic species.

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Author Contributions

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Conflicts of interest

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

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Point of view

Misguided policy may jeopardize a diverse South brazilian environmental protection area

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Abstract: The Devonian Escarpment (DEEPA), located in the south of Brazil, represents an important area of environmental preservation composed by grasslands, gallery forests, as well as rock outcrops and archaeological sites. A law project (LP 527/2016), which suggests a reduction of the DEEPA area in approximately 70% of its original area (from 393,579 to 125,895 ha), is currently being processed in the Paraná State House of Representatives. Such reduction seems to be related to economic interests (mainly agriculture and mining) in the state of Paraná. If approved, LP 527/2016 will allow farmers to deliberately expand their activities, with the suppression of natural forest as main consequence. Additionally, loss of faunal diversity, contamination of water and soils, and alteration in nutrient cycles are expected, due the intensive use of agrochemicals. In addition to the direct environmental consequences, we expect the disappearance of areas of high geological interest, reducing local geodiversity, as well as substantial economic losses with ecotourism. Brazil is a signatory to the United Nations Convention on Biological Diversity, where it undertakes to develop strategies to prevent biodiversity loss and ecosystem degradation by 2020. An approval of the LP would be contradictory, considering that there are few natural vegetation areas in this region of the country. We emphasize that the proposal of such projects goes against the sustainability in the country and disregard the scientific knowledge generated until then. Thus, it is necessary to develop regional and federal political objectives that guarantee economic development in a balanced way, considering the local bio and geodiversity, not the proposal of mechanisms that destroy them.

Keywords: *Environmental law, Grassland, Devonian Escarpment, Biodiversity loss, Geodiversity.*

Política equivocada pode colocar em risco uma área de preservação ambiental do sul do Brasil

Resumo: A Escarpa Devoniana (APAED), localizada no sul do Brasil, representa uma importante área de preservação ambiental composta por campos, matas de galeria, além de afloramentos rochosos e sítios arqueológicos. Atualmente, tramita na câmara dos deputados do estado do Paraná um projeto de lei estadual (PL 527/2016) que sugere a redução da área da APAED em aproximadamente 70% de sua área original (de 393.579 para 125.895 ha). Tal redução parece estar relacionada com interesses econômicos (principalmente agropecuária e mineração) no estado do Paraná. Caso aprovado, o PL 527/2016 permitirá que agricultores expandam suas atividades deliberadamente, tendo como consequência principal a supressão de áreas de floresta nativa. Com isso, espera-se perda de diversidade faunística, contaminação de água e solos e alteração nos ciclos de nutrientes, dado o aumento no uso de agroquímicos. Além das consequências ambientas diretas, podemos esperar o desaparecimento de elevado interesse geológico, reduzindo a geodiversidade local, além de substanciais perdas econômicas com ecoturismo. O Brasil é um dos países signatários da Convenção da Diversidade Biológica das Nações Unidas, onde se compromete a desenvolver estratégias que evitem a perda da biodiversidade e a degradação de ecossistemas até 2020. Assim, a aprovação desse projeto de lei é, no mínimo, contraditória considerando que existem poucas áreas de vegetação natural nessa região do país. Destacamos que a proposta de tais projetos vai contra a sustentabilidade no país e desconsideram o conhecimento científico gerado até então. Dessa forma, é necessário o desenvolvimento de objetivos políticos regionais e federais que garantam o desenvolvimento econômico de forma equilibrada, considerando a bio e geodiversidade local, e não a proposta de mecanismos que as destruam.

Palavras-chave: *Lei ambiental, Campos Gerais, Escarpa Devoniana, Perda da biodiversidade, Geodiversidade.*

Introduction

Protected areas are proposed to preserve and protect the biodiversity and its components, such as habitats, species, populations, and ecosystem services (Margulis & Pressey 2000), and their effectiveness has been widely shown (e.g. Frederico et al. 2018). In Brazil, the protection of natural areas is guaranteed by the National System of Conservation Units of Nature (SNUC in Portuguese – federal law 9.985/2000), whose main objectives are, among others, to protect endangered species, and preserve and recover the diversity of natural ecosystems. However, Brazilian natural resources have repeatedly been targeted with misguided policies (Bernard et al. 2014, Azevedo-Santos et al. 2015, Tófoli et al. 2016, Azevedo-Santos et al. 2017, Tófoli et al. 2017, Alves et al. 2018, Silveira et al. 2018). Currently, more than 20 legislative proposals are circulating in the Brazilian Congress to loosen regulations governing activities such as building roads and hydroelectric dams or expanding agricultural businesses (Tollefson 2016, Ferrante & Fearnside 2018). For example, the constitutional amendment 65/2012 (i.e. PEC 65/2012) has been proposed with the aim to weaken the licensing process of large developments by loosening the current power that environmental agencies have to suspend a project based on its Environmental Impact Assessment (EIA). As a consequence, EIA would no longer be necessary and mitigation and compensation, now required and supervised by the licensing authority, would be voluntary (El Bizri et al. 2016).

Other political proposals intend to reduce the size and/or the protected status from conservation units in specific regions of Brazil. According to SNUC, there are two main categories of conservation units: Integral Protection Units (IPU - a restricted category in terms of use of natural resource) and Sustainable Use Units (SUU), whose main objective is to reconcile the conservation of nature with the sustainable use of a portion of its natural resources. Last year, a law project (LP 8107/2017) was edited by the Brazilian National Congress aiming to alter the conservation status of the Jamanxim National Forest (i.e. a subcategory of IPU) in Pará state. According to this LP (which is still being processed in the House of Representatives), about 27% of the area of this national forest will be transformed in Environment Protection Area (EPA), a subcategory of SUU which allows the anthropic occupation and sustainable exploitation of natural resources. If approved, an area of approximately 350,000 ha of the Amazonian forest will be less protected and more likely to be deforested. The main argument for this action is to regularize illegal occupations that are already transforming the forest into a pasture and/or cropland.

Another recent nationwide law project (LP 3.751/2015) aims to revoke the legal status of the recently established IPU for which land ownership conflicts are not resolved within 5 years of PA creation. According to Silveira et al. (2018), if this law goes further, all future and current protected areas will be affected, including those located in the Amazon and in the two Brazilian hotspots for biodiversity: Atlantic Forest and Cerrado (Myers 2000). Clearly, those policies have a bias towards production and economy-driven activities.

The strategy for "boosting the economy" at the cost of loosening environmental protection is not restricted only to federal regulations; municipalities and states also play a big role on these actions (i.e. Azevedo-Santos et al. 2015, Tófoli et al. 2016, Alves et al. 2018). Currently, there is one regional law project, in Paraná State (South Brazil), that intends to reduce an environmental protection area (the Devonian Escarpment EPA) by legal means that are environmentally hostile. This project will reduce the degree of protection in one conservation unit, with the intention of favoring agriculture, such as crop and livestock production.

Overview of the Devonian Escarpment EPA

The Devonian Escarpment EPA (hereafter DEEPA) was created in 1992 under a state decree (Paraná state decree no. 1231/1992) whose primary objective was to protect the bio- and geodiversity of a natural area of approximately 393.579 ha, with a unique set of vegetation with high rates of biodiversity and endemism. This area is mainly composed by grasslands, gallery woods, rocky outcrops, and canyons and patches of Araucaria forest (a forest dominated by the gymnosperm *Araucaria angustifolia*, a species endangered of extinction; Maack 1948, Paraná 2004, Thomas 2013). This EPA is a crucial area for biodiversity preservation because it comprises within its limits four State Parks, five Private Reserve of Natural Heritage, a significant portion of the Campos Gerais National Park, and integrates important Brazilian river basins (such as the Paranapanema and Paraná Rivers) through a river network composed by the Tibagi, Iguaçu and Ribeira Rivers (Paraná 2004, Oliveira 2014). Furthermore, it is situated at the limit of two biomes: the Atlantic Forest and the Cerrado (i.e. Brazilian Savannah). Both biomes have high rates of aquatic and terrestrial biodiversity, but also are amongst the most threatened habitat in the planet. The landscapes of those biomes are highly fragmented and have been considered hotspot for biodiversity conservation, since they support many endemic and threatened species (Myers 2000, Conte et al. 2016, Paulitsch 2017).

The geodiversity of the DEEPA is an expression of a vast assemblage of products and processes related to the geological contrast between the Paraná Basin and its basement rocks, notably the Devonian sandstones of the Furnas Formation. Stones from this lithostratigraphic unit support the topographic step known as the "Devonian Escarpment", reaching altitudes as high as 1,290 m. Geological (mineralogy of sandstones and tectonic structures), geomorphological and hydrological factors led to an expressive hydrographic network, but also to a peculiar karst system in non-carbonate rocks. The geodiversity of the DEEPA hosts the principal regional aquifer and unique ecological conditions with newly discovered species, including troglobiont organisms (Cardoso et al. 2014). The regional geodiversity framework is complemented with a large variety of soils that sustain the typical grassland landscape, its agricultural use and regional identity (Melo et al. 2007, Guimarães et al. 2009, Melo et al. 2011, Guimarães et al. 2017).

Although extremely important for geo- and biodiversity conservation, this area has already been jeopardized by many anthropogenic pressures since its creation, such as cattle livestock, mining exploration and reforestation with exotic tree species, but mainly by agricultural practices developed in the region. The DEEPA is included in an area that is known to be the largest producer of grains in the Paraná state (SEAB 2015). In fact, the state of Paraná currently is the 2nd larger producer of soybean in Brazil, and its crop areas are exponentially increasing since 1970, reaching approximately 5,000,000 ha of planted area in 2015, with the potential to increase in next years (SEAB 2015). Because of this exacerbating production, the removal of native vegetation and replacement by corn and soybean crops is constant (SEAB 2015). Only 3% of the original Araucaria forest remains, and its deforestation is continuously increasing (e.g. between 2014 and 2015, 1,777 ha of this region was deforested, representing approximately 89% of all deforestation in Paraná) (SOS Mata Atlântica 2013). This habitat fragmentation has effects on the regional fauna, which depends directly on these areas as a refuge, places for breeding, foraging (Cleary et al. 2016, Jeliazkov et al. 2016) and pollination (Solé-Senan et al. 2017).

Intensification of agriculture has been pointed out as one of the greatest causes of biodiversity loss in agroforestry systems, mainly in developing countries where the access to advanced methods and technology are limited (Pereira et al. 2012, Tilman et al. 2017, Djagba et al. 2018). The effects of this anthropogenic impact are acknowledged to be twice as many harmful to biodiversity in tropical ecosystems (Barlow et al. 2016) and are noticeable in all organization levels (i.e. from microorganisms to ecosystems services; Storkey et al., 2013, Solé-Senan et al. 2017). Additionally, we can expect soil quality degradation and water contamination due to the use of fertilizers and pesticides. (Reynolds et al. 2015, Djagba et al. 2018). In the DEEPA region, for example, besides the suppression of native vegetation, the agriculture intensification may also contribute with the contamination of water bodies by agrochemicals, which has been reported to cause mass death of aquatic organisms and affect water quality and supply to its surrounding municipalities (Ayres 2006, Oliveira 2014).

Unsustainable Policy

The DEEPA is already struggling to overcome all the above mentioned anthropogenic impacts, and policymakers are insisting on putting this vital environment at serious risk again. Currently, an unsustainable law project (LP 527/2016) is being proposed for the DEEPA, which plans to reduce the area of this protection area by 70% (Figure 1). The LP claims the federal law 9.985/2000, which guarantees the sustainable use of the environment, to justify the resizing of the protection area. However, article no. 225 of the Brazilian Federal Constitution prohibits the use of an area, even for sustainable use, if its protection is not guaranteed. It seems evident that the term "sustainability" has been intentionally misinterpreted for economic benefits.

The 527/2016 LP aims to reduce de DEEPA area from 393,579 ha to 125,895 ha (Figure 1). Its central argument is the technical limitation of the original State decree to precisely delimit the protection area boundaries. However, the new proposal is anchored in the following criteria: the landowner would have to keep its area as an EPA only if the areas of rocks outcrops and natural forests exceed the areas of production (i.e. if the natural area comprises more than 50% of the property). Otherwise, the landowner would be allowed to use its area deliberately, such as suppress natural vegetation of the remaining area to increase its productive area. Therefore, such areas destroyed by years of inadequate use would be ignored (rather than recovered), and the remaining preserved areas could be reduced by up to 50%.

Furthermore, the new proposal completely ignores the endangered gymnosperm *Araucaria angustifolia*, treats the grasslands as 'consolidated' areas by agriculture activities, and the rich fauna that inhabits the DEEPA is completely unconsidered. The original DEEPA Management Plan recorded 92 mammals, 337 birds, 60 reptiles, 51 amphibians, and 92 fish species, representing 63%, 47%, 39%, 40%, and 15% of the Paraná state' species, respectively. Since this area is poorly studied, its species richness should be much higher. Moreover, the resized DEEPA's map excludes a series of outstanding geosites, with representatives of the geoheritage of the Campos Gerais region, and also archaeological points of interest, mainly with thousand years art rock and settlements. It also worth to mention that in the DEEPA's region there are two of the most visited parks of Paraná state (i.e. Vila Velha State Park and Guartelá State Park). Those parks are important to the economy of the region because they contribute significantly to the increase in revenues generated by ecotourism in the southern region of Brazil (SETU 2012, PRTUR 2014).

Landscape Consequences of the Law Project Approval

Currently, there are 25,707 fragments remnants of the Atlantic forest and Cerrado forest with its area ranging from 0.08 ha to 11,779,18 ha, and 1,423 remnants of the Grasslands ranging from 0.08 ha to 50 ha in the original area of the DEEPA. The forest fragments accounted for 23%, and Grasslands 0.04% of the total area of the DEEPA (Figure 2 and Table 1).

Grassland areas (i.e. Campos Gerais) are critically in danger of disappearing within the DEEPA. Currently, they represent only 0.04% of the total DEEPA's area, with a size always smaller than 50 ha. This means that grasslands, which are usually neglected in conservation plans (Overbeck et al. 2015), are losing area to planted pasture, agriculture and forest plantations (see Table 1). The grasslands, in its various physiognomies, have been under severe pressure with the expansion of agricultural, livestock and silvicultural activities. At the same time, due to their great scenic beauty, some regions of the Campos Gerais are highly sought for tourism and recreational purposes. The current conservation status of grasslands in Paraná state also results from historical processes of use and occupation of the soil, being related to the agricultural expansion since the cycle of the first colonizers this region. The remnants of native grasslands are mostly in areas of rugged terrain, which are not amenable to agricultural mechanization, and the

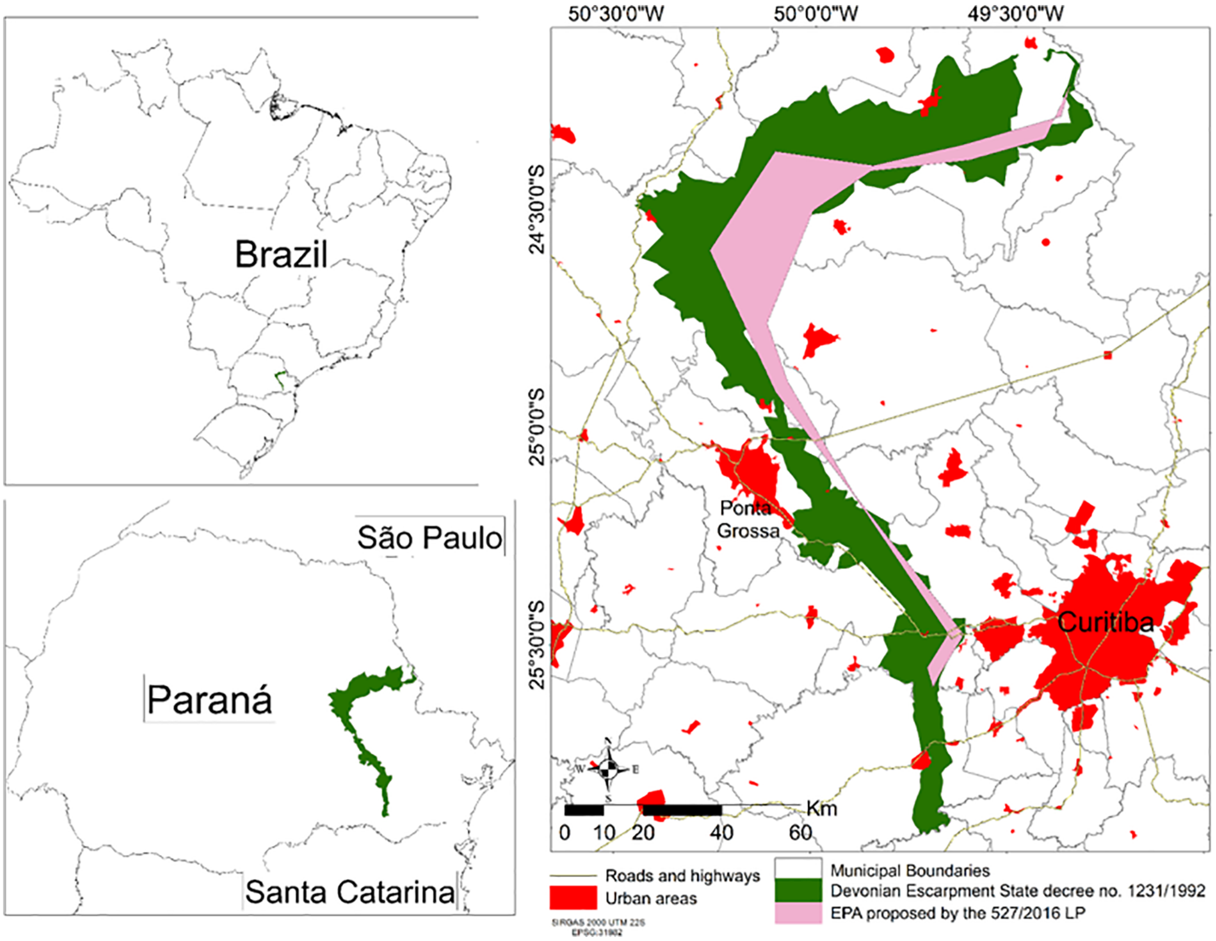

Figure 1. The original area of the DEEPA (in green) and the resized DEEPA proposed by the 527/2016 LP (in pink) in Paraná State, South Brazil.

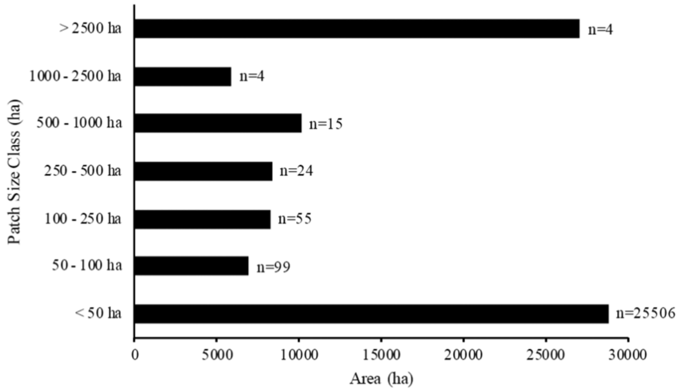

Figure 2. Distribution of the fragments remnants of the Atlantic forest and Cerrado (Brazilian Savannah) areas in the DEEPA. Based on the available data of fragments database from MapBiomas 2.3 (Available at: http://mapbiomas.org).

Table 1. Area occupied and percentage (%) by land cover classes inside the current area of the DEEPA. Based on the available data of fragments database from MapBiomas 2.3 (Available at: http://mapbiomas.org). Categories are based on MapBiomas 2.3. Forest Formations = represents Atlantic forest and Cerrado patches; Forest Plantation = forestry patches; Grassland = natural grasslands.

Class	Area (ha)	%
Forest Formations	95,392.62	23.0
Forest Plantation	51,182.43	12.3
Grasslands	201.41	0.04
Pasture	67,641.6	16.3
Agriculture	197,782.6	47.6
Non-vegeted areas	98.41	0.02
Urban Infrastructure	15,53.31	0.37
Water bodies	886.94	0.21

less fragmented portions are found within the state (e.g. Vila Velha and Guartelá State Parks) and federal Conservation Units (e.g. Campos Gerais National Park) (Dalazoana & Moro, 2011).

Most of the remaining fragments in the DEEPA are small patches (patches < 50 ha accounts for 99.2% of all forest fragments; Figure 2). The area of a patch is closely linked to its ability to accommodate greater species diversity since larger areas can offer better conditions for species-habitat interactions, consequently promoting the establishment of viable populations of local species (Metzger et al. 2009). With the expected reduction of the DEEPA, Atlantic forest, Cerrado savannas and grasslands patches will likely be reduced and/or eliminated from the landscape. The smaller patches can be negatively influenced by species-habitat interactions, resulting in local biodiversity loss (Fahrig 2003). This decrease in biological diversity is linked to ecological changes such as the imbalance of the higher rate of species extinction and the lower immigration rate of species from the neighboring fragments (Kageyama et al. 1998, Laurance & Vasconceloz 2009).

Suppressing small patches brings even more significant damage to biodiversity. The presence of small fragments is an important starting point for reducing the spatial isolation of the existing large fragments (e.g. Ribeiro et al. 2009, Ferreira et al. 2014, Ferreira et al. 2018, comparing isolation indices of fragments larger or smaller than 50 ha in all the Atlantic Forest remnants). Additionally, small fragments have ecological benefits to the landscape because they can act as steppingstones for species movements, increasing the connectivity among larger fragments. Also, small fragments increase the genetic flow through the surrounding matrix and promote the patch colonization process, the efficiency of these small fragments as steppingstones are related to the permeability of the matrix (Baum et al. 2004). The more suitable the matrix, the more support it offers to species that move among the patches (Gascon et al. 1999, Murphy & Lovett-Doust 2004).

The reduction of this critical environmental protection area will put at risk the remaining forest fragments and grasslands that preserve the geodiversity and the endemic biodiversity of the region. The DEEPA currently has a regulatory function that controls the different types of land use. The rural properties included inside the DEEPA has to keep a Permanent Preservation Area (PPA) of the forest as a function of the river width (i.e. the wider the river, the larger the PPA of riparian vegetation). With the reduction of this EPA and exclusion of rural properties of the EPA category, the area outside the ranges of the new protection area proposed by the LP will be regulated by the recently modified Brazilian Native Vegetation Protection Law (law 12651/2012), whose effects for preserving biodiversity were widely criticized by environmentalist and the scientific community (e.g. Bernard et al. 2014, Soares-Filho et al. 2014 and Vieira et al. 2018).

According to the Brazilian Native Vegetation Protection Law (BNVPL), the Permanent Preservation Area of rural property is a function of the size of the area and not of the river width (as it is in the EPA category), which disrupt the function of ecological corridors provided by PPAs included in the EPA category. For example, a large rural property could have a PPA of 30m and the neighboring property, if smaller, could have a PPA of 5m; in this scenario, many ecological functions would be disrupted such as the ecological corridors, the availability of shelter for animals and, also, the genetic flow among populations. Moreover, the BNVPL authorize that the PPAs, which were being used for agriculture, livestock or any economic use, to maintain its activities without having to restore it. Also, it does not mention the restoration of escarpments, mountains and other high elevation and slope places, which is a threat to the geodiversity of the DEEPA. Elevations areas preserve landforms sustained by geologic formations that resisted to denudation, climatic changes and tectonic forces for many geological eras, and, therefore, conserve important fossils that preserve the evolutionary history of the South American Plate.

Concluding Remarks

Despite all these alerts, it would not be the first time that the Brazilian government ignores scientific knowledge. The freeze of the public spending on nature protection for the next 20 years, as mentioned by Magalhães (2017), will lead to irreversible damage to what is still left of the Campos Gerais region. If approved, the LP 527/2016 will transform the DEEPA at current sustainable use into an unsustainable non-regulated area, allowing farmers to expand their croplands deliberately. The United Nations declared the current decade the ´Decade of Biodiversity´ (Tscharntke et al. 2012), and the Convention on Biological Diversity (CBD) set targets (Aichi targets) to prevent biodiversity loss and degradation of ecosystems until 2020. Brazil is one of the signatory countries and committed to achieving all Aichi targets, including the target no. 11, which aims to conserve, at least, 17% of terrestrial areas of particular importance for biodiversity. Currently, the Atlantic Forest (which includes the DEEPA) has less than 10% of its area preserved through conservation units (MMA 2017). The approval of the LP 527/2016 will reduce the length of the protection area instead of increasing it, which is contradictory to the CBD international agreement that Brazil committed to taking part in. While South American and European countries are going towards conservation and creation of new preservation areas (e.g. Spain is removing dams from rivers to restore its ecosystems, and Chile is amplifying its national parks in the Patagonia region; Schiermeier 2018; La Nación 2017), Brazil is going on the opposite direction. Ultimately, a key challenge is to develop regional and federal political goals that ensure the biodiversity conservation, not to destroy it.

Author Contributions

Gustavo Henrique Zaia Alves: Conceptualized the idea and wrote main manuscript.

Regiane da Silva Santos: Contributed with the research about the overview of the Devonian Escarpment and critical revision adding intellectual content.

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Gislaine Iachstel Manetta: Contributed equally to manuscript preparation; contribution to critical revision by adding intellectual content.

Hugo José Message: Contributed equally to manuscript preparation; contribution to critical revision by adding intellectual content.

Laryssa H. R. Pazianoto: Contributed equally to manuscript preparation; contribution to critical revision by adding intellectual content.

Evanilde Benedito: Contributed equally to manuscript preparation; contribution to critical revision by adding intellectual content.

Gilson Burigo Guimarães: Contributed to critical revision by adding intellectual content in regard to geological aspects and geodiversity.

Edivando Vitor do Couto: Build the map and ran the landscape analysis in MapBiomas 2.0; also, contributed to critical revision by 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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Short Communication

Diet and trophic niche overlap of *Boana bischoffi* and *Boana marginata* (Anura: Hylidae) in southern Brazil

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Abstract: The species *Boana bischoffi* and *Boana marginata* are endemic to the Atlantic Forest, where they often occur in sympatry. There is a large gap in the knowledge of natural history of both species. In this study, we aimed to describe and compare the diet composition of *B. bischoffi* and *B. marginata* in the southern domain of the Brazilian Atlantic Forest. We analyzed the gastrointestinal contents of 43 individuals of *B. bischoffi* and 30 individuals of *B. marginata*. Both showed a high trophic niche overlap (0.90 O_{jk}). The most important prey categories for both species belonged to the orders Araneae and Coleoptera. The species niche breadth (Bsta) varied from 0.35 to 0.42, suggesting a generalist feeding behavior for both species. Our data provide unprecedented information on these species' food composition, contributing to a better knowledge of the natural history of neotropical anurans.

Keywords: *Trophic ecology, Atlantic forest, Niche breadth, Amphibians.*

Dieta e sobreposição de nicho trófico de *Boana bischoffi* e *Boana marginata* (Anura: Hylidae) no sul do Brasil

Resumo: As espécies *Boana bischoffi* e *Boana marginata*, são endêmicas da Mata Atlântica onde frequentemente ocorrem em simpatria. Existe uma grande lacuna no conhecimento da história natural de ambas as espécies. Nesse estudo, nosso objetivo foi descrever e comparar a composição da dieta de *B. bischoffi* e *B. marginata* no limite sul da Mata Atlântica brasileira. Analisamos o conteúdo gastrointestinal de 43 indivíduos de *B. bischoffi* e 30 indivíduos de *B. marginata*. Ambas apresentaram elevada sobreposição de seus nichos tróficos (0,90 O_{jk}). As categorias de presas mais importantes para ambas as espécies pertenciam as ordens Araneae e Coleoptera. A amplitude de nicho das espécies (Bsta) variou de 0,35 a 0,42, sugerindo comportamento alimentar generalista para ambas. Nossos dados trazem informações inéditas sobre a composição alimentar destas espécies, contribuindo para um maior conhecimento da história natural dos anuros neotropicais.

Palavras-chave: *Ecologia trófica, Mata Atlântica, Amplitude de nicho, Anfíbios.*

Introduction

Information on species trophic ecology is fundamental to describe their ecological niches (Sih 2001), which, in turn, are necessary to understand the functioning of communities and ecosystems (Solé & Rödder 2010). Several studies have been carried out to investigate the diet of neotropical anurans (López et al. 2009, Sabagh et al. 2012, Barbosa et al. 2014, Kittel & Solé 2015, Oliveira et al. 2017), however, few evaluate the trophic relationship of resource-sharing species (Sabagh et al. 2010, Oliveira et al. 2015, Moser et al. 2017). Studies on the trophic ecology of coexisting species can provide important information about the use of food resources, thus helping to understand ecological interactions (Begon et al. 2007).

The Hylidae family has 714 known species (Frost 2018) and, in the extreme south of Brazil, they feed mainly on arthropods of the orders Coleoptera, Araneae and Hemiptera (Kwet 2001). The tree frogs *Boana bischoffi* (Boulenger, 1887) and *Boana marginata* (Boulenger, 1887) are endemic to the Atlantic Forest biome (Marcelino et al. 2009) and occur in sympatry in the southern Brazilian plateau. Both species have a similar biology but *B. bischoffi* occurs both in forest and open areas, while *B. marginata* occurs exclusively in closed forest areas (Kwet et al. 2010).

Morphologically and phylogenetically close species tend to use resources in a similar way (Ricklefs & Trevis 1980) and understanding how this occurs is essential to answer questions about community structure (Lawor 1980). Effective and widely used parameters to understand the species trophic relation are the trophic niche breadth and niche overlap (Pianka 1974, Sabagh & Carvalho-E-Silva. 2008, Sabagh et al. 2010). In this study, we describe and compare the food composition of *B. bischoffi* and *B. marginata*, as well as their trophic niches, to provide information about the natural history of these hylids.

Material and Methods

1. *Study site*

The study was carried out at the southern boundary of the Atlantic Forest, in a region of Mixed Ombrophilous Forest (or Araucaria Forest). Samplings were carried out in a well-preserved portion of Araucaria Forest, inside a Federal Conservation Unit, the National Forest of São Francisco de Paula (29°25'22.4"S; 50°23'11.2"W, 907 a.n.m.), municipality of São Francisco de Paula, state of Rio Grande do Sul, Brazil. The reproductive site sampled was formed by a lake connected to a small stream, where both *B. bischoffi* and *B. marginata* were found (Figure 1). The area has an annual precipitation of 2162 mm distributed evenly throughout the year, subtemperate climate and with an annual average temperature of 14.5 °C (Backes 1999, Maluf 2000).

2. *Data Collection*

Captures were made through active search (Crump & Scott Jr 1994) at night from October to November 2015. The individuals found were manually captured and immediately packed in a refrigerated styrofoam box to reduce their digestion's physiological activities (Oliveira et al. 2015). The collections were carried out with the competent Federal Organ authorization, SISBIO (authorization # - 45861-1). At the laboratory, the animals were euthanized with topical anesthetic (xylocaine), fixed in 10% formaldehyde and preserved in 70% alcohol. This procedure was in accordance with the institutional license obtained for experimentation with animals (# PPCEUA07.2016). Subsequently, each individual was dissected for the removal of gastrointestinal contents (stomach and intestine considered as a single sample). This material was kept in 70% alcohol until the screening process with a stereomicroscope. In addition to food content, we also collect muscle tissue from individuals for stable isotope analysis, which was part of a complementary study developed by our staff. All collected individuals are housed in the scientific collection of the Laboratory of Ecology of Terrestrial Vertebrates (*Boana bischoffi*: CHLEVT 1248-1275, 1324, 1325, 1327, 1328, 1342-1352; *Boana marginata*: CHLEVT 1364-1382, 1384-1392, 1703). Extracted contents were identified until the lowest possible taxonomic level, which was limited by prey digestion level. In general, prey presented a high fragmentation degree and, for this reason, the maximum taxonomic resolution reached mostly the level of order. For each prey category, the number, volume (detailed below) and frequency of occurrence were calculated. Some fragments whose identification was not possible were grouped in the category "others" and only their volume was quantified. Volume was calculated by using the area (mm²) occupied by each item with a graph paper support attached to the bottom of the Petri dish, where we evenly spread each item, maintaining a regular height of 1 mm (Hellawell & Abel 1971). In order to calculate each item volume (V), the area value (mm²) was multiplied by its height (1 mm) (Oliveira et al., 2015).

3. *Data Analysis*

To measure the importance of each prey category, we used the Index of Relative Importance (IRI): IRI = (% N +% P) % FO, where %N is the relative abundance of each prey in the diet; %P is the mass percentage of each prey in the diet; and %FO is the relative frequency of occurrence of prey (Pinkas et al. 1971, Krebs 1999). The higher the value of IRI, the greater is the importance of each prey in the diet. To analyze the dimension of the trophic niches breadth, we calculated the Levin's Niche Breadth Index (B) (Krebs 1999). This index is defined by: B = 1/Σpi2, where p is the individual proportion of a given resource i (taxon) found in the diet. To facilitate comparisons between species, the Standardized Index of Levins (Bsta) was calculated using the following equation: Bsta = (B-1) / (n-1), where n represents the number of resources recorded. Values close to zero are attributed to a specialist diet, while values close to one are attributed to a generalist diet.

To analyze if the trophic niche overlaps between the species, with regards to the degree of similarity between their diets, we used the Trophic Niche Overlap Index of Pianka (Ojk) (Pianka, 1974), defined by the following equation: $O_{jk} = \sum_{n=1}^{0} p_{ij} \times p_{ik} \Big/ \sqrt{\sum_{n=1}^{0} p_{ij}^{2} \times \sum_{n=1}^{0} p_{ik}^{2}}$, where Ojk is the niche overlap index between the species j and k; pij is equivalent to the proportion of the resource type i relative to the total of resources used by the species j; pik is the proportion of resource i relative to the total of resources used by the species k; and n is the total number of resource categories used by the species j and k. The index ranges from 0 to 1, when there is no overlap or a complete overlap between the species diets, respectively (Krebs, 1999).

Results

We analyzed the gastrointestinal contents from 43 individuals of *B. bischoffi* and 30 individuals of *B. marginata.* Of these, only two individuals (both *B. marginata*) had their digestive tracts empty. We identified a total of 149 food items, distributed in 18 prey categories. We identified 15 prey categories for *B. bischoffi* and 12 for *B. marginata*. According to the Index of Relative Importance (IRI), the two most important prey for both *B. bischoffi* and *B. marginata* were Araneae (IRI% = 45.2 and 29.1, respectively) and Coleoptera (IRI% = 25, 7 and 58.6, respectively) (Table 1). The "others" group represented 15.9% of the total volume in *B. bischoffi* and 38.9% in *B. marginata*. In both species, we found plant material, representing 2.30% of the total volume of *B. bischoffi* and 5.10% of *B. marginata* (Table 1). Only three prey categories were consumed exclusively by *B. marginata*: Odonata, Isopoda and Formicidae. The number of exclusive prey was higher for *B. bischoffi*: Lepidoptera, Lepidoptera (larva), Dermaptera, Diptera (larva), Pseudoescorpionida and Diplopoda. However, the two species had a high overlap in their diet, according to the Pianka Niche Overlap Index (0.90 Ojk). In relation to niche breadth, the Levins' Standardized Trophic Niche Index (Bsta) was higher for *B. bischoffi* (0.42) than for *B. marginata* (0.35) (Table 1).

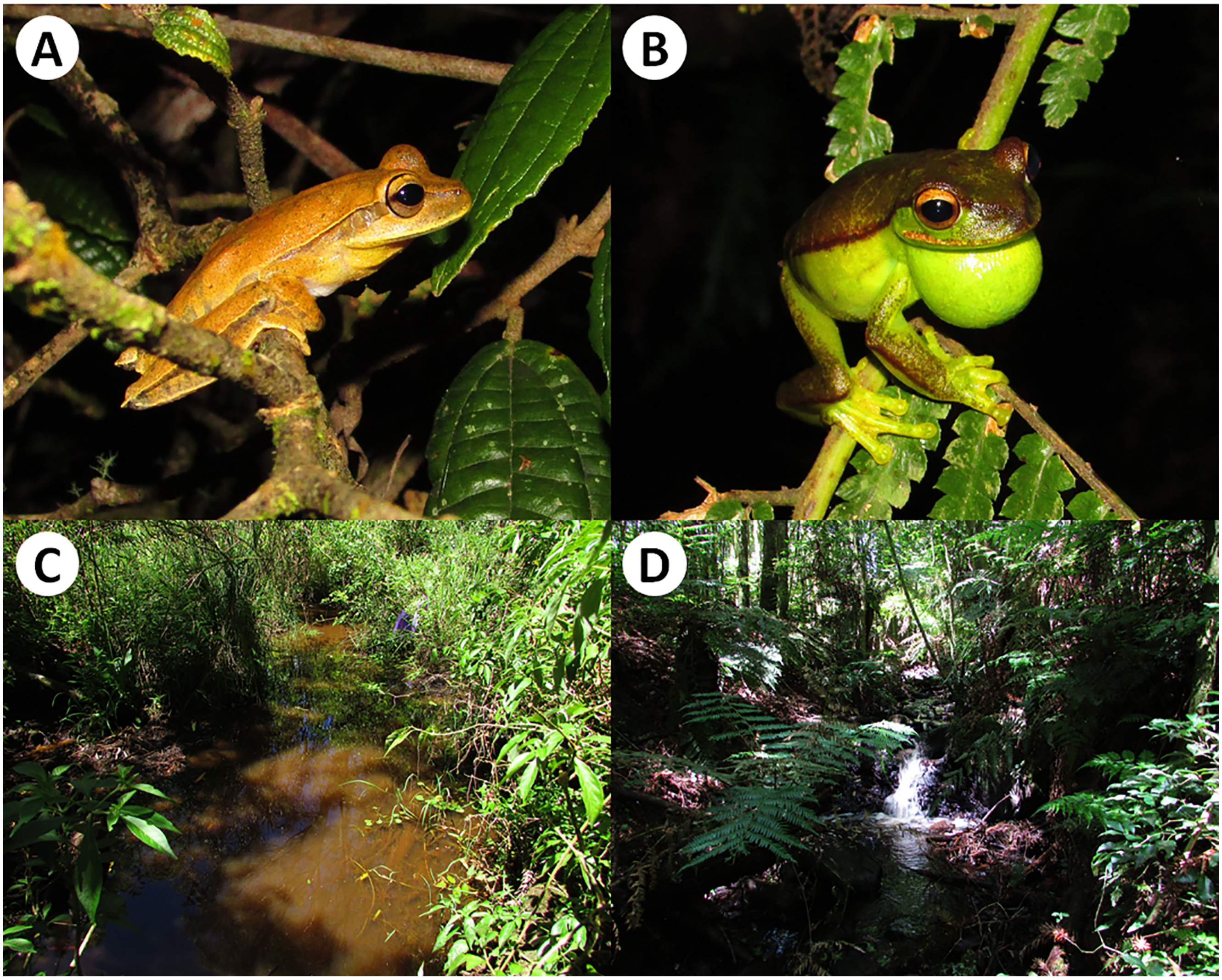

Figure 1. Anurans and sampled environments in the Araucaria Forest at the National Forest of São Francisco de Paula, municipality of São Francisco de Paula, state of Rio Grande do Sul, Brazil. A, *Boana bischoffi*, CHLEVT 1250, adult male, SVL 40.0 mm; B, *Boana marginata*, CHLEVT 1379, adult male, SVL 50.0 mm; C and D lentic and lotic environments, respectively.

Discussion

Boana bischoffi and *B. marginata* exhibited similar composition in their diets. In addition, the most important prey were the same for both species. This similarity in their diets was expected due to phylogenetic proximity between them (Faivovich et al., 2004). Furthermore, it is likely that both species were exposed to the same prey supply since they were collected in the same study site, although in possible different microhabitats. Unfortunately, we do not have data on the prey availability in the habitat, and therefore our results should be interpreted with caution. The taxonomic level of Order could be a limitation for our explanations (Tavares-Cromar & Williams 1996, Bundy et al. 2011, Pombo et al. 2013). Nevertheless, some basic aspects of food-web, as the mean chain length and linkage complexity vary little within the degree of taxonomic resolution (Thompson & Townsend 2000), and food webs are best explained by coarser taxonomic ranks, from kingdom to class (Eklöf et al. 2012).

The orders Araneae and Coleoptera are considered very abundant arthropods in the environment (Baretta 2007) and were also recorded in abundance in the study area (Oliveira M. pers. comm.). The importance of Araneae and Coleoptera has also been recorded for several other neotropical anurans (e.g., Maneyro et al. 2004, Miranda et al. 2006, Duré et al. 2009, Rosa et al. 2011, Oliveira et al. 2015, Moser et al. 2017). In addition, spiders and beetles were also the most important prey for other species of the same genus, such as *Boana leptolineata*, in a region that is similar and close to the studied environment (Barbosa et al. 2014). Coleopterans are a group of highly diverse organisms and very abundant in the environment, especially during the warmer months (Teixeira et al. 2009), making them prey of easy consumption.

Spiders are also a very important prey category for the whole Hylidae family. For species of the genus *Scinax*, for example, the order Araneae represented the most important prey category in their diets (Sabagh et al. 2010, Kittel & Solé 2015). In addition, for other

Table 1. Prey categories found in the gastrointestinal contents of *B. bischoffi* and *B. marginata*. N = number of individuals, V = total volume (in mm^3) occupied by prey category, FO = prey category's frequency of occurrence, IRI = Index of Relative Importance, (%) = percentage values over total.

Prey categories	*Boana bischoffi*				*Boana marginata*			
	%N	%V	%FO	IRI%	%N	%V	%FO	IRI%
Araneae	26.50	13.40	54.80	45.20	21.60	13.30	35.70	29.08
Coleoptera	20.40	14.40	35.70	25.72	37.20	21.30	42.80	58.62
Diptera	10.20	12.30	19.00	8.87	7.80	1.70	14.30	3.18
Acarina	5.10	0.10	11.90	1.28	7.80	0.02	14.30	2.62
Hemiptera	5.10	8.20	9.50	2.62	2.00	1.00	3.60	0.25
Coleoptera (larva)	9.20	7.20	23.80	8.09	5.90	3.40	10.70	2.34
Orthoptera	5.10	6.20	11.90	2.79	3.90	0.40	7.10	0.73
Hymenoptera	5.10	4.80	11.90	2.44	2.00	0.60	3.60	0.21
Lepidoptera	2.00	2.00	4.80	0.40	0.00	0.00	0.00	0.00
Lepidoptera (larva)	3.00	3.80	7.10	1.01	0.00	0.00	0.00	0.00
Blatodea	2.00	3.10	4.80	0.51	3.90	5.80	7.10	1.62
Dermaptera	2.00	2.00	4.80	0.40	0.00	0.00	0.00	0.00
Diptera (larva)	2.00	3.70	4.80	0.56	0.00	0.00	0.00	0.00
Odonata	0.00	0.00	0.00	0.00	3.90	7.00	3.60	0.91
Isopoda	0.00	0.00	0.00	0.00	2.00	1.30	3.60	0.27
Hymenoptera Formicidae	0.00	0.00	0.00	0.00	2.00	0.04	3.60	0.17
Pseudoscorpionida	1.00	0.10	2.40	0.05	0.00	0.00	0.00	0.00
Diplopoda	1.00	0.10	2.40	0.06	0.00	0.00	0.00	0.00
Plant material	0.00	2.30	0.00	-	0.00	5.10	0.00	-
Other	0.00	15.90	0.00	-	0.00	38.90	0.00	-
Niche breadth	0.42				0.35			
Niche overlap	0.90							

species of *Boana*, such as *B. albopunctata* (De Araújo et al., 2007), *Boana raniceps* (Sabagh et al. 2010), *B. leptolineata* (Barbosa et al. 2014) and *B. pulchella* (Da Rosa et al. 2011), spiders have proved to be an important part of these species' food composition. The presence of spiders in the diet of these hylids is probably related to the fact that predator and prey use the same micro-habitat, suspended in shrubs, facilitating the encounter of prey (De Araújo et al. 2007, Barbosa et al. 2014).

Boana bischoffi and *B. marginata* demonstrated a similar trophic niche breadth, with generalist characteristics. The niche breadths recorded in this study were similar to those recorded for *Aplastodiscus perviridis* (Bsta = 0.27, Oliveira et al. 2017) and *B. pulchella* (Bsta = 0.31, Oliveira 2014), but smaller than those recorded for *B. leptolineata* (Bsta = 0.51, Barbosa et al. 2014), *B. raniceps* (Bsta = 0.64, Sabagh et al. 2010) and *Scinax acuminatus* (Bsta = 0.48, Sabagh et al. 2010). In general, *B. bischoffi* and *B. marginata* have large niches in comparison to other species [e.g., *Rhinella arenarum* and *R. dorbignyi* (Isacch & Barg 2002), *R. icterica* (Sabagh & Carvalho-e-Silva 2008), *Physalaemus biligonigerus* (Oliveira et al. 2015), *P. gracilis* and *P. lisei* (Moser et al. 2017) and *Aplastodiscus perviridis* (Oliveira et al. 2017)]. Our results suggest a more generalist behavior of the studied hylids species, feeding on similar quantities from a greater prey variety (Sabagh et al. 2010, Barbosa et al. 2014).

The high niche overlap recorded for *B. marginata* and *B. bischoffi* was expected since congeneric species that share the same habitat tend to have similar diets (Duellman & Trueb 1986, Guidali et al. 2000, Sabagh et al. 2010). The high niche overlap was also recorded for *Dendropsophus nanus* and *D. sanborni* (87.2%, Menin et al. 2005), *Physalaemus lisei* and *P. gracilis* (98%, Moser et al. 2017), and *Phyllomedusa burmeisteri* and *Pithecopus rohdei* (82%, Lima et al. 2010). The trophic overlap of hylids from different genera, such as *Boana raniceps* and *Scinax acuminatus* (60%, Sabagh et al. 2010), was considerably lower compared to hylids from the same genus.

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Author contributions

Camila Moser: Collected the data, participated in the screening, analyses and article elaboration

Mateus de Oliveira: Collected the data, participated in the screening, analyses and article elaboration

Fernanda Avila: Collected the data, participated in the screening, analyses and article elaboration

Diogo Dutra-Araújo: Collected the data and participated in the article elaboration

Renata K. Farina: Collected the data and participated in the screening.

Alexandro Marques Tozetti: Participated in the analyses and article elaboration

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

Ichthyofauna of headwater streams from the rio Ribeira de Iguape basin, at the boundaries of the Ponta Grossa Arch, Paraná, Brazil

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Abstract: The rio Ribeira de Iguape basin (Paraná State) is highly relevant for the preservation of remnants of the Atlantic Forest. Its high levels of diversity and fish endemism explain the river dynamics promoted by the Ponta Grossa Arch, a geological structure that promotes headwater capture and isolation between the upper rio Paraná drainage, the rio Iguaçu and the rio Ribeira de Iguape. Here, our objective was to provide an unprecedented inventory for headwater streams of the rio Ribeira de Iguape basin at the boundaries of the Ponta Grossa Arch. We found 29 species of fish representing four orders and nine families. Siluriformes was the richest order followed by Characiformes. Nine species presented high abundance of sampled individuals, contributing with 87.8% of the collections. Four species appeared in less than 25% of the sampled sites (occasional), and six species appeared in more than 50% (constant). We identified three non-described species and one non-native (*Coptodon rendalli*). Two species had their geographical distribution extended and the presence of *Astyanax bifasciatus*, endemic to the Iguaçu river basin, ratifies recent events of headwater capture between coastal drainages and those that flow into the continent. Studies of biogeographic divisors are necessary to explain the origin and dispersion processes of species in order to direct studies on diversity and preventive management actions. *Coptodon rendalli* (Tilapia) is an alarming record on the introduction of species in the region.

Keywords: *Altitudinal gradient, biogeography, coastal drainages, ichthyology, invasion.*

Ictiofauna de riachos de cabeceira da bacia do rio Ribeira de Iguape, nos limites do Arco de Ponta Grossa, Paraná, Brasil

Resumo: A bacia do rio Ribeira de Iguape (Estado do Paraná) é altamente relevante para a preservação de remanescentes da Mata Atlântica. Os seus altos níveis de diversidade e de endemismo de peixes explicam a dinâmica fluvial promovida pelo Arco de Ponta Grossa, estrutura geológica que promove eventos de captura de cabeceira e de isolamento entre as drenagens do alto rio Paraná, do rio Iguaçu e do rio Ribeira de Iguape. Aqui, nosso objetivo foi fornecer um inventário inédito para riachos de cabeceira da bacia do rio Ribeira de Iguape nos limites do Arco de Ponta Grossa. Encontramos 29 espécies de peixes representando quatro ordens e nove famílias. Siluriformes foi a ordem mais rica seguida por Characiformes. Nove espécies apresentaram alta abundância de indivíduos amostrados, contribuindo com 87,8% das coletas. Quatro espécies apareceram em menos de 25% dos locais amostrados (ocasionais), e seis espécies apareceram em mais de 50 % (constantes). Identificamos três espécies não descritas e uma (*Coptodon rendalli*) não nativa. Duas espécies tiveram sua distribuição geográfica ampliada e, a presença de *Astyanax bifasciatus*, endêmica para a bacia do rio Iguaçu, ratifica eventos recentes de captura de cabeceira entre drenagens costeiras e aquelas que fluem para dentro do continente. Estudos de divisores biogeográficos são necessários para explicar os processos de origem e dispersão de espécies a fim de direcionar estudos sobre diversidade e ações de manejo preventivas. *Coptodon rendalli* (Tilápia) é um registro alarmante sobre a introdução de espécies na região.

Palavras-chave: *Biogeografia, drenagens costeiras, ictiologia, invasão, gradiente altitudinal.*

Introduction

The most direct means of accessing the biodiversity of a biome or locality is to carry out an inventory of species. The elaboration of any taxonomic list is not trivial, because much effort and time are spent in the specific techniques of sampling for a particular group of organisms as well as gaining the knowledge required about systematics, taxonomy, ecology, and natural history (Silveira et al. 2010). Recognizing the species of fish is essential to design effective conservation and management strategies of aquatic ecosystems (Oliveira et al. 2014; Agostinho et al. 2016; Melo et al. 2016; Ferreira et al. 2017). Therefore, ichthyofaunistic inventories are crucial to robust analyzes of biodiversity involving ecological and biogeographic aspects.

South America is home to many freshwater fishes, with approximately 20 orders, 69 families, 739 genera and 5,160 valid species (Reis et al. 2016). However, knowledge of the actual quantity of species and their geographical distribution is far from complete (Ota et al. 2015). Specifically to the Paraná State, where are numerous river networks, recent inventories of fish (Baumgartner et al. 2012; Pagotto et al. 2012; Frota et al. 2016a; Cavalli et al. 2018) have been improving the knowledge about the state's large sub-basins, yet economically biased by hydroelectric projects financing. Headwater regions of the rivers, with lower profitability of the electric sector, remain without substantial samplings, even though they have a high rate of endemism and are ecologically threated by human occupation (Baumgartner et al. 2012).

Recent studies have provided important records of fish species in headwater streams of the Paraná State, but all of them are limited to the systems of the upper rio Paraná (*e.g.,* Delariva & Silva 2013; Hoffmann et al. 2015; Cetra et al. 2016; Frota et al. 2016a; Cavalli et al. 2018; Claro-García et al. 2018) and the rio Iguaçu (*e.g.,* Frota et al. 2016b; Larentis et al. 2016; Delariva et al. 2018), which converges to the rio de la Plata system. Many headwater streams located in Atlantic Coastal basins of the Paraná State are unlisted and little is known about which species reside there.

Among the coastal drainages of the Paraná State, the rio Ribeira de Iguape basin is an ecologically relevant region for preserving remaining areas of the Atlantic Forest with high endemism of fish (Oyakawa et al. 2006; Menezes et al. 2007; Oyakawa & Menezes 2011). The headwaters of the rio Ribeira de Iguape basin stand out the Ponta Grossa Arch. In this important geological structure, recent tectonic activities (see Franco-Magalhães et al. 2010) have resulted in an accelerated fluvial dynamism between drainages of the upper rio Paraná, rio Iguaçu, and rio Ribeira de Iguape. This fact has been proven by the sharing of species by headwater capture events, and or serving as an efficient biogeographic barrier (Ribeiro 2006; Frota et al. 2016a; Morais-Silva et al. 2018). Considering this backdrop, our objective was to provide an inventory for headwater streams' fish of the rio Ribeira de Iguape basin at the boundaries of the Ponta Grossa Arch.

Material and Methods

1. *Study area*

The rio Ribeira de Iguape basin is in the São Paulo and Paraná states, draining 25,000 km^2 and including 25 municipalities in São Paulo and 15 in Paraná. One-third of this area is in Paraná State, which is formed by the confluence of the rios Ribeirinha and Açungui, 85 km to the north of the capital, Curitiba. Toward to its mouth in the Atlantic Ocean, in the municipality of Iguape (SP), the rio Ribeira de Iguape travels 470 km through a fairly rugged relief with strong altitudinal gradients, extending from the coastal plain, close to sea level, to the peaks of the mountain, range with more than 1,000 m of altitude. The rio Ribeira de Iguape basin includes one of the largest remaining areas of Brazil's Atlantic Forest, which despite its critical nature still holds significant biodiversity and endemism (Oyakawa et al. 2006).

The streams of the Atlantic Forest are characterized by beds formed by rocks, presenting clear waters, strong currents, relatively low temperatures, and high concentration of dissolved oxygen (Oyakawa et al. 2006). Streams can be divided into three main types according to their topographical location: 1) mountain streams or headwater streams, 2) lowland streams, and 3) coastal streams. Headwater streams, like those studied here, are characterized by great slope of the relief with thicker bottom substrate and relatively straight channel (Oyakawa et al. 2006).

The basin of the rio Ribeira de Iguape in the Paraná State is bordered to the north by the rio Itararé basin, the rio Tibagi basin to the west (both belong to the upper rio Paraná system), and to the south by the rio Iguaçu basin (Figure 1A). The headwaters of the rio Ribeira de Iguape basin in the Paraná State are located on the boundaries of the Ponta Grossa Arch. This arch involves a large deformational structure with NW-SE trend axis. Its elevation (Figure 1A) varies between more than 1,500 m in the center and SE, and 600 m in NW (Franco-Magalhaes et al. 2010).

2. *Data collection*

The specimens were collected under a permanent license to collect zoological material, number 14028-1, granted by the Brazilian Institute of Environment and Renewable Natural Resources (IBAMA, in Portuguese). All the 32 streams were sampled (Table 1; Figure 1B) on six collection trips: October 2014, October 2015, July 2016, October 2016, January 2017, and April 2017. A 100-m stretch was defined at each site, which were georeferenced (Table 1) and sampled by electrofishing. This technique employs two electrified dip nets, producing an electric discharge between 200 and 400 V and current of 2 A, that stun the fish temporarily (Lobón-Cervia 1991). The specimens were anesthetized in benzocaine hydrochloride and fixed in 10% formalin in the field. After a few days, the fishes were transferred to 70% ethanol in the laboratory and deposited in the Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura (NUP).

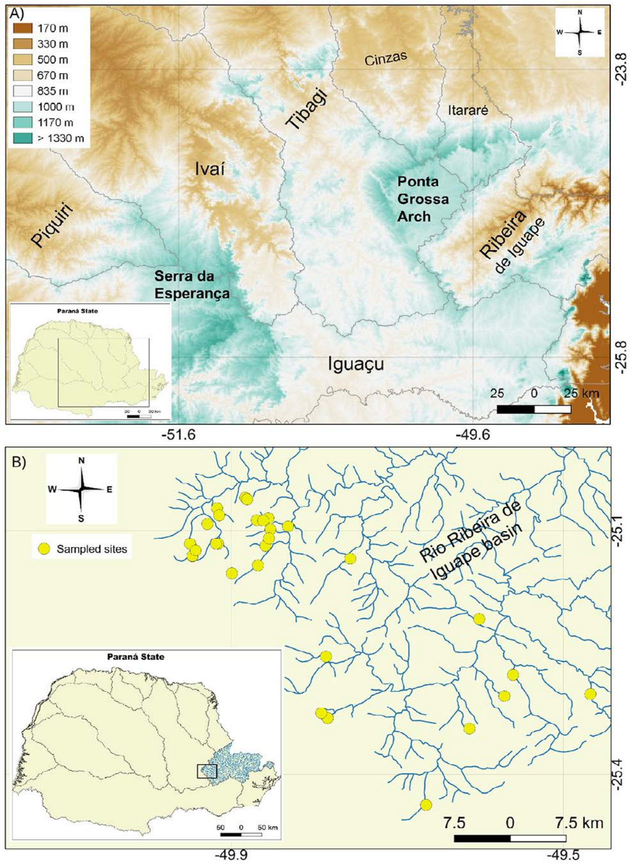

Figure 1. Map of the study area showing: (A) hypsometric map of Paraná State with highlight to the Ponta Grossa Arch, (B) the location of the rio Ribeira de Iguape basin within of the Paraná State, and sampled sites in headwater streams.

Fish species were classified based on Eschmeyer & Fong (2018) and species determinations were accomplished by comparison of the specimens with original descriptions or with type specimens of species reported from rio Ribeira de Iguape basin by Oyakawa et al. (2006), Menezes et al. (2007) and Oyakawa & Menezes (2011). Whenever possible, the determinations of the species were checked by specialists of each taxonomic group. The species were categorized by their frequency of occurrence (hereafter, constancy), which was calculated for all sampled streams according to Dajoz (1983). Species with a constancy up to 25% were classified as occasional, between 25 and 50% as accessory, and higher than 50% as constant. In addition, the species were categorized by origin into autochthonous and allochthonous, respectively, to distinguish native and introduced species to the rio Ribeira de Iguape basin, following Oyakawa et al. (2006), Menezes et al. (2007), and Oyakawa & Menezes (2011).

Results

There were collected a total of 5,617 individuals distributed in four orders, nine families, and 29 species (Tables 1 and 2; Figures 2 and 3). Siluriformes (Figure 4) was the most representative order in number of species and families (16 species and four families), followed by Characiformes (nine species and three families). Among the families, Loricariidae and Characidae, both with seven species, presented higher richness contributing together with approximately 48.3% of all collected species, followed by Heptapteridae (five species) with approximately 17.3% of all collected species (Figure 5).

Table 1. Information on the headwater streams sampled in the rio Ribeira de Iguape basin, Paraná State, Brazil. Numbers in parentheses after each species name indicates abundance.

Sample point	Remarks	Altitude (meters)	Coordinates	Municipality	Species caught
1	Rio da Mata, affluent of rio Açungui, rio Ribeira de Iguape basin	~745	25º19'49.5"S, 49º47'2.4"W	Ponta Grossa	*Astyanax laticeps* (2), *Astyanax bifasciatus* (39), *Bryconamericus microcephalus* (15), *Cambeva davisi* (13), *Characidium pterostictum* (39), *Chasmocranus lopezae* (11) *Harttia kronei* (22), *Isbrueckerichthys duseni* (35), *Pimelodella transitoria* (17) and *Rineloricaria kronei* (2)
2	Rio Santa Cruz, affluent of rio Açungui, rio Ribeira de Iguape basin	~728	25º19'28.1"S, 49º47'29.7"W	Campo Largo	*Astyanax bifasciatus* (4), *Astyanax laticeps* (7), *Bryconamericus microcephalus* (8), *Cambeva davisi* (9), *Characidium pterostictum* (2), *Chasmocranus lopezae* (11), *Deuterodon iguape* (4), *Geophagus iporanguensis* (1), *Harttia kronei* (3), *Isbrueckerichthys duseni* (23), *Kronichthys lacerta* (5), *Phalloceros harpagos* (33), *Pimelodella transitoria* (14), *Rhamdia quelen* (1) and *Rineloricaria kronei* (9)
3	Rio do Jacui, affluent of rio Santa Cruz, rio Ribeira de Iguape basin	~801	25º15'17.8"S, 49º47'9.4"W	Ponta Grossa	*Astyanax laticeps* (34), *Cambeva davisi* (8), *Characidium pterostictum* (13), *Harttia kronei* (13), *Isbrueckerichthys duseni* (15), *Kronichthys lacerta* (7), *Phalloceros harpagos* (16) and *Rhamdia quelen* (3)
4	Rio Caçador, affluent of rio Conceição, rio Ribeira de Iguape basin	~727	25º8'4.2"S, 49º45'23.9" W	Ponta Grossa	*Cambeva davisi* (2), *Characidium pterostictum* (15), *Harttia kronei* (44), *Isbrueckerichthys duseni* (47), *Kronichthys lacerta* (2), *Neoplecostomus ribeirensis* (2) and *Phalloceros harpagos* (1)

5	Rio Ribeirão, affluent of rio Açungui, rio Ribeira de Iguape basin	~639	25º12'31.5S, 49º36'5.2"W	Campo Largo	*Cambeva davisi* (2), *Chasmocranus lopezae* (6), *Corydoras ehrhardti* (5), *Deuterodon iguape* (41), *Geophagus iporanguensis* (1), *Harttia kronei* (26), *Hypostomus interruptus* (1), *Isbrueckerichthys duseni* (7), *Kronichthys lacerta* (4), *Neoplecostomus ribeirensis* (1), *Rhamdia quelen* (1) and *Rhamdioglanis transfasciatus* (10)
6	Rio do Cerne, affluent of rio Açungui, rio Ribeira de Iguape basin	~684	25º16'38.9"S, 49º33'38.6"W	Campo Largo	*Astyanax laticeps* (34), *Cambeva davisi* (2), *Corydoras ehrhardti* (10), *Geophagus iporanguensis* (13), *Harttia kronei* (14), *Hypostomus interruptus* (1), *Isbrueckerichthys duseni* (20), *Kronichthys lacerta* (6), *Phalloceros harpagos* (12), *Rhamdia quelen* (3) and *Rineloricaria kronei* (31)
7	Rio Açungui, affluent of rio Ribeira de Iguape, rio Ribeira de Iguape basin	~809	25º26'14.6"S, 49º39'55.3"W	Campo Largo	*Astyanax bifasciatus* (15), *Astyanax laticeps* (44), *Cambeva davisi* (9), *Chasmocranus lopezae* (2), *Harttia kronei* (5), *Isbrueckerichthys duseni* (3), *Pimelodella transitoria* (18), *Rhamdia quelen* (2), *Kronichthys lacerta* (1), *Phalloceros harpagos* (3) and *Rineloricaria kronei* (1)
8	Rio Retiro, affluent of rio Açungui, rio Ribeira de Iguape basin	~904	25º20'36.6"S, 49º36'47.5"W	Campo Largo	*Astyanax laticeps* (13), *Isbrueckerichthys duseni* (6), *Phalloceros harpagos* (1) and *Rhamdia quelen* (3)
9	Riacho Boa Vista, affluent of rio Açungui, rio Ribeira de Iguape basin	~843	25º18'12.5"S, 49º34'15.9"W	Campo Largo	*Cambeva davisi* (21), *Phalloceros harpagos* (17) and *Rhamdia quelen* (1)
10	Rio Conceição, affluent of rio Ribeira de Iguape, rio Ribeira de Iguape basin	~773	25º18'3.5"S, 49º28'3.2"W	Campo Magro	*Astyanax laticeps* (44), *Chasmocranus lopezae* (3), *Corydoras ehrhardti* (16), *Geophagus iporanguensis* (4), *Harttia kronei* (10), *Hoplias* aff. *malabaricus* (1), *Isbrueckerichthys duseni* (38), *Kronichthys lacerta* (2), *Phalloceros harpagos* (7), *Parotocinclus maculicauda* (3), *Rhamdia quelen* (3) and *Rineloricaria kronei* (5)
11	Rio da Barra, affluent of rio Conceição, rio Ribeira de Iguape basin	~959	25º17'57.3"S, 49º24'39.2"W	Campo Magro	*Astyanax laticeps* (17), *Cambeva davisi* (2), *Harttia kronei* (71), *Isbrueckerichthys duseni* (50), *Neoplecostomus ribeirensis* (10), *Phalloceros harpagos* (1) and *Rhamdia quelen* (1)
12	Rio Córrego Fundo, affluent of rio Piedade, rio Ribeira de Iguape basin	~875	25º15'44.5"S, 49º23'57.2"W	Itaperuçu	*Astyanax laticeps* (9), *Astyanax bifasciatus* (1), *Cambeva davisi* (1), *Corydoras ehrhardti* (1), *Geophagus iporanguensis* (4), *Isbrueckerichthys duseni* (52), *Neoplecostomus ribeirensis* (5) and *Rhamdia quelen* (9)
13	Unnamed river, affluent of Ribeirão Grande, rio Ribeira de Iguape basin	~858	25º3'34.14"S, 49º52'58.83"W	Ponta Grossa	*Astyanax lacustris* (42), *Astyanax laticeps* (1), *Astyanax* sp. 1 (5), *Cambeva davisi* (48), *Geophagus iporanguensis* (24), *Hypostomus interruptus* (1), *Isbrueckerichthys duseni* (6) and *Phalloceros harpagos* (228)
14	Unnamed river	~795	25º7'7.19"S, 49º51'31.59"W	Ponta Grossa	*Astyanax* sp. 1 (2) and *Isbrueckerichthys duseni* (13)
15	Unnamed river, affluent of Arroio Passo do Nenê, rio Ribeira de Iguape basin	~765	25º5'55.23"S, 49º51'10.39"W	Ponta Grossa	*Astyanax* sp. 1 (1), *Geophagus iporanguensis* (2), *Hoplias* aff. *malabaricus* (1), *Hypostomus interruptus* (4), *Isbrueckerichthys duseni* (51), *Phalloceros harpagos* (15) and *Rhamdia quelen* (4)
16	Unnamed river, affluent of Arroio Itaguá, rio Ribeira de Iguape basin	~851	25º4'20.07"S, 49º55'1.74"W	Ponta Grossa	*Cambeva davisi* (22), *Chasmocranus lopezae* (1), *Coptodon rendalli* (10), *Geophagus iporanguensis* (95), *Hoplias* aff. *malabaricus* (7), *Hypostomus interruptus* (66), *Isbrueckerichthys duseni* (4), *Kronichthys lacerta* (3), *Phalloceros harpagos* (1,637), *Pimelodella transitoria* (2) and *Rhamdia quelen* (4)
17	Unnamed river, affluent of Arroio Itaguá, rio Ribeira de Iguape basin	~854	25º4'51.63"S, 49º54'52.29"W	Ponta Grossa	*Cambeva davisi* (18), *Geophagus iporanguensis* (2) and *Phalloceros harpagos* (318)

18	Unnamed river, affluent of Ribeirão Grande, rio Ribeira de Iguape basin	~839	25º3'40.84"S, 49º52'51.01"W	Ponta Grossa	*Astyanax lacustris* (13), *Astyanax laticeps* (3), *Cambeva davisi* (42), *Geophagus iporanguensis* (14), *Hypostomus interruptus* (10), *Isbrueckerichthys duseni* (2) and *Phalloceros harpagos* (344)
19	Unnamed river	~882	25º6'58.25"S, 49º56'58.47"W	Ponta Grossa	*Cambeva davisi* (16)
20	Unnamed river, affluent of rio da Areia, rio Ribeira de Iguape basin	~863	25º5'29.87"S, 49º55'43.42"W	Ponta Grossa	*Astyanax laticeps* (35), *Astyanax* sp. 1 (4), *Cambeva davisi* (81), *Geophagus iporanguensis* (2), *Hoplias* aff. *malabaricus* (3) and *Rhamdia quelen* (2)
21	Unnamed river, affluent of rio Guarituba, rio Ribeira de Iguape basin	~714	25º5'40.83"S, 49º49'53.79"W	Ponta Grossa	*Astyanax lacustris* (10), *Cambeva davisi* (4), *Cambeva* cf. *cubataonis* (14), *Corydoras ehrhardti* (25), *Deuterodon iguape* (1), *Geophagus iporanguensis* (36), *Hoplias* aff. *malabaricus* (1), *Hypostomus interruptus* (58), *Isbrueckerechthys duseni* (8), *Kronichthys lacerta* (7), *Parotocinclus maculicauda* (58), *Phalloceros harpagos* (34), *Rhamdia quelen* (4) and *Rineloricaria kronei* (19)
22	Unnamed river, affluent of rio São Pedro, rio Ribeira de Iguape basin	~923	25º7'52.41"S, 49º56'48.14"W	Ponta Grossa	*Astyanax laticeps* (26), *Cambeva davisi* (11), *Characidium pterostictum* (33), *Chasmocranus lopezae* (5), *Deuterodon iguape* (58), *Imparfinis* sp. (6), *Kronichthys lacerta* (9), *Neoplecostomus ribeirensis* (2) and *Rhamdioglanis transfasciatus* (2)
23	Unnamed river, affluent of rio São Pedro, rio Ribeira de Iguape basin	~923	25º7'51.36"S, 49º56'48.38"W	Ponta Grossa	*Astyanax laticeps* (17), *Cambeva davisi* (8), *Characidium pterostictum* (40), *Chasmocranus lopezae* (5), *Deuterodon iguape* (116), *Imparfinis* sp. (17) and *Isbrueckerichthys duseni* (16)
24	Unnamed river	~894	25º7'26.95"S, 49º56'34.61"W	Ponta Grossa	*Astyanax laticeps* (13) and *Cambeva davisi* (11)
25	Unnamed river, affluent of Arroio Três Barras, rio Ribeira de Iguape basin	~859	25º6'58.33"S, 49º54'57.75"W	Ponta Grossa	*Astyanax laticeps* (2), *Cambeva davisi* (51), *Chasmocranus lopezae* (4), *Cnesterodon iguape* (1), *Deuterodon iguape* (11), *Geophagus iporanguensis* (1), *Harttia kronei* (3), *Isbrueckerichthys duseni* (20), *Kronichthys lacerta* (2), *Neoplecostomus ribeirensis* (10) and *Phalloceros harpagos* (5)
26	Unnamed river	~784	25º6'34.07"S, 49º51'16.97"W	Ponta Grossa	*Astyanax* sp. 2 (6) and *Callichthys callichthys* (1)
27	Unnamed river	~828	25º5'13.75"S, 49º52'8.94"W	Ponta Grossa	*Astyanax laticeps* (3), *Astyanax* sp. 2 (1), *Cambeva davisi* (17) and *Isbrueckerichthys duseni* (6)
28	Unnamed river, affluent of Arroio Três Barras, rio Ribeira de Iguape basin	~868	25º6'57.95"S, 49º55'6.06"W	Ponta Grossa	*Astyanax laticeps* (2), *Astyanax* sp. 1 (5), *Cambeva davisi* (44), *Chasmocranus lopezae* (3) and *Isbrueckerichthys duseni* (22)
29	Unnamed river, affluent of Ribeirão da Cruz, rio Ribeira de Iguape basin	~789	25º5'3.91"S, 49º51'19.16"W	Ponta Grossa	*Cambeva davisi* (1), *Geophagus iporanguensis* (1), *Hoplias* aff. *malabaricus* (2), *Hypostomus interruptus* (14), *Isbrueckerichthys duseni* (8), *Phalloceros harpagos* (12), *Rhamdia quelen* (2) and *Rineloricaria kronei* (10)
30	Unnamed river, affluent of Ribeirão da Cruz, rio Ribeira de Iguape basin	~798	25º5'15.63"S, 49º51'41.46"W	Ponta Grossa	*Astyanax laticeps* (2), *Astyanax* sp. 1 (7), *Coptodon rendalli* (1), *Geophagus iporanguensis* (44), *Hypostomus interruptus* (11), *Isbrueckerichthys duseni* (1) and *Phalloceros harpagos* (26)
31	Unnamed river	~943	25º9'8.25"S, 49º53'57.53"W	Ponta Grossa	*Astyanax* sp. 2 (2), *Geophagus iporanguensis* (4), *Hoplias* aff. *malabaricus* (2), *Kronichthys lacerta* (2) and *Phalloceros harpagos* (7)
32	Unnamed river	~834	25º'33.31"S, 49º52'4.43"W	Ponta Grossa	*Cambeva davisi* (2), *Characidium pterostictum* (5), *Isbrueckerichthys duseni* (7) and *Phalloceros harpagos* (3)

Table 2. Fish species collected in headwater streams from the rio Ribeira de Iguape basin, at the boundaries of the Ponta Grossa Arch, Paraná State, Brazil.

Ordem	Family	Species	Origin/Constancy	Voucher
Characiformes	Characidae	*Astyanax lacustris* (Lütken, 1875)	Autochthonous/Occasional	NUP19288
		Astyanax laticeps (Cope, 1894)	Autochthonous/Constant	NUP 17407, NUP 17418, NUP 17444, NUP18324, NUP 18948
		Astyanax bifasciatus Garavello & Sampaio, 2010	Autochthonous/Occasional	NUP18323, NUP 18354
		Astyanax sp. 1	Autochthonous/Occasional	NUP 19290, NUP19302, NUP 19305
		Astyanax sp. 2	Autochthonous/Occasional	NUP19304
		Bryconamericus microcephalus (Miranda Ribeiro, 1908)	Autochthonous/Occasional	NUP17413, NUP 17420
		Deuterodon iguape Eigenmann, 1907	Autochthonous/Occasional	NUP 17419, NUP18305, NUP 18900
	Erythrinidae	*Hoplias* aff. *malabaricus* (Bloch, 1794)	Autochthonous/Occasional	NUP19303
	Crenuchidae	*Characidium pterostictum* Gomes, 1947	Autochthonous/Occasional	NUP17410, NUP 17428, NUP 17442, NUP 18897
Siluriformes	Callichthyidae	*Callichthys callichthys* (Linnaeus, 1758)	Autochthonous/Occasional	NUP18924
		Corydoras ehrhardti Steindachner, 1910	Autochthonous/Occasional	NUP18315, NUP 18899, NUP 18911, NUP 18938, NUP 18955
	Heptapteridae	*Chasmocranus lopezae* Miranda Ribeiro, 1968	Autochthonous/Accessory	NUP 17415, NUP 17426, NUP18304, NUP 18902, NUP 18940
		Imparfinis sp.	Autochthonous/Occasional	NUP19294
		Pimelodella transitoria Miranda Ribeiro, 1907	Autochthonous/Occasional	NUP17416, NUP 17427, NUP 18919
		Rhamdia quelen (Quoy & Gaimard, 1824)	Autochthonous/Accessory	NUP18915, NUP 18925, NUP 18945, NUP 18952
		Rhamdioglanis transfasciatus Miranda Ribeiro, 1908	Autochthonous/Occasional	NUP18312, NUP 18901
	Loricariidae	*Harttia kronei* Miranda Ribeiro, 1908	Autochthonous/Accessory	NUP 17408, NUP 17424, NUP17445, NUP 18905, NUP 18947
		Hypostomus interruptus (Miranda Ribeiro, 1918)	Autochthonous/Accessory	NUP18307, NUP 19300
		Isbrueckerichthys duseni (Miranda Ribeiro, 1907)	Autochthonous/Constant	NUP 17411, NUP18358, NUP 18896, NUP 18904, NUP 19289
		Kronichthys lacerta (Nichols, 1919)	Autochthonous/Accessory	NUP 17421, NUP 17441, NUP 18302, NUP 18914, NUP 18921
		Neoplecostomus ribeirensis Langeani, 1990	Autochthonous/Occasional	NUP18353, NUP 18949, NUP 18956
		Parotocinclus maculicauda (Steindachner, 1877)	Autochthonous/Occasional	NUP18342, NUP 18943
		Rineloricaria kronei (Miranda Ribeiro, 1911)	Autochthonous/Occasional	NUP18322, NUP 18903, NUP 18909, NUP 18923, NUP 18935
	Trichomycteridae	*Cambeva* cf. *cubataonis* (Bizerril, 1994)	Autochthonous/Occasional	NUP19291
		Cambeva davisi (Haseman, 1911)	Autochthonous/Constant	NUP18332, NUP 18927, NUP 18933, NUP 18950, NUP 18957
Cichliformes	Cichlidae	*Coptodon rendalli* (Boulenger, 1897)	Allochthonous/Occasional	NUP19301
		Geophagus iporangensis Haseman, 1911	Autochthonous/Accessory	NUP18316, NUP 19306
Cyprinodontiformes	Poeciliidae	*Cnesterodon iguape* Lucinda, 2005	Autochthonous/Occasional	NUP19295
		Phalloceros harpagos Lucinda, 2008	Autochthonous/Constant	NUP 17417, NUP 18894, NUP 18908, NUP 18918, NUP 18944, NUP19307

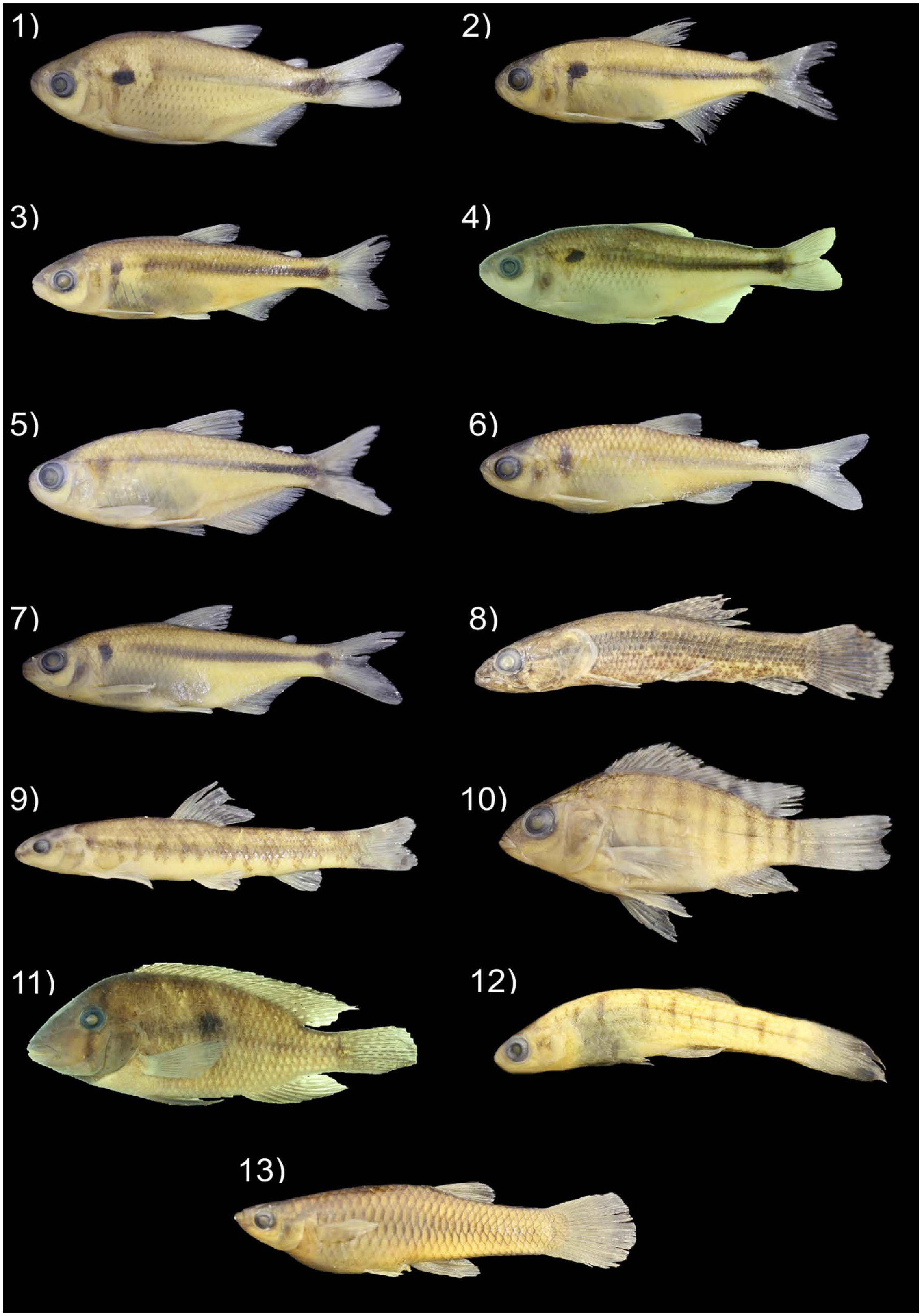

Figure 2. Representative individuals of the Characiformes, Cichliformes and Cyprinodontiformes registred to the headwater streams from the rio Ribeira de Iguape basin, at the Ponta Grossa Arch boundaries, Paraná State, Brazil. Their catalogue numbers in the Coleção Ictiológica do Nupélia (NUP) and standard lengths are presented after the names of species. 1) *Astyanax lacustris*, NUP 19288, 61.1 mm; 2) *Astyanax laticeps*, NUP 18324, 40.4 mm; 3) *Astyanax bifasciatus*, NUP 18354, 75.0 mm; 4) *Astyanax* sp. 1, NUP 19302, 72.2 mm; 5) *Astyanax* sp. 2, NUP 19304, 48.2 mm; 6) *Bryconamericus microcephalus*, NUP 17413, 47.8 mm; 7) *Deuterodon iguape*, NUP 18305, 71.4 mm; 8) *Hoplias* aff. *malabaricus*, NUP 19303, 56.0 mm; 9) *Characidium pterostictum*, NUP 17410, 53.7 mm; 10) *Coptodon rendalli*, NUP 19301, 34.8 mm; 11) *Geophagus iporangensis*, NUP 18316, 120.4 mm; 12) *Cnesterodon iguape*, NUP 19295, 24.5 mm; 13) *Phalloceros harpagos*, NUP 17417, 31.3 mm.

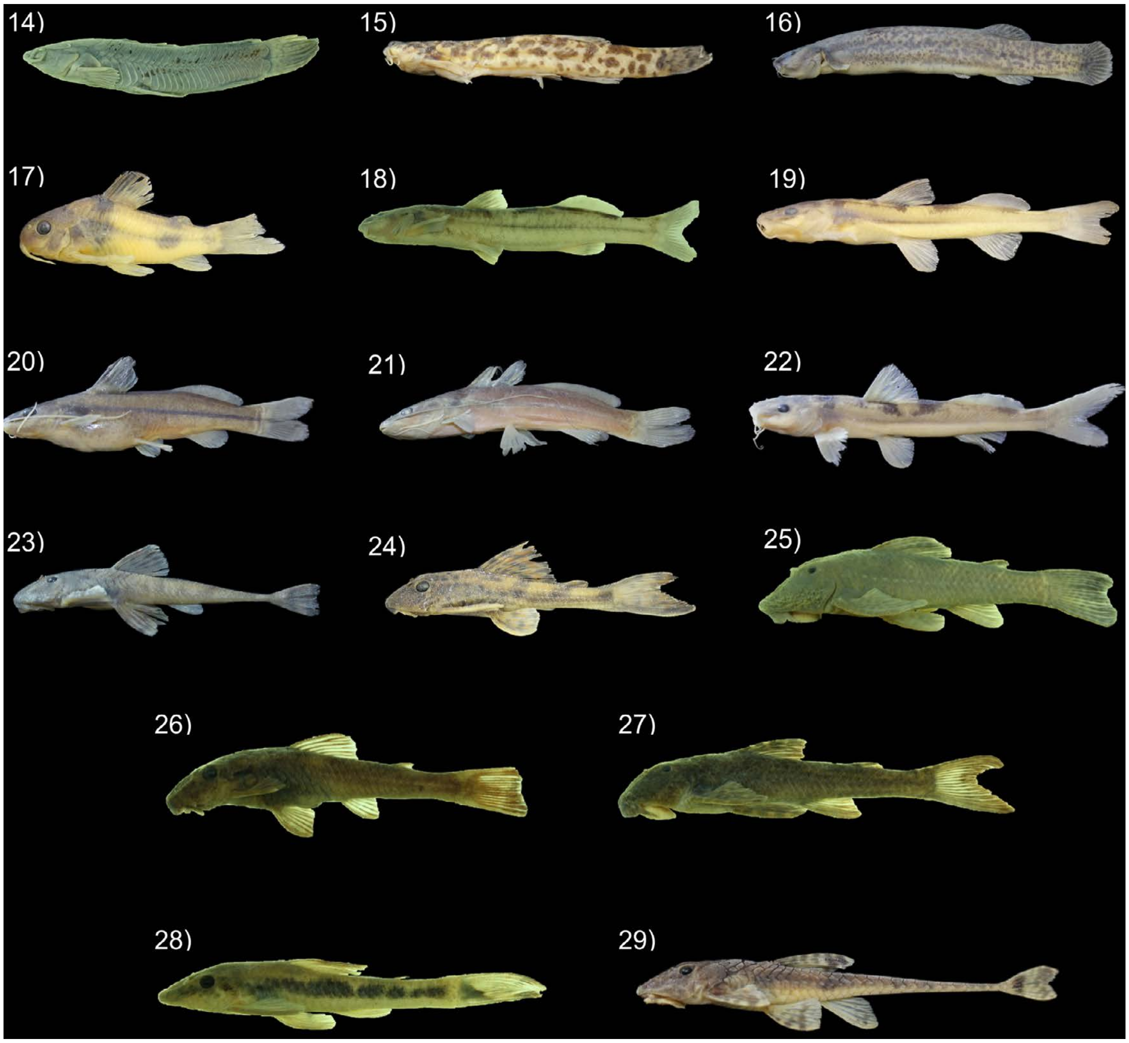

Figure 3. Representative individuals of the Siluriformes registred to the headwater streams from the rio Ribeira de Iguape basin, at the Ponta Grossa Arch boundaries, Paraná State, Brazil. Their catalogue numbers in the Coleção Ictiológica do Nupélia (NUP) and standard lengths are presented after the names of species. 14) *Callichthys callichthys*, NUP 18924, 75.9 mm; 15) *Cambeva* cf. *cubataonis*, NUP 19291, 41.4 mm; 16) *Cambeva davisi*, NUP 18332, 94.9 mm; 17) *Corydoras ehrhardti*, NUP 18315, 38.5 mm; 18) *Chasmocranus lopezae*, NUP 18304, 103.6 mm; 19) *Imparfinis* sp., NUP19294, 63.5 mm; 20) *Pimelodella transitoria*, NUP 17416, 100.9 mm; 21) *Rhamdia quelen*, NUP 18915, 90.3 mm; 22) *Rhamdioglanis transfasciatus*, NUP 18312, 118.3 mm; 23) *Harttia kronei*, NUP 17445, 68.8 mm; 24) *Hypostomus interruptus*, NUP 18307, 57.8 mm; 25) *Isbrueckerichthys duseni*, NUP 18358, 111.1 mm; 26) *Kronichthys lacerta*, NUP 18302, 51.5 mm; 27) *Neoplecostomus ribeirensis*, NUP 18353, 68.2 mm; 28) *Parotocinclus maculicauda*, NUP 18342, 42.0 mm; 29) *Rineloricaria kronei*, NUP 18322, 109.6 mm.

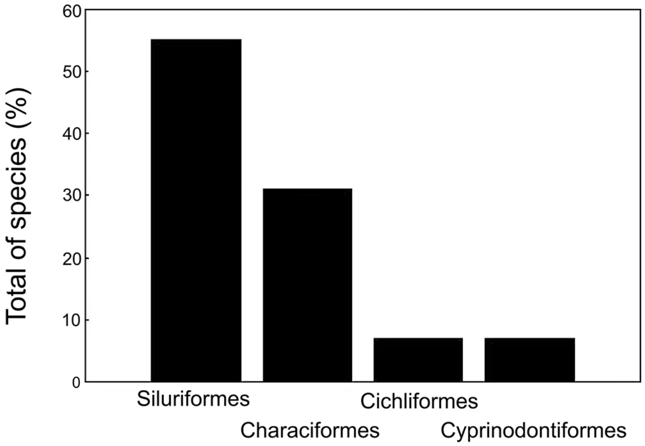

Figure 4. Percentages of the total number of collected species in each order in the headwater streams from rio Ribeira de Iguape basin, Paraná State, Brazil.

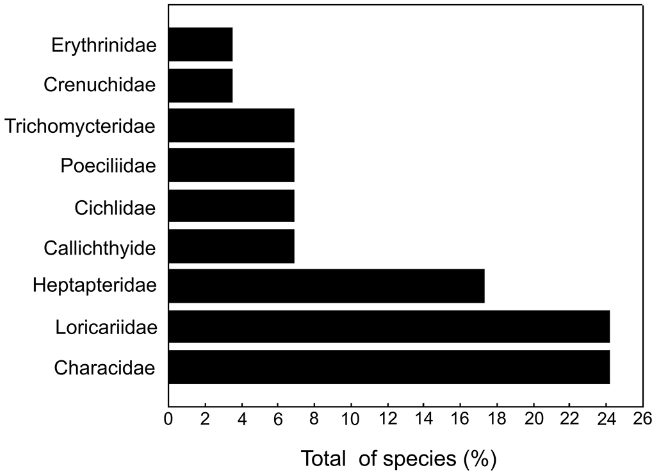

Figure 5. Percentages of the total number of collected species in each family in the headwater streams from rio Ribeira de Iguape basin, Paraná State, Brazil.

Regarding the abundance of the individuals sampled (Figure 6), *Phalloceros harpagos* Lucinda, 2008 (2,720 individuals), *Isbrueckerichthys duseni* (Miranda Ribeiro, 1907) (460 individuals), *Cambeva davisi* (Haseman, 1911) (445 individuals), *Astyanax laticeps* (Cope, 1894) (308 individuals), *Geophagus iporangensis* Haseman, 1911 (248 individuals), *Deuterodon iguape* Eigenmann, 1907 (231 individuals), *Harttia kronei* Miranda Ribeiro, 1908 (211 individuals), *Hypostomus interruptus* (Miranda Ribeiro, 1918) (166 individuals), and *Characidium pterostictum* Gomes, 1947 (147 individuals) were the most abundant species, contributing with approximately 87.8% of all collected individuals. On the other hand, the other 20 species did not have 100 individuals collected.

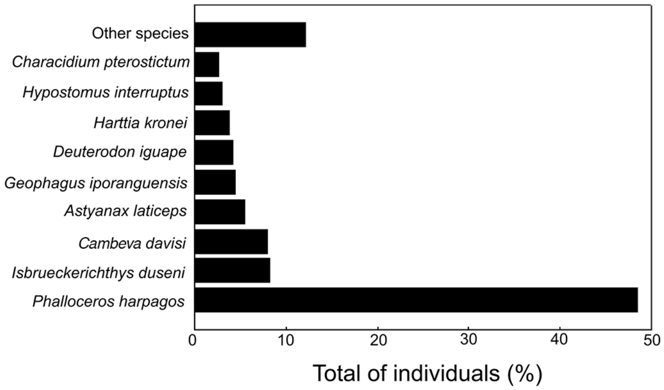

Figure 6. Percentages of number of individuals of each species with more than 100 individuals collected in the headwater streams from the rio Ribeira de Iguape basin, Paraná State, Brazil.

As for constancy (Table 2), most of the species were categorized as occasional (19 species). Four species (*Astyanax laticeps, Isbrueckerichthys duseni, Phalloceros harpagos*, and *Cambeva davisi*) were categorized as constant, and the six (*Chasmocranus lopezae* Miranda Ribeiro, 1968, *Rhamdia quelen* (Quoy & Gaimard, 1824), *Harttia kronei, Hypostomus interruptus, Kronichthys lacerta* (Nichols, 1919), and *Geophagus iporangensis*) were categorized as accessory. There were collected three putatively undescribed species: *Astyanax* sp. 1, *Astyanax* sp. 2, and *Imparfinis* sp. Individuals collected of *Cnesterodon iguape* Lucinda, 2005 and *Cambeva* cf. *cubataonis* (Bizerril, 1994) represent an expansion to their geographical distribution beyond the type locality. The new record of *Astyanax bifasciatus* Garavello & Sampaio, 2010 to the rio Ribeira de Iguape basin may give more evidence of ichthyofaunistic exchange among basins in the region. Considering the origin of species (Table 2), only *Coptodon rendalli* (Boulenger, 1897) was classified as allochthonous.

Discussion

This inventory recorded 29 fish species for the headwaters streams of the rio Ribeira de Iguape basin (nearly 39.7% of the total recorded in Oyakawa et al. 2006, a key publication for this basin). The highest number of species in Siluriformes and Characiformes follows the pattern of the Neotropical region of fish diversity (Castro 1999, Lowe-McConnell 1999, Buckup et al. 2007) and meets the samplings already carried out in the rio Ribeira de Iguape basin (*e.g.,* Oyakawa et al. 2006; Oyakawa & Menezes 2007; Cetra et al. 2012; Barrella et al. 2014).

Headwaters streams of the Atlantic Forest present high velocity of water flow and a mosaic of large rocks, which favors species with morphology to fixation and support (Oyakawa et al. 2006; Ferreira et al. 2010), as the sucker mouths of Loricariidae, and spines in the fins of Heptapteridae and in the operculum Trichomycteridae. Environments with fast-flowing and rocky riverine habitats (like those ones we sampled here) are local incubators of the diversification and biological specialization, promoting high rates of diversification of lineages and phenotypes, especially for Loricariidae (Roxo et al. 2017). The Atlantic Coastal basins contain low-diversity lineages of loricariids and with early-branching restricted to these drainages (Roxo et al. 2014), reinforcing the importance of the rio Ribeira do Iguape basin to the evolution and conservation of Loricariidae (Roxo et al. 2012). Characidae is widely recognized as the family with the most number of species in the Neotropical region, and the fourth among all the actinopterygian (Mirande 2018). The high diversity of mesohabitats in streams of the Atlantic Forest (backwaters separated by rapids) allow species adapted to live in the water column as the characids (Oyakawa et al. 2006; Ferreira et al. 2010).

Oyakawa et al. (2006) mentioned *Phalloceros caudimaculatus* (Hensel, 1868), however, in the systematic review of *Phalloceros* Eigenmann, 1907, Lucinda (2008) described that *P. harpagos* presents a wider geographical distribution, covering the rio Paraná-Paraguay basin and the coastal drainages of the rio Itaboapana (Espirito Santo State) and rio Araranguá (Santa Catarina State), and restriced *P. caudimaculatus* to southern of South America. The individuals sampled here were *P. harpagos* and had a wide distribution in practically all types of environments of the rio Ribeira de Iguape basin, from those with high contents of dissolved oxygen and with strong flow, to backwaters, with low dissolved oxygen and higher temperatures (Oyakawa et al. 2006). Due to the wide range of environmental conditions that *P. harpagos* supports, the viviparity, and the coexistence of juveniles and adults in the same habitats (Mazzoni et al. 2011), its abundance recorded here reflected a discrepant dominance of this species.

In general, occasional species were more numerous in the streams sampled here. Probably at least part of the explanation is the influence of the altitudinal gradient. Studies about elevational gradients frequently report a decrease in species richness with increasing altitude (Fu et al. 2004; Jaramillo-Villa et al. 2010), and an increase of the number of endemic species in headwaters regions (Carvajal-Quintero et al. 2015). Our sampling showed that some constant or accessory species (*e.g. Harttia kronei, Hypostomus interruptus, Isbrueckerichthys duseni* and *Cambeva davisi*) also present the highest frequencies of abundance. These species has morphological structures adapted to the fixation in structures underwater an withstand the force of the water flow, like species of *Characidium*, and members of Heptapteridae, Loricariidae, and Trichomycteridae (Oyakawa et al. 2006). Thereby, these species can colonize environments at higher altitudes.

The putatively undescribed species collected highlighted a gap in the taxonomic knowledge about headwater regions, meeting the biodiversity shortfalls, which are the gaps between existing evolutionary knowledge and the complete knowledge of a particular biological domain at a certain point in time (Hortal et al. 2015). These shortfalls are a direct consequence of the complexity generated by an evolutionary system, in which the rate of production of new entities exceeds the maximum rate at which we can describe them.

It should be noted that many species are disappearing without being known, cataloged or formally described, what was called by Brown & Lomolino (1998) as Linnean shortfall. A similar case of biodiversity shortfall refers to the lack of knowledge of the actual geographical distribution of organisms, named Wallacean shortfall (Lomolino 2004). The two species (*Cnesterodon iguape* and *Cambeva* cf. *cubataonis*) that have expanded their geographic distributions beyond their type locality showed this gap, which may be strongly dangerous in relation to the historical patterns of biogeographic data analysis (Meyer et al. 2015). Thus, our samplings support an inherent concern related to the probable biodiversity shortfalls existing in the ichthyofauna of the headwaters streams from the rio Ribeira de Iguape basin.

The presence of *Astyanax bifasciatus*, an endemic species of the rio Iguaçu system (Garavello & Sampaio 2010; Baumgartner et al. 2012), corroborates recent events of headwater captures between coastal drainages and those that flow into the interior of the continent. Studies of biogeographic divisors are necessary in attempts to explain the processes of origin and dispersion of species (Barton 1988). Watershed dividers, such as high mountains (Ponta Grossa Arch, for example), are often seen as effective dispersion barriers for fish, and fish distributions are used to assign river basin boundaries (Ingenito & Buckup 2007). Clearly, the fish composition of the rio Ribeira de Iguape basin is very different from the neighboring basins (see Ingenito et al. 2004; Hoffmann et al. 2015; Cetra et al. 2016; Claro-García et al. 2018). The formation of the Ponta Grossa Arch represents a barrier for fish populations and, in addition, due to their recent tectonic activities (Franco-Magalhaes et al. 2010), some species have been shared between the rio Ribeira de Iguape basin and neighboring basins of the upper rio Paraná and rio Iguaçu systems (Ribeiro 2006, Morais-Silva et al. 2018), constituting a characteristic pattern recognized by Ribeiro (2006) as "Pattern C". This pattern represents recent vicariant events between the upland crystalline shield rivers and the adjacent coastal drainages leading to the sharing of fish species and the formation of truly hybrid zones. Thus, the limits of the Ponta Grossa Arch bring interesting patterns of geographic distribution of the ichthyofauna with great effects in biogeographical and phylogeographic studies (Morais-Silva et al. 2018).

The presence of the allochthonous species *Coptodon rendalli* (popularly known as "tilapia") is an alarming record! Numerous fish farms are in full swing and breed non-native species that present a high risk of invasion in natural environments such as tilapia. Several authors have affirmed the invading potential of fish farms and the negative effects of new introductions (*e.g.*, Orsi & Agostinho 1999, Daga et al. 2015, Daga et al. 2016, Lima et al. 2018). Tilapias significantly change native aquatic communities due to predation of eggs and larvae, aggressive competition for space and food (Sanches et al. 2012), and alteration of the substrate for nest building and predation of zooplankton, which inevitably increases the levels of eutrophication causing changes in the limnological parameters and consequently affecting the native species (Figueredo & Giani 2005; Córdova-Tapia et al. 2015). Thus, Brazil should adopt restriction and control measures for species with high invasion potential, as well as invest in technologies to prevent fish farm leakage in order to control the introduction of tilapia in natural environments (Padial et al. 2017; Alves et al. 2018, Cassemiro et al. 2018; Gubiani et al. 2018).

Conclusions

Progress in Neotropical ichthyology depends on the biotic inventory of poorly sampled areas for identification of fish diversity (Schaefer 1998). For this inventory, we provide samplings in headwater streams of the rio Ribeira de Iguape basin in a location of high altitudes with interesting biogeographic patterns and inserted in Conservation Units in the Paraná State. Again, Atlantic Forest streams show a high degree of endemism in relation to their fish, therefore, the data obtained here may provide support for future biogeographic, ecological and conservationist studies.

Author Contributions

Augusto Frota: contributed to data acquisition, analysis and interpretation of data, drafting of the manuscript and wrote the paper.

Hugo José Message: contributed to analysis and interpretation of data, and critical revision for adding substantive intellectual content.

Rachel Calil de Oliveira: contributed to data acquisition and drafting of the manuscript.

Evanilde Benedito: contributed to analysis and interpretation of data, and critical revision for adding substantive intellectual content.

Weferson Júnio da Graça: contributed to data acquisition, analysis and interpretation of data, and critical revision for adding substantive 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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Short Communication

Composition, abundance and biomass of a fish assemblage in a southern Brazilian coastal stream during polyhaline/euhaline condition

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Abstract: The coastal streams of southernmost Brazil, Rio Grande do Sul state, are marked by a period of regular marine intrusion resultant from intense oceanic winds. In the present study we aimed to investigate the species composition, abundance and relative biomass of the ichthyofauna in the lower stretch of a coastal stream during summer, a period of regular marine intrusion. Estreito is a coastal hydrological complex composed by lakes, swamps and a perennial stream, located at the central-south portion of the coastal plain of Rio Grande do Sul state. During the summer of 2018, the ichthyofauna of lower Estreito stream was sampled by beach hauls applied in 17 random points distributed in a stretch of ca. 2km. Measurements at the sampled stretch revealed salinities between 19.3 to 31.3 ppt, characterizing the studied system as polyhaline/euhaline during summer. The sample of 4,533 specimens revealed the occurrence of 20 species, being the great majority marine-dwelling. The most abundant species were the anablepid *Jenynsia lineata* (70.3%), the cichlid *Geophagus brasiliensis* (19.3%) and the the mugilid *Mugil curema* (7.5%). The highest relative biomass was recorded for *J. lineata*, followed by *M. curema* and *G. brasiliensis*. The dominance of marine-dwelling species in the assemblage composition and the high abundance of limnic-estuarine *J. lineata* corroborate previous studies conducted in other washouts of Rio Grande do Sul.

Keywords: *Jenynsia lineata, Mugil curema, Geophagus brasiliensis, ichthyofauna dynamics, salinity, washout.*

Composição, abundância e biomassa de uma assembléia de peixes em um arroio costeiro do sul do Brasil durante condição polihalina/euhalina

Resumo: Os arroios costeiros do extremo sul do Brasil, estado do Rio Grande do Sul, são marcados por um período de regular intrusão marinha resultante de intensos ventos oceânicos. No presente estudo nós tivemos como objetivo investigar a composição de espécies, abundância e biomassa relativa da ictiofauna no trecho inferior de um arroio costeiro durante o verão, período de regular intrusão marinha. Estreito é um complexo hidrológico costeiro composto por lagoas, pântanos e um arroio perene, localizados na porção centro-sul da planície costeira do Rio Grande do Sul. Durante o verão de 2018, a ictiofauna do baixo arroio Estreito foi amostrada por arrastos de praia aplicados em 17 pontos aleatórios distribuídos em um trecho de cerca de 2 km. Medidas no trecho amostrado revelaram salinidades entre 19.3 e 31.3 ppt, caracterizando o sistema estudado como polialino/euhalino durante o verão. A amostra de 4.533 espécimes revelou a ocorrência de 20 espécies, sendo a grande maioria habitantes marinhos. As espécies mais abundantes foram o anablepídeo *Jenynsia lineata* (70,3%), o ciclídeo *Geophagus brasiliensis* (19,3%) e o mugilídeo *Mugil curema* (7.5%). A maior biomassa relativa foi registrada para *J. lineata*, seguido por *M. curema* e *G. brasiliensis*. A dominância das espécies marinhas na composição da assembléia e a alta abundância da espécie límnica-estuarina *J. lineata* corrobora estudos prévios conduzidos em outros sangradouros do Rio Grande do Sul.

Palavras-chave: *Jenynsia lineata, Mugil curema, Geophagus brasiliensis, dinâmica da ictiofauna, salinidade, sangradouro.*

Introduction

Coastal streams are considered marine-freshwater ecotones, hosting diverse fish assemblages composed of freshwater, estuarine and marine dwelling species (Whitfield 1999, Bastos et al. 2013). These systems, also known as "washouts", are abundant in the southernmost Brazilian coast (Rio Grande do Sul state), draining wetlands and lagoons distributed along a stretch of ca. 620 km of sandy deposits. The coastal streams or washouts of Rio Grande do Sul coastal plain (RSCP) are strongly influenced by climatic parameters such as precipitation and evaporation, which act directly on systems dynamics of discharge and connection to the sea (Figueiredo & Calliari 2006). Thus, coastal streams represent singular scenarios for studies on the ichthyofauna occurring in transient environments.

In view of the high representiveness of coastal stream or washouts in RSCP, few studies concerning the ichthyofauna in these systems were conducted. Bastos et al. (2013, 2014) surveyed the ichthyofauna occurrying in three coastal streams located in the southern segment of RSCP. Oliveira et al. (2014) investigated the role of mullets in transport of marine nutrients into the freshwater food webs in one of the coastal streams studied by Bastos et al. (2013, 2014). In this same system, Oliveira et al. (2018) verified the use of freshwater habitats by juveline mullets through the analysis of otolith chemistry.

"Estreito" is a hydrological complex which covers an area of ca. 453 hectares in the central-south portion of RSCP (São José do Norte municipality). This complex is composed by interconnected coastal lakes, perennial and intermittent peripheral swamps and a perennial coastal stream connected to the Atlantic Ocean (Gianuca & Tagliani 2012). The Estreito complex is recognized for its importance for the conservation of the local biodiversity (Burger & Ramos 2006, Gianuca & Tagliani 2012) and therefore it is inserted in a Permanent Protection Area, which implies in a protected territory with restrictions to human use. However, the pine silviculture established in the surroundings has been causing profound changes in the landscape, including the extinction of coastal streams (Gianuca & Tagliani 2012). During summer, Estreito is submitted to frequent and intense oceanic winds, causing frequent marine intrusions in the fluvial system (Oliveira & Calliari 2006). Herein we aimed to investigate the species composition, abundance and relative biomass of the fish assemblage occurring in the lower Estreito stream during its period of regular of marine intrusion. Despite being a typical limnic system, we hypothesize a higher representativeness of marine species when compared to limnic species, which will reflect on higher richness, abundance and biomass of marine-related taxa.

Material and Methods

Sampling campaigns were performed during the summer of 2018 in 17 random points distributed on a stretch of ca. 2 km of the lower Estreito coastal stream (Figure 1). Bottom varies from sandy to muddy and vegetation is composed by *Ruppia maritima* L. stands. Maximum depth does not exceed two meters. Fishes were captured using a beach seine net (10 x 2.5 m, 5 mm mesh size). Seventeen seine hauls were applied, each covering a distance of approximately 40 meters. Salinity was measured using a multiparameter water quality checker (Horiba®, model U50) at each sampling point. Captured fishes were euthanized in clove oil solution, fixed in 10% formalin, and conserved in 70% ethanol in the Ichthyological Collection of Genetics Laboratory at the Universidade Federal do Rio Grande (CILG). Collection was authorized by the Brazilian environmental agency "Instituto Chico Mendes para Conservação da Biodiversidade" (ICMBio) (license nº 56947-1). All the adopted procedures are in accordance with the protocols of the institutional committee for ethics in animal use (CEUA-FURG). Specimens were identified according to Heemstra & Randall (1993), Fischer et al. (2004) and Froese & Pauly (2018).

Relative abundance was calculated as the ratio between species-specific abundance and total abundance, with values presented as percentages of the total. Summary statistics (mean, standard deviation and range) of total length (in millimeters) was calculated for the species with a sample size greater than 10 individuals. For species with smaller sample size, the range or absolute values were presented. The biomass (in grams) of each species was obtained by weighing the formolized specimens, drained from conservative. Relative biomass of each especies was calculated as the ratio between species-specific biomass and total biomass.

Results and Discussion

Salinity at the sampled points varied from 19.3 to 31.3 ppt, characterizing the system as polyhaline/euhaline during the period of study. A total of 4,533 specimens belonging to 20 species, 14 families and 10 orders were collected (Table 1). The richest order and family were Gobiiformes and Gobiidae, comprising five and three species respectively. The one-sided livebearer *Jenynsia lineata* (Jenyns, 1842) was the most abundant species, followed by the pearl cichlid *Geophagus brasiliensis* (Quoy & Gaimard, 1824), and the white mullet *Mugil curema* Valenciennes, 1836 (Table 1). The highest relative biomass was recorded for *J. lineata*, followed by *M. curema* and *G. brasiliensis* (Table 1). As expected, the fish assemblage found in lower Estreito stream during the period of regular marine intrusion was composed mainly by marine-dwelling species, which comprised 70% of the species richness. By the other hand, marine-dwelling species encompassed only 9.8% of total abundance and 33.1% of total biomass. The species *Elops saurus* Linnaeus, 1766, *Platanichthys platana* (Regan, 1917), *Odontesthes argentinensis* (Valenciennes, 1835), *Awaous tajasica* (Lichtenstein, 1822), *Ctenogobius stigmaticus* (Poey, 1860), *Gobionellus oceanicus* (Pallas, 1770), *Epinephelus marginatus* (Lowe, 1834), *Menticirrhus littoralis* (Holbrook, 1847), *Citharichthys spilopterus* Günther, 1862 and *Paralichthys orbignyanus* (Valenciennes, 1839) were not recorded by Bastos et al. (2013, 2014) in the coastal streams located further south. These species, however, were recorded in larger marine-freshwater ecotonal systems of RSCP, comprised by the estuaries of coastal lagoons and rivers (Ramos & Vieira 2001, Loebmann & Vieira 2005, Burns et al. 2010). Our specimens of marked goby *C. stigmaticus* represent the second record for Rio Grande do Sul. This species had only previously been recorded in the state for the estuary of the Patos lagoon (Burns et al. 2010. The dusky grouper *E. marginatus* is considered as threatened in Rio Grande do Sul state (FZB 2014). The habits, number of collected specimens, relative abundance, summary statistics of total length and relative biomass of all recorded species are shown in Table 1.

The high representativeness of marine-dwelling species was expected in view of the regular marine intrusion and consequent high levels of salinity during the sampling period. However, a higher

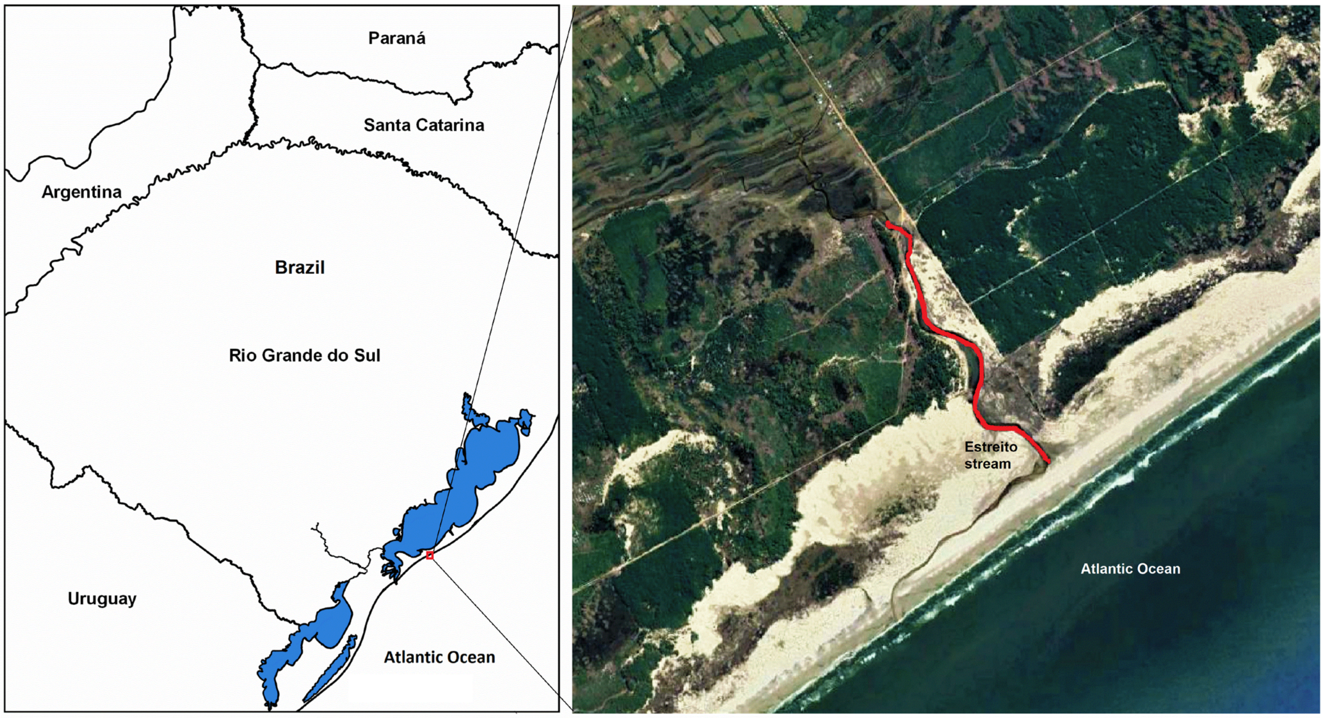

Figure 1. Location of Estreito stream and the sampled stretch (in red).

occurrence of strictly limnic species was also expected due to the connection with limnic systems such as lakes and swamps, located at a short distance from the sampled stream stretch. Instead, a unique strictly limnic species (*Hyphessobrycon igneus* Miquelarena, Menni, López & Casciotta, 1980) was recorded. Comparatively, Bastos et al. (2014) found more than half of the assemblages in three coastal streams located at the southern coastal plain of Rio Grande do Sul were composed by limnic species. Interestingly, the higher proportion of freshwater species found in their study remained even during summer, when marine intrusion events were detected. Nevertheless, salinity in these systems was much lower than that recorded in Estreito stream, ranging from 0 to 2. Thus, it is possible that the high salinity limits the occurrence of strictly limnic species in the lower Estreito stream, at least during summer.

Despite the high representativeness in the species composition, marine-dwelling species contributed little to abundance, except for the white mullet *M. curema*, the third species in order of abundance and the second in order of relative biomass in our sample. Bastos et al. (2014) found a similar pattern of abundance for marine species, where the majority of species were few abundant and higher abundances were recorded only for mullets (*M. curema* and *M. liza*). *Mugil curema* is a catadromous species commonly found in shallow marine waters, coastal lagoons and washouts (Fischer et al. 2004). The use of coastal streams habitats by juvenile mullets is well documented in RSCP (Bastos et al. 2014, Oliveira et al. 2014, 2018) and the exclusive occurrence of juveniles of *M. curema* in our sample, based on the available data for size at sexual maturity (Froese & Pauly 2018), reinforce these evidences. Thus, it seems that *M. curema* is the only marine species to use intensively lower Estreito stream during the period of regular marine while the other marine-dwelling species are occasional inhabitants in the system.

The one-sided livebearer *J. lineata* was the most abundant species in the present study, and concentrated half of the total biomass. *Jenynsia lineata* is a limnic-estuarine species (Assumpção et al. 2016) with a punctual record in marine waters (Calviño & Alonso 2016). *Jenynsia lineata* was one of the most abundant species in the systems studied by Bastos et al. (2013). There are evidences that *J. lineata* is especially abundant in systems with marine influence and also in typical freshwater environments (Garcia et al. 2003, Bastos et al. 2003). Mai et al. (2005) also verified that juveniles of *J. lineata* showed higher growth rate and survival in intermediate salinities. Therefore, lower Estreito stream fits as a suitable environment for the occurrence and development of *J. lineata*.

The cichlid *G. brasiliensis* was the second species in order of abundance and the third in order of relative biomass in our study. *Geophagus brasiliensis* presented low abundance or was absent in Bastos et al. (2013) summer samples. The high abundance of *G. brasiliensis* in lower Estreito stream is remarkable considering that it is a typical limnic species (Assumpção et al. 2016). Cichlids are typically freshwater inhabitants and few species can tolerate brackish waters (Froese & Pauly, 2018). Evidences of salinity tolerance by *G. brasiliensis* were obtained through controlled essays, where the species was submitted to increased salinity treatments (De Graff & Coutts 2010, Gutierre et al. 2014). Notwithstanding, the species has been found in estuarine systems (e.g. Garcia et al. 2001, Benincá et al. 2012), but those records were not accompanied by appropriate data on salinity. Thus, the tolerance of *G. brasilensis* to high salinity in natural environment (19.3-31.3 ppt) and its high relative abundance under this condition is herein recorded.

In the present study we verified the predominance of marine-related species in a coastal stream under a regime of regular marine intrusion in RSCP. Meanwhile, the highest abundance and biomass corresponded to

Table 1. Fish species recorded in lower Estreito stream (Rio Grande do Sul state, southern Brazil) during polyhaline/euhaline conditions (summer of 2018), habits (marine (M), estuarine (E) and limnic (L)), number of captured specimens (N), relative abundance (N%), summary statistics of total length in millimeters (mean ± one standard deviation (range)), absolute biomass (W) and relative biomass (W%) in grams.

Taxon	**Habit**	**N (N%)**	**Total length (mm)**	**W (W%)**
Elopiformes				
Elopidae				
Elops saurus Linnaeus, 1766	M, E	2 (0.04)	(70-74)	0.6 (0.02)
Clupeiformes				
Clupeidae				
Platanichthys platana (Regan, 1917)	E, L	7 (0.15)	(39-56)	5.7 (0.15)
Characiformes				
Characidae				
Hyphessobrycon igneus Miquelarena, Menni, López & Casciotta, 1980	L	1 (0.02)	27	0.7 (0.02)
Atheriniformes				
Atherinopsidae				
Atherinella brasiliensis (Quoy & Gaimard, 1824)	M, E	9 (0.20)	(36-49)	2.6 (0.07)
Odontesthes argentinensis (Valenciennes, 1835)	M, E, L	16 (0.35)	56 ± 8 (41-156)	30.1 (0.77)
Cyprinodontiformes				
Anablepidae				
Jenynsia lineata (Jenyns, 1842)	E, L	3187 (70.31)	35 ± 8 (10-73)	1953.0 (49.86)
Poeciliidae				
Phalloceros caudimaculatus (Hensel, 1868)	E, L	16 (0.35)	26 ± 3 (19-30)	3.0 (0.08)
Gobiiformes				
Eleotridae				
Eleotris pisonis (Gmelin, 1789)	M, E, L	10 (0.22)	101 ± 13 (80-120)	112.1 (2.86)
Dormitator maculatus (Bloch, 1792)	M, E, L	1 (0.02)	85	6.3 (0.16)
Gobiidae				
Awaous tajasica (Lichtenstein, 1822)	E, L	3 (0.07)	(45-93)	7.5 (0.19)
Ctenogobius stigmaticus (Poey, 1860)	M	27 (0.60)	66 ± 8 (40-78)	55.1 (1.41)
Gobionellus oceanicus (Pallas, 1770)	M, E, L	2 (0.04)	(110-145)	16.1 (0.41)
Cichliformes				
Cichlidae				
Geophagus brasiliensis (Quoy & Gaimard, 1824)	E, L	874 (19.28)	29 ± 14 (14-197)	648.9 (16.57)
Perciformes				
Carangidae				
Trachionotus marginatus Cuvier, 1832	M	28 (0.62)	46 ± 10 (28-66)	35.1 (0.90)
Serranidae				
Epinephelus marginatus (Lowe, 1834)	M	4 (0.09)	(77-89)	27.9 (0.71)
Sciaenidae				
Menticirrhus littoralis (Holbrook, 1847)	M	1 (0.02)	77	3.7 (0.09)
Micropogonias furnieri (Desmarest, 1823)	M, E	3 (0.07)	(129-138)	84.1 (2.15)
Mugiliformes				
Mugilidae				
Mugil curema Valenciennes, 1836	M, E, L	338 (7.46)	57 ± 14 (10-110)	920.9 (23.51)
Pleuronectiformes				
Paralichthyidae				
Citharichthys spilopterus Günther, 1862	M, E, L	2 (0.04)	(49-53)	1.9 (0.05)
Paralichthys orbignyanus (Valenciennes, 1839)	M, E	2 (0.04)	(46-53)	1.8 (0.05)
Total		4533 (100)		3917. 1 (100)

a limnic/estuarine species with high toleration to increased salinity. Our data corroborate previous studies in similar southward systems, leading to the recognition of a possibly recurrent pattern in coastal streams or washouts of RSCP. More studies based on larger spatial and temporal coverage may contribute to the seasonal dynamics and diversity of the ichthyofauna in these peculiar environments.

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Author Contributions

Fernando Marques Quintela: Substantial contribution to concept and design of the study; contribution to data collection; contribution to manuscript preparation.

Fabiano Corrêa: Contribution to data analysis and interpretation; contribution to critical revision, adding intelectual content.

Adriana Gava: Substantial contribution to concept and design of the study; contribution to data collection; contribution to critical revision, adding intelectual content.

Conflict of interest

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

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