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Article

Oligochaeta (Annelida: Clitellata) in the Juruena River, MT, Brazil: species indicators of substrate types

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Abstract: Oligochaeta assemblages are important components in freshwater environments, where their abundance and composition may indicate aspects related to water quality and sediment. The lack of information about these communities in Brazilian aquatic environments, as well as the application of native species as bioindicators of lotic environmental, stimulated the conception of this paper. Thus, the aim was to study the community of aquatic Oligochaeta in selected stretches of the Juruena River (MT), thereby generating grounds for future environmental monitoring action in lotic ecosystems. For this purpose, samples were analyzed at bimonthly intervals during the period from January to November 2009, in stretches of the Juruena River (Amazon River Basin) located in the State of Mato Grosso (MT). Two methods were used to collect the organisms: a) "D" network in small clusters of fixed macrophytes in the sediment on the river banks; and b) Ekman-Birge dredge in fine sediment. Preliminary results were 584 organisms distributed in 22 taxa. Of these, 22 valid species were identified. This number corresponds to approximately 25% of the aquatic oligochaete species registered in Brazil. Of these species, *Limnodrilus hoffmeisteri*, *Dero nivea* and *Pristina rosea* can be associated with organic enrichment conditions and/or some level of environmental degradation.

Keywords: *Freshwater oligochaetes, bioindicators, lotic environments, freshwater environments.*

Oligochaeta (Annelida: Clitellata) do Rio Juruena, MT, Brasil: espécies indicadoras em diferentes substratos

Resumo: Comunidades de oligoquetos constituem importante componente em ambientes de água doce, onde sua abundância e composição podem indicar aspectos relacionados à qualidade da água e do sedimento. A carência de informações sobre estas comunidades em ambientes aquáticos brasileiros, bem como a aplicação de espécies nativas como bioindicadores de ambientes lóticos estimularam o desenvolvimento do presente trabalho. Assim, o objetivo foi estudar a comunidade de oligoquetos aquáticos em trechos selecionados do Rio Juruena (MT), gerando subsídios para futuras ações de monitoramento ambiental em ecossistemas lóticos. Métodos: Para isso foram analisadas amostras efetuadas em intervalos bimestrais durante o período de janeiro a novembro 2009, em trechos do Rio Juruena (Bacia Hidrográfica do Rio Amazonas) localizado no Estado de Mato Grosso (MT). Para coleta dos organismos foram utilizados dois métodos: a) rede em "D" em pequenos agrupamentos de macrófitas fixas no sedimento nas margens do rio; e b) draga Ekman-Birge em sedimento arenoso. Os resultados preliminares 584 organismos distribuídos em 22 táxons. Dentre estes, foram identificadas 22 espécies válidas. Esse número corresponde aproximadamente 25% das espécies de oligoquetos aquáticos registradas no Brasil. Destas espécies, *Limnodrilus hoffmeisteri*, *Dero nivea* e *Pristina rosea* podem ser associadas à condições de enriquecimento orgânico e/ou algum nível de degradação ambiental.

Palavras-chave: *Oligoquetos aquáticos, bioindicadores, ambientes lóticos, ambientes de água doce.*

Introduction

Oligochaeta are some of the most abundant groups in continental aquatic macrofauna and play an important role in the process of decomposition and cycling of organic matter in freshwater ecosystems (Ragonha & Takeda, 2014, Cesar & Henry 2017). These organisms are found in almost all fresh aquatic environments (Cesar & Henry 2017), living in sediment and water columns (Rodriguez & Reynoldson 2011), and in association with other organisms (Corbi et al. 2004, Alves & Gorni 2007, Gorni & Alves 2007, Gorni & Alves 2008, Oda 2015).

In addition, these worms have limited mobility and are influenced by the habitat characteristics in which they are found (Behrend et al. 2012). Thus, the richness and abundance of Oligochaeta is directly related to environmental variables (Marchese & Drago 1999, Jablonska, 2014), such as availability of food resources (Martins & Silveira, Alves 2011); dissolved oxygen (Dornfeld et al. 2006); type of substrate (Moretto et al. 2013); water temperature (Nascimento & Alves 2009), thus being considered indicators of specific habitats.

However, although common in freshwater environments (Timm et al. 2001), knowledge about Oligochaeta fauna in Brazilian fresh waters is still fragmented and incomplete (Alves et al. 2008, Takeda et al. 2017). This lacuna is mainly due to the concentration of studies related to the spatial distribution of benthic invertebrate fauna, with emphasis on insect larvae (Roque & Trivinho-Strixino 2001, Sanseverino & Nessimian, 2001), the great extent of the still unexplored parts of the Brazilian hydrographic basins (Joly et al. 2011) and the low financial investment in scientific research in the country (Agostinho et al. 2005, Magurran 2011).

However, since the 1980s, taxonomic identification keys developed specifically for South America (Brinkhurst & Marchese 1989) and Brazil (Righi 1984) encouraged new research on the Oligochaeta, addressing aspects of their ecology (Petsch et al. 2015, Rodrigues et al. 2016), their geographic distribution (Gorni & Alves, 2008, Gomes et al. 2017), their composition in anthropologically disturbed environments (Behrend et al. 2012, Rosa et al. 2014) and their use as test organisms in ecotoxicological experiments (Corbi et al. 2015, Lobo & Espindola 2016).

However, the lack of information about these organisms in Brazilian aquatic environments is still evident in many regions (Gomes et al. 2017), as well as the use of native species as bioindicators of the quality of the country's aquatic ecosystems. Thus, the main objective of this paper was to study the aquatic Oligochaeta community in selected stretches of the Juruena River (MT), providing information for future action of environmental monitoring in lotic ecosystems.

Material and Methods

1. *Study area*

For the analysis of the Oligochaeta assemblages, samples were analyzed at bimonthly intervals during the period from January to November 2009. For the regular samplings, five sites were selected from the Juruena River (Amazon River Basin), located in the State of Mato Grosso (MT) (Figure 1).

The region is demarcated by humid tropical climates to contrasting seasons. Regionally the rainy season has its beginning, usually in the month of September until the month of April. From December to March are characterized by a increase in regional rainfall (Tardy, 1986). The basin is mainly sheltered by Ombrophilous Forest (Instituto Brasileiro de Geografia Estatística, 1992). In the upper portion of the basin, close to the Juruena, the vegetation is classified as "Cerrado". The soils in the basin are largely composed of red-yellow Acrisols (29%), red-yellow Oxisols (27%) and Arenosols (18%) (Empresa Brasileira de Pesquisa Agropecuária, 1980).

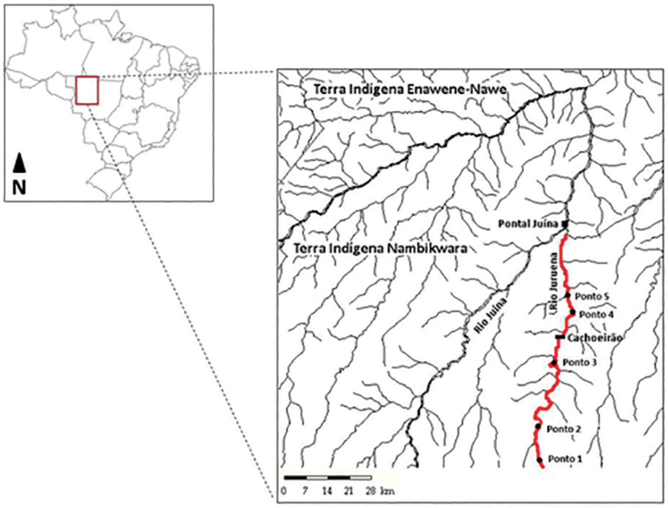

Figure 1. Map showing the location of the study area with highlight of the sampled section.

The headwaters of Rio Juruena are situated at the Parecis Plateau, in a savanna ecosystem ("cerrado"). In this area the water is totally transparent and poor in nutrients (N, P, and Ca). Biochemical oxygen demand (BOD) and fecal coliform bacteria are low, demonstrating that anthropic contributions are insignificant. Macrophytes are not abundant and are distributed in discrete and sparse stands, sometimes covering a sand plateau onshore.

The collection sites were selected considering: i) prevailing habitats, ii) easy access, and iii) adequate sites for the use of benthic fauna samplers (details of the geographical location of the sites are shown in Table 1).

2. *Data collection*

The collection of organisms followed the methodology described by Dowing (1984) and Peckrasky (1984). Thus, two methods of collecting zoobenthos were used: a) the D-net sampler in small groups of fixed macrophytes on the river margin; and b) Ekman-Birge dredge in fine sediment to collected sediment and associated organisms.

The net, with 0.30 m mesh openings, was dragged 1.0 m by the macrophyte roots, making a capture area of 0.3 m^2. The dredge, with an area of 0.0225 m^2, was launched in locations close to the margins in depths of up to 3 m. As an adopted procedure, two samples (replicas) were made with the net and dredge at each sampling site (a total of 50 sample units). Still at the sites, the collected samples were washed in a sieve with a mesh of 0.21 mm opening, fixed in 10% formalin and preserved in 70% alcohol. In the laboratory, the samples were washed again on a 0.021mm mesh granulometric sieve. The organisms were screened in a WILD® stereomicroscope with a maximal increase of 30 times.

Table 1. Relative abundance of aquatic Oligochaeta species in Juruena River, and geographic coordinates of the sampling sites. +: ≤ 10%; ◊: 10 < 50%; ■: ≥ 50%.

Species/Coordinates	**Sample site**				
	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
	13° 22’ 01” S **59° 00’ 45” W**	**13° 15’ 56" S** **59° 01’ 10" W**	**13° 04’ 27” S** **58° 58’ 32” W**	**12° 54’ 13” S** **58° 54’ 47” W**	**12° 51’ 22” S** **58° 55’ 41” W**
Allonais chelata		+	+	+	+
Allonais inaequalis	+	+		◊	+
Aulodrilus pigueti	+	+			
Aulophorus costatus	+		+		+
Aulophorus lodeni		+			
Branchiura sowerbyi	+	+	+		
Brinkhurstia americana	■	■	■	■	■
Dero digitata					+
Dero pectinata			+		+
Dero sawayai				+	
Dero nivea				+	
Haplotaxis aedeochaeta	+	+	+		+
Limnodrilus hoffmeisteri	+	+			
Nais communis	+		+	◊	+
Nais elinguis	◊	+	+	+	+
Nais variabilis		+	+	+	+
Narapa bonettoi	+				
Pristina leidyi	+	+		+	+
Pristina rosea	+	+	+	+	+
Pristina menoni		+			+
Slavina evelinae	+	+	+		+

For the identification of Oligochaeta, taxonomic criteria adopted by Brinkhurst & Jamieson (1971), Righi (1984), Brinkhurst & Marchese (1989), Pinder & Brinkhurst (1994) and Timm (2009) were followed. The list of species in synonymy was based on the catalog proposed by Christoffersen (2007).

All biological material identified was deposited in the Laboratory of Ecology and Aquatic Ecotoxicology (LEEA) linked to the Department of Hydraulics and Sanitation, School of Engineering of São Carlos, University of São Paulo.

In order to verify the sensitivity of the species to the different habitats, fixed macrophytes, mainly Podostemacean species on the river margin (collected with a D-net sampler) and fine sediment (Ekman-Birge dredge) we applied Indicator Species Analysis (ISA) (Dufrene & Legendre, 1997) (alpha = 0.05). This analysis combines species relative abundance with their relative frequency of occurrence in the various groups of samplers. This analysis was made using the ‘’indicspecies” package (De Caceres & Legendre, 2009) in R software (R Core Team, 2017), with 10,000 permutations.

Results

A total of 584 organisms were identified in 22 valid species comprising 11 genera. This number corresponds to less than 1% of the aquatic species described in the world and approximately 1/4 of the species registered in Brazil (Christoffersen 2007, Martin et al. 2008). The relative abundance of the Oligochaeta species are shown in Table 1. The specie *Brinkhurstia americana* showed a relative abundance greater than 50% of the total fauna in all the points sampled.

The Indicator Species Analysis (ISA) reveals five indicator species of fixed macrophytes (*Aulophorus costatus*, *Dero pectinata*, *Dero digitata*, *Dero sawayai* and *Dero nivea*), and four indicator species from fine sediment (*Aulodrilus pigueti*, *Limnodrilus hoffmeisteri*, *Aulophorus lodeni* and *Narapa bonetoi*) (Table 2).

Discussion

Listed below are the species, according to the nomenclature proposed by Timm (2017) with respective records from the Brazilian territory and ecological considerations.

Allonais chelata

Distribution: **São Paulo:** collected in urban streams by Alves and Lucca (2000) and associated with aquatic macrophytes (Alves & Gorni 2007). **Pernambuco:** Gurjaú reservoir (Marcus 1944). **Pará:** Tapajós river, Trombetas river, São Manuel River, Cuminá river, Salgado lake, Cupari river by Marcus (1942) and by Du Bois-Reymond Marcus (1947,

Table 2. Indicator Species in two collected habitats on the Juruena River (fixed macrophites and fine sediment). IV (%): species indicator value obtained by 10,000 permutations (using the Monte Carlo Method).

Taxa	Habitat	IV (%)
Aulodrilus pigueti	Fine sediment	36.1
Aulophorus costatus	Fixed macrophytes	29.7
Aulophorus lodeni	Fine sediment	20.9
Dero digitata	Fixed macrophytes	20
Dero pectinata	Fixed macrophytes	25.1
Dero sawayai	Fixed macrophytes	20
Dero nivea	Fixed macrophytes	20
Limnodrilus hoffmeisteri	Fine sediment	36.1
Narapa bonettoi	Fine sediment	20.9

1949a, 1949b). **Amazonas:** Grande Curuay lake by Marcus (1944) and Du Bois-Reymond Marcus (1944, 1947). **Rio Grande do Sul:** areas of irrigated rice fields (Sternert et al. 2012).

Allonais inaequalis

Distribution - **São Paulo**: associated with gastropods of the species *Pomacea bridgesii* (Gorni & Alves 2006); associated with aquatic macrophytes (Alves; Gorni, 2007); and sponges of the species *Metania spinata* (Gorni & Alves 2008a). It was also collected in the sediment of urban streams (Alves et al. 2006; Sanches et al. 2016). **Rondônia:** Cuniã lake by Gomes et al. (2017).

Aulodrilus pigueti

Distribution: **Paraná:** Patos Lake and Ivinhema River (Montanholi-Martins & Takeda 2001); in the Paraná River (Montanholi-Martins & Takeda, 1999); in different floodplain habitats of the Paraná River (Ragonha & Takeda, 2014, Petsch et al. 2015), in several reservoirs in the state of Paraná (Moretto et al. 2013); in the Paraná River, in the Ilha Grande National Park, between the states of Mato Grosso do Sul and Paraná (Ragonha et al. 2013); in tributaries of the Paraná River (Ragonha et al. 2014); in Ivinhema River and Baía River (Behrend et al. 2009) and Iguaçu River (Behrend et al. 2012). **São Paulo**: marginal lagoon of the Mogi-Guaçu River (Alves & Strixino 2000, 2003). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000). **Piauí:** collected in the Poti River by Sales et al. (2014). **Rio Grande do Sul:** in areas of irrigated rice fields (Sternert et al. 2012).

Aulophorus costatus

Distribution - **Paraná**: Patos Lake and Ivinhema tributary (Montanholi-Martins & Takeda 2001). **São Paulo:** in the city of São Paulo (Marcus 1942, 1943); associated with the gastropod *Pomaceae bridgesii* (Gorni & Alves 2006); associated with submerged macrophytes (Alves & Gorni 2007); in reservoirs (Pamplin et al. 2005). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000). **Rondônia:** Cuniã Lake by Gomes et al. (2017).

Aulophorus lodeni

Distribution – **São Paulo:** In the Infernão lagoon associated with the rhizosphere of the *Scirpus cubensis* macrophyte (Correia & Trivinho-Strixino 1998), associated with the *Scirpus*, *Eichhornia* and *Salvinia* macrophytes in the Infernão lagoon by Trivinho-Strixino et al. (2000). **Pará:** in the Cupari River (Marcus 1942, Du Bois-Reymond Marcus 1947, 1949a, 1949b).

Branchiura sowerbyi

Distribution - **São Paulo:** Tietê River (Marcus 1942, 1943, Du Bois-Reymond Marcus, 1949a); in the Salto Grande eutrophic Reservoir (Dornfeld et al. 2006); Americana Dam by Pamplin et al. (2006); in the Monjolinho dam in the city of São Carlos (Fusari & Fonseca-Gessner 2006); Tietê River reservoirs by Pamplin et al. (2005) and by Suriani et al. (2007) and in a marginal lagoon of the Mogi-Guaçu River (Alves & Strixino 2000, 2003). **Paraná:** in several reservoirs in the state of Paraná (Moretto et al. 2013) and in the Iguaçu River (Behrend et al. 2012). **Piauí:** Poti River by Sales et al. (2014).

Brinkhurstia americana

Distribution - **São Paulo:** urban streams (Alves & Lucca 2000, Alves et al. 2006; Sanches et al. 2016); in the Ribeirão das Anhumas reservoir (Corbi & Trivinho-Strixino 2002); sediments of the Ponte Nova and Bariri reservoirs (Pamplin et al. 2005). **Paraná:** Ivinhema River and associated with the macrophyte *Eichhornia azurea* in the Patos lake (Montanholi-Martins & Takeda 2001), in the Paraná River (Montanholi-Martins & Takeda 1999); in Ivinhema River and Baía River (Behrend et al. 2009); in Iguaçu River (Behrend et al. 2012) and in several reservoirs in the state of Paraná (Moretto et al. 2013). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000).

Dero digitata

Distribution - **São Paulo:** associated with the gastropod *Pomaceae bridgesii* (Gorni & Alves 2006); associated with aquatic macrophytes (Alves & Gorni,2007); in the Tietê River dam (Pamplin et al. 2005) and urban streams (Alves &, Lucca 2000, Rosa et al. 2014). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000). **Pará:** Tapajós, Cururu, Acará, São Manuel, Cupari and Juruena rivers and in the cities of Fordlandia, Belterra, Santarém and Itaituba (Marcus 1942, 1944, Du Bois-Reymond Marcus 1947, 1949a, 1949b). **Amazonas:** Grande Curuay Lake (Marcus 1944, Du Bois-Reymond Marcus 1944, 1947). **Rondônia:** Cuniã Lake (Gomes et al. 2017). **Paraná:** Iguaçu River (Behrend et al 2012); in different habitats of the Paraná River floodplain (Ragonha & Takeda 2014), in several Paraná state reservoirs (Moretto et al. 2013) and associated with the macrophytes *Hydrilla verticillata* and *Egeria najas* collected in the Paraná River and Leopoldo Backwater (Behrend et al. 2013). **Rio Grande do Sul:** in areas of irrigated rice fields (Sternert et al. 2012).

Dero pectinata

Distribution – **Paraná:** Patos Lake, in the floodplain of the Paraná River (Montanholi-Martins & Takeda 2001), in different floodplain habitats of the Paraná River (Ragonha & Takeda 2014). **São Paulo:** was collected in reservoirs (Marcus 1943, Pamplin et al. 2005), in sediment of Ribeirão das Anhumas reservoir (Corbi & Trivinho-Strixino, 2002); in an urban stream (Alves & Lucca 2000, Alves et al. 2006) and in Tietê river reservoirs (Suriani et al. 2007). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000).

Dero sawayai

Distribution: **São Paulo**: in streams (City of São Paulo) and Rio Claro by Marcus (1943), associated with gastropods (Gorni & Alves, 2006), associated with submerged macrophytes (Alves & Gorni 2007); associated with the sponge *Metania spinata* (Gorni, Alves, 2008a) and in urban impacted streams (Sanches et al. 2016). **Paraná:** associated with the macrophytes *Hydrilla verticillata* and *Egeria najas* collected in the Paraná River and the Leopoldo Backwater (Behrend et al. 2013), in the Iguaçu River (Behrend et al. 2012) and the Paraná River in the Ilha Grande National Park, between the states of Mato Grosso do Sul and Paraná (Ragonha et al. 2013); in different floodplain habitats of the Paraná River (Ragonha & Takeda, 2014); in tributaries of the Paraná River (Ragonha et al. 2014); in Baía River in artificial substrates (Fujita et al. 2015) and Ivinhema and Baía rivers (Behrend et al., 2009). **Minas Gerais:** associated with decomposing leaves of *Eichhornia azurea* in Manacás Lake (Martins et al. 2011). **Ceará:** was found among individuals of the species *Stolella agilis* f. *iheringi* (Marcus 1942, 1943). **Alagoas:** was found in the city of Satuba, in an artificial tank (Marcus 1943, 1944). **Pernambuco:** São Francisco River (Marcus 1943, 1944). **Rio Grande do Sul:** in areas of irrigated rice fields (Sternert et al. 2012).

Dero nivea

Distribution: **São Paulo**: macrophyte rhizosphere (Correia &Trivinho-Strixino 1998); marginal lagoon of the Mogi-Guaçu River (Alves & Strixino 2000); associated with macrophytes in Infernão Lagoon (Trivinho-Strixinoet al. 2000); in the sediments of the Ribeirão das Anhumas reservoir (Corbi & Trivinho-Strixino 2002), in Tietê river reservoirs (Pamplin et al. 200, Suriani et al. 2007); associated with gastropods (Gorni & Alves 2006), in submerged macrophytes (Alves & Gorni 2007); associated with the sponge *Metania spinata* (Gorni & Alves 2008a) and in urban impacted streams (Sanches et al. 2016). **Rio Grande do Sul**: in areas of irrigated rice fields (Sternert et al. 2012). **Rondônia:** Cuniã Lake (Gomes et al. 2017).

Haplotaxis aedeochaeta

Distribution - **Paraná**: Detected in the Ivinhema River (Montanholi-Martins; Takeda, 2001); Parana River (Montanholi-Martins & Takeda 1999); in floodplain areas of the Parana River (Ragonha & Takeda, 2014, Petsch et al. 2015), in Rio Ivinhema (Behrend et al. 2009), and in tributaries of the Paraná River (Ragonha et al. 2014).

Limnodrilus hoffmeisteri

Distribution: **São Paulo:** in cement tanks of the University of São Paulo, in streams of the Jardim Europa and Rio Tietê (Marcus 1942), in urban streams (Alves & Lucca 2000, Alves et al. 2006, Rosa et al. 2014, Sanches et al. 2016); in marginal lagoon of the Mogi-Guaçu River, by Alves and Strixino (2000, 2003), in the Tietê River reservoir (Pamplin et al. 2005, Suriani et al. 2007), in the Salto Grande eutrophic Reservoir (Dornfeld et al. 2006), in the American Dam (Pampli et al. 2006), in the dam Monjolinho (Fusari & Fonseca-Gessner 2006), in the Monjolinho reservoir (Fusari et al. 2006); in Monte Alegre Lake (Cleto-Filho & Arcifa 2006), in streams of the Intervales State Park (Alves et al. 2008); Galharada and Serrote streams (Gorni & Alves 2008b), in mesohabitats of Galharada stream (Gorni & Alves 2012). **Paraná:** Iguaçu River (Behrend et al. 2012) and Paraná River, in the Ilha Grande National Park, between the states of Mato Grosso do Sul and Paraná (Ragonha et al. 2013); in the Paraná River (Montanholi-Martins & Takeda, 1999); in different floodplain habitats of the Paraná River (Ragonha & Takeda 2014), in several reservoirs in the state of Paraná (Moretto et al. 2013); in the Paraná River tributaries (Ragonha et al. 2014) and Ivinhema and Baía rivers (Behrend et al. 2009). **Rio Grande do Sul:** Quadros lagoon, with muddy sediment and weakly brackish water (Marcus 1944). **Minas Gerais:** occurred in a stream of the Atlantic Forest (Rosa et al. 2015); in the São Pedro stream (Martins et al. 2008) and in an urban stream (Frizzera & Alves 2012). **Piauí:** Poti River by Sales et al. (2014).

Nais communis

Distribution: **São Paulo**: associated with the sponge *Ephydatia crateriformis* (Marcus, 1943), the sponge *Radiospongilla amazonenses* (Corbi et al. 2005) and the sponge *Metania spinata* (Gorni & Alves 2008a); associated with the macrophytes (Trivinho-Strixino et al. 2000, Alves & Gorni 2007); sediment of urban streams (Alves & Lucca 2000); associated with gastropods (Gorni & Alves 2006, Martins & Alves 2008); sediment of the Monjolinho River (Alves et al. 2006); associated with bryophytes of the genus *Fissidens* sp. and *Philonotis* sp. (Gorni & Alves 2007); Campo do Meio and Galharada streams (Gorni; & Alves 2008b, Gorni & Alves 2012), streams of the Intervales Park (Alves et al. 2008); was detected in impacted urban streams (Rosa et al. 2014, Sanches et al. 2016). **Minas Gerais**: detected in first order streams of preserved areas (Rodrigues et al. 2013) and in an urban stream (Frizzera & Alves 2012). **Paraná**: Paraná River, in the Ilha Grande National Park, between the states of Mato Grosso do Sul and Paraná (Ragonha et al. 2013), in the Iguaçu River (Behrend et al. 2012); in different floodplain habitats of the Paraná River (Ragonha & Takeda 2014, Petsch et al. 2015); associated with the macrophytes *Hydrilla verticillata* and *Egeria najas* collected in the Paraná River and Leopoldo Backwater (Behrend et al. 2013); in tributaries of the Paraná River (Ragonha et al.,2014); Baía River, in artificial substrates (Fujita et al. 2015) and Ivinhema and Baía rivers (Behrend et al. 2009). **Alagoas**: was found by Marcus (1944) in the Paulo Afonso waterfall. **Pará:** near Belterra by Marcus (1942) and Du-Bois Reymond Marcus 1947, 1949a, 1949b).

Nais elinguis

Distribution - **São Paulo:** associated with the gastropod *Pomaceae bridgesii* (Gorni & Alves 2006).

Nais variabilis

Distribution - **São Paulo:** associated with Odonata larvae *Elasmothemis cannacrioides* and *Mnesarete* sp. (Corbi et al. 2004); associated with submerged aquatic macrophytes (Alves & Gorni,2007); leaf litter of Galharada stream (Gorni & Alves 2008b, Gorni & Alves 2012) and was detected in impacted urban streams (Sanches et al. 2016). **Minas Gerais**: it was detected in first order streams of preserved areas (Rodrigues et al. 2013). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000).

Narapa bonettoi

Distribution - **Paraná**: Ivinhema River (Montanholi-Martins; Takeda, 2001; Takeda et al. 2001); the Parana River (Montanholi-Martins & Takeda 1999); Ivinhema and Baía River (Behrend et al. 2009); in several reservoirs in the state of Paraná (Moretto et al. 2013), in floodplain areas of the Paraná River (Ragonha & Takeda 2014, Petsch et al. 2015) and in tributaries of the Paraná River (Ragonha et al. 2014) **São Paulo:** Gouveia stream (Alves et al. 2006; Alves et al. 2006). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000).

Pristina leidyi

Distribution - **São Paulo:** associated with colonies of *Ephydatia crateriformis* sponges, in the Araguá River sediment (Marcus 1943); (Alves & Gorni 2007), associated with the *Metania spinata* sponge (Gorni & Alves 2008a), associated with gastropods of the species *Pomacea bridgesii* (Gorni & Alves 2006), associated with submerged macrophytes (Gorni & Alves 2008b), in mesohabitats of the Galharada stream (Gorni & Alves 2012) and was detected in impacted urban streams (Sanches et al. 2016). **Paraná:** in Ivinhema River (Behrend et al. 2009); Iguaçu River (Behrend et al. 2012); in tributaries of Paraná River (Ragonha et al. 2014) and Baía River, in artificial substrates (Fujita et al. 2015). **Minas Gerais:** occurred associated with decomposing leaves of *Eichhornia azurea* in Manacás Lake (Martins et al. 2011); in first order streams of preserved areas (Rodrigues et al. (2013) and associated with bryophytes (Rodrigues et al. 2016). **Alagoas:** in a creek of the city of Garça Torta (Du Bois-Reymond Marcus 1947). **Pernambuco:** São Francisco river (Marcus 1944). **Rio Grande do Sul**: in areas of irrigated rice fields (Stenert et al. 2012). **Amazonas**: near Humaitá by Marcus, (1943) and Du Bois- Reymond Marcus (1947, 1949a, 1949b).

Pristina rosea

Distribution - **São Paulo:** Pinheiros River and the campus of the University of São Paulo associated with bromeliads (Marcus 1943), in the Tietê River associated to the plant of the genus *Calathea* sp. (Marcus 1944); in urban streams and a rural stream (Alves et al. 2006); in the Monjolinho reservoir, organically enriched (Fusari & Fonseca-Gessner 2006); associated with bryophytes of the genus *Fissidens* sp. and *Philonotis* sp. (Gorni & Alves 2007); in streams of low order of the Campos do Jordão State Park, characterized by cold waters, rapids and stony bed (Gorni & Alves 2008b), in mesohabitats of the Galharada stream (Gorni & Alves 2012) and was evidenced in impacted urban streams (Gorni et al., 2017). **Minas Gerais**: occurred in first-order streams of preserved areas (Rodrigues et al. 2013); associated with bryophytes (Rodrigues et al. 2016) and in a stream of the Atlantic Forest (Rosa et al. 2015). **Pernambuco:** city of Recife, in pools of water (Marcus 1944) and in Tegipió (Marcus 1943).

Pristina menoni

Distribution: **São Paulo:** urban streams (Alves et al. 2006, Sanches et al. 2016), associated to bryophytes of the genus *Fissidens* sp. and *Philonotis* sp. collected in the Jacaré Pepira River (Gorni & Alves 2007). **Paraná:** Ivinhema River (Montanholi-Martins & Takeda 2001). **Minas Gerais:** associated with bryophytes (Rodrigues et al. 2016). **Rondônia:** Cuniã Lake (Gomes et al. 2017).

Slavina evelinae

Distribution - **São Paulo:** in cement tanks of the University of São Paulo, associated to *Ficus elastica* (Marcus 1942); Canindé lake (Marcus 1944), macrophyte rhizosphere (Correia &Trivinho-Strixino 1998), in the sediment of the Ribeirão das Anhumas reservoir (Corbi & Trivinho-Strixino 2002), in the Tietê river reservoir sediment (Pamplin et al. 2005, Suriani et al. 2007); in submerged macrophytes (Alves & Gorni 2007) and was evidenced in impacted urban streams (Sanches et al. 2016). **Paraná:** in Ivinhema and Baía rivers (Behrend et al. 2009); Paraná River, between the states of Mato Grosso do Sul and Paraná (Ragonha et al. 2013); in different floodplain habitats of the Paraná River (Ragonha &Takeda 2014); in the Iguaçu River (Behrend et al. 2012); associated with the macrophytes *Hydrilla verticillata* and *Egeria najas* collected in the Paraná River and the Leopoldo Backwater (Behrend et al. 2013); found in floodplain areas of the Paraná River (Petsch et al. 2015) and in the Paraná River tributaries (Ragonha et al. 2014). **Ceará:** associated with bryozoans of the species *Stolella agilis* (Marcus 1942). **Rio Grande do Sul**: in areas of irrigated rice fields (Stenert et al. 2012). **Mato Grosso do Sul:** Negro River (Takeda et al. 2000). **Minas Gerais:** urban streams (Frizzera & Alves 2012). **Pernambuco:** Near São Bartolomeu (Marcus 1942, 1943). **Pará:** in several rivers of the state (Marcus 1942, Du Bois-Reymond Marcus 1947, 1949a, 1949b).

Based on species distribution patterns and habitat preference (ISA) in the Juruena River, we concluded that *Dero* species are associated with marginal regions of aquatic ecosystems, mainly occurring as aquatic macrophytes. This condition demonstrates the susceptibility of this species group to samplers such as D-net. *Limnodrilus hoffmeisteri*, *Aulodrilus pigueti, Narapa bonettoi* and *Aulophorus lodeni* were related with depositional zones, where fine sediments occur. *L. hoffmeisteri* are also registered as being associated to organic enrichment conditions and/or some degree of environmental degradation.

Thus, in order to increase the efficiency of future environmental quality monitoring programs carried out in the region, cautious monitoring of these species in other parts of the Amazon River Basin is advisable.

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Author Contributions

Guilherme Rossi Gorni: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation;

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Nathalie Aparecida de Oliveira Sanches: Contribution to data collection; Contribution to manuscript preparation.

Vanessa Colombo-Corbi: Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Juliano José Corbi: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Conflicts of interest

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

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Collaborative research and the hunting in the Brazilian Cerrado: The case of Xerente Indigenous Land

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Abstract: This paper presents a study of the hunting activities on Xerente Indigenous Land (XIL) in the Brazilian Cerrado. We used data from a Hunting Monitoring Program from eight villages to evaluate the effects of village age, distance from village to town and season (wet and dry) on the composition of hunted species, animals and biomass killed and availability of preferred game-species. We also assessed for any indication of fauna depletion around the villages. Distance from village to town and season were the only variables which affected composition. Preferred species occured more often in villages closer to the town. However, these villages present strong indications of local game-species depletion. The hunters' great capacity for spatial movement can eliminate this effect. Animals and biomass killed was high in the dry season. Game-species composition between seasons can avoid the overexploitation of some sensitive species, such as low-land tapir. These results are important in the design of local management and conservation plans. Our results also contribute to further understanding the impacts of hunting in open Neotropical environments.

Keywords: *game fauna, indigenous, Cerrado, conservation, participatory monitoring.*

Pesquisa Colaborativa e a Caça no Cerrado Brasileiro: O caso da Terra Indígena Xerente

Resumo: Este artigo apresenta um estudo sobre a atividade de caça na Terra Indígena Xerente (TIX), Cerrado brasileiro. Utilizamos dados do Programa de Monitoramento de Caça em oito aldeias para avaliar os efeitos da idade da aldeia, a distância da aldeia para cidade e a estação (úmida e seca) sobre a composição de espécies caçadas, animais e biomassa abatidos e a disponibilidade de espécies preferidas para o consumo. Também avaliamos a indicação de depleção da fauna ao redor das aldeias. Distância da aldeia para a cidade e estação foram as únicas variáveis que afetaram a composição das espécies. Espécies preferidas ocorreram mais frequentemente em aldeias mais próximas à cidade. No entanto, essas aldeias apresentam fortes indicativos de depleção local das espécies. Entretanto, a grande capacidade de locomoção dos caçadores pode eliminar esse efeito. Animais e biomassa abatidos foram maiores durante a estação seca. A composição das espécies cinegéticas entre as estações pode evitar a exploração excessiva de algumas espécies sensíveis, como a anta. Esses resultados são importantes na elaboração de planos locais de manejo e conservação. Nossos resultados também contribuem para o melhor entendimento dos impactos da caça em ambientes neotropicais abertos.

Palavras-chave: *fauna cinegética, indígenas, Cerrado, conservação, monitoramento participativo.*

Introduction

Indigenous people of the Neotropics depend on the exploitation of natural resources for their livelihoods, needing to hunt for their subsistence and maintenance of their social structures (Fragoso et al. 2000, Nasi et al. 2008, Luzar et al. 2012, Shepard 2014, Iwamura et al. 2014). The manner in which this exploitation is performed is related to several factors, such as ecological drivers (e.g., species abundance), physical environment (spatial and climate), logistical apparatus (e.g., effort expended in the hunt), available technology (e.g., firearms use) and cultural constraints (e.g. taboos and food preferences) (Hill et al. 1997, Read et al. 2010, Luzar et al. 2012, Shepard et al. 2012). There is an extensive literature about indigenous hunting which has approached these factors, identifying the impacts on exploited wildlife as well as on the social organization of these societies (e.g. Alvard et al. 1997, Hill et al. 1997, Leeuwenberg & Robinson 1999, Souza-Mazurek et al. 2000, Fragoso et al. 2000, Koster 2008, Constantino et al. 2008, Read et al. 2010, Shepard et al. 2012, Iwamura et al. 2014, Constantino 2015, 2016).

These factors may explain the hunting efficiency and availability of game species. The distance between settlement and kill site is used as an indicator of the abundance of the exploited species (Constantino 2015). If hunters kill most of their prey near to villages, it is assumed that exploited populations are representative and still available (Read et al. 2010, Strong et al. 2010, Constantino 2015), however if hunts occur far from villages, this indicates that exploited animal populations are depleted closer to villages (Hill et al. 1997, Souza-Mazurek et al. 2000, Constantino 2015). The availability of prey may be related to the climate. The Ache hunters and gatherers from Paraguay show an increased rate of finding main game-species during the dry season (Hill et al. 1997).

Village age and size, environmental integrity of the surroundings, hunting pressure history and distance to urban centers, may be relevant drivers in affecting the availability of preferred game species for consumption (Escamilla et al. 2000, Souza-Mazurek et al. 2000, Jerozolimski & Peres 2003, Constantino et al. 2008, Constantino 2016). If there are indications these factors are driving decreases in the availability of game species, this may disturb the social organization of these societies and their interaction with the animals and environment (Milner-Gulland et al. 2003, Shepard et al. 2012, Iwamura et al. 2014).

In a synthetic framework these individual and synergistic factors are important in the understanding of wildlife exploitation by indigenous people. However, previous studies typically focused on forest environments, with few studies conducted in open environments, such as Brazilian Cerrado Savanna. For example, studies with the indigenous Xavante people observed that their hunting activity intensifies during the dry period, and that fire is an important tool in their hunting activities (Leeuwenberg & Robinson, 1999, Fragoso et al. 2000, Welch et al. 2013). Another example comes from a study on the spatial patterns of indigenous hunting communities that occupy savanna and forest environments in Guyana. One of the results found was that the communities from savanna environments travel greater distances in their hunts compared to those from forest environments (Read et al. 2010).

The Xerente indigenous group inhabits the Cerrado biome of northern Brazil, and for the duration of one year took part in hunting participatory monitoring (HMP) (de Paula et al. 2017) in order to build a sustainable program for fauna exploitation. In this study we used the results of collaborative-research to evaluate the impact potential of some anthropogenic and environmental variables on hunted wildlife. In accordance with the literature, we evaluated the effects of village age and size, distance from village to town and season on the composition of hunted species and availability of preferred game-species. We then assessed if there were any indications of fauna depletion around the villages. To determine this, we evaluated if the distance from village to town affects the occurrence of hunting events, and if these hunt events occur further from villages. In addition, we evaluated whether season affected the number and biomass of killed species.

Material and Methods

1. *Study area*

The Xerente Indigenous Land (XIL) (9°34’37,4” S, 43°06’38,3” W) is located in Tocantínia municipality, Tocantins State, Brazil, covering an area of approximately 2,884 km^2 (Figure 1). The Xerente belong to the *Jê* linguistic Family and *Akwẽ* language speakers, comprising the *Jê-Centrais* indigenous group. Their population in 2016 reached 3860 (Fundação Nacional do Índio 2016, unpublished data), currently distributed throughout 62 villages. The region is located within the Cerrado biome, and the XIL presents several phytophysiognomies: dense *cerrado*, typical and thin, rupestrian fields and path (savanna environment), *cerradão* and gallery forest (forest environment) (Ribeiro & Walter 2008). The region’s climate is humid with moderate water deficit, an average annual temperature of 28°C, and average annual precipitation between 1,700 to 1,800 mm (SEPLAN 2008). The pluviometric distribution presents climatic seasonality, alternating between the rainy season (November to April) and the extremely dry season (May to October).

The XIL within proximity to seven towns and is surrounded by a mosaic of anthropogenic landscapes including pasture, monocultures, and hydroelectric infrastructure (see Figure 1). XIL is the main protected area in the region, protecting significant amounts of natural vegetation. However, access to this area is facilitated by unpaved state roads (see Figure 1) connecting towns and serving as an outflow route for the region’s agricultural output.

Data collection

1. *Hunting data*

Hunting data was collected through the HMP carried out in ten villages during a one year period (Mar. 2014 to Feb. 2015) (de Paula et al. 2017*)*. Fifty-two hunters/monitors (91% of all hunters in the 10 monitored villages) contributed to the program, recording data about their own hunting activities on standardized monitoring sheets. For this study, we included data from only eight villages, as we used a base village with 10 or more recorded hunting events. Only species contributing at least 10 individuals or 1% of the killed biomass were included, with the exception of *Tapirus terrestris* (lowland tapir) which represented only nine kills but 35% of total killed biomass. Thus, 11 large and medium-sized mammals and one bird were chosen: *Cuniculus paca* (paca), *Dasyprocta* sp. (agouti), *Dasypus novemcinctus* (nine-banded armadillo), *Hydrochoerus hydrochaeris* (capybara), lowland tapir, *Mazama americana* (red brocket deer), *Mazama gouazoubira* (brown brocket deer), *Nasua nasua* (coati), *Ozotoceros bezoarticus* (pampas

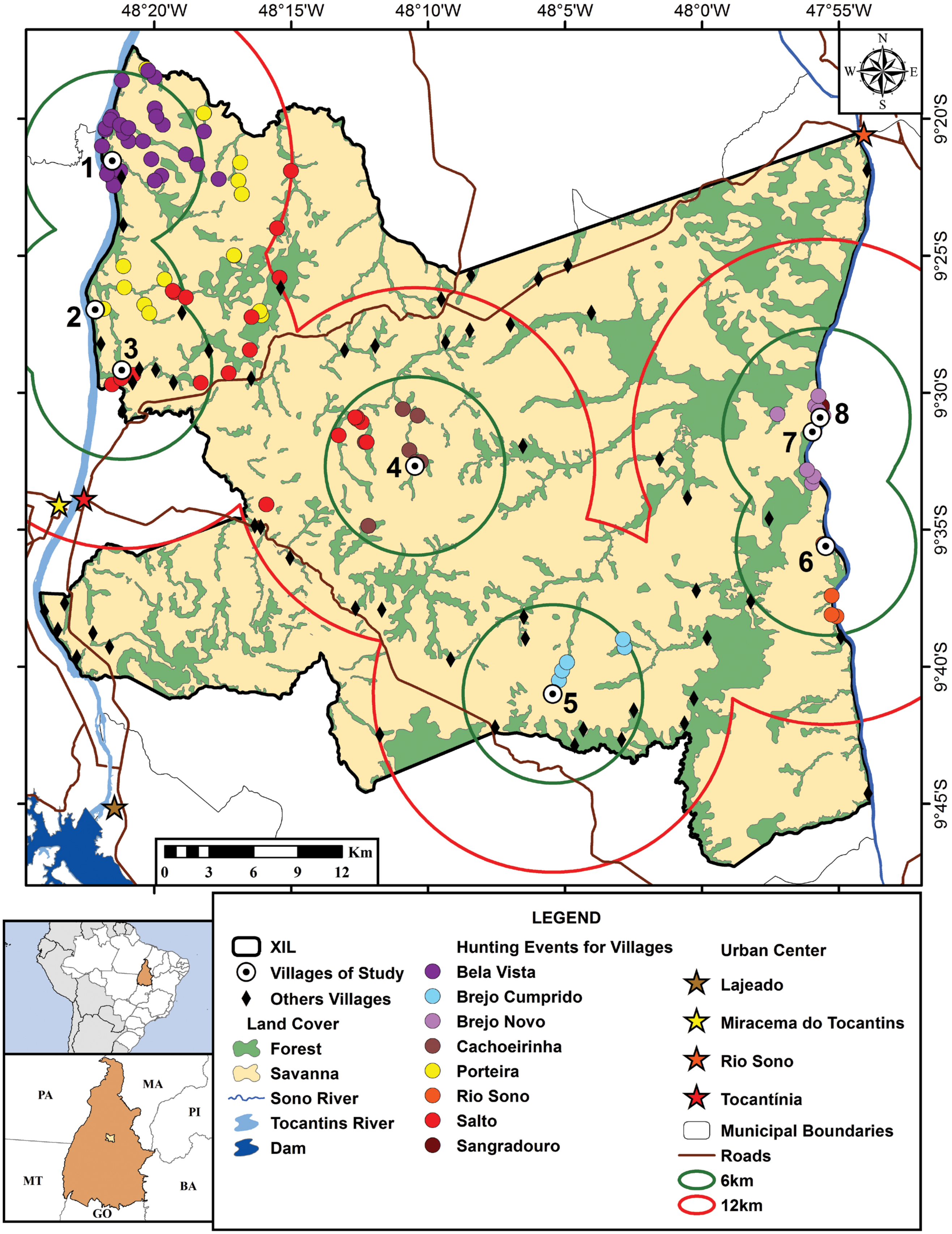

Figure 1. Locality of Xerente Indigenous Land and locations of hunting events. Numbers indicate the villages selected for the study: (1) Bela Vista; (2) Porteira; (3) Salto; (4) Cahoeirinha; (5) Brejo Cumprido; (6) Rio Sono; (7) Sangradouro; (8) Brejo Novo.

deer), *Pecari tajacu* (collared peccary), *Penelope superciliaris* (rusty margined guan) and *Tayassu pecari* (white-lipped peccary). A total of 317 hunting events were included, for a total of 419 dead animals and an estimated 5583.85 kilograms of killed biomass. Opportunistic hunts (see de Paula et al. 2017) were not considered.

2. *Village data*

Distance (km) between villages and the town of Tocantínia were measured with ArcGis 9.3. Distances were classified into the following categories: near: 8-13 Km; intermediate: 18-22 Km; farther: 31-48 Km (Supplementary Material Table S1). This town was chosen because it is the main urban center influencing XIL. The Aage and size (number of families) of each village was collected in field (Supplementary Material Table S1).

3. *Preferred species*

We used semi-structured questionnaires whit hunters and non-hunters (n = 104) to identify preferences based on taste categories: A: high level of preference; B: medium level of preference; C: low level of preference; D: very low level of preference. Species were then classified according to the category cited with the greatest frequency (%). Furthermore, we classified all large prey (≥ 30 kg) as highly preferred, based on the principle that hunters always shoot this size class due to the high rate of meat return (Alvard 1993, Jerozolimski & Peres 2003).

4. *Hunting event locations*

Monitors recorded the locations of 135 (42.5%) hunting events. These locations were plotted in a Landsat 8 satellite with orbit 222 and point 67 (USGS 2014) of A3 size and 1: 115 000 scale through participatory mapping (Read et al. 2010, Luzar et al. 2011, Constantino 2015). To facilitate the hunter's orientation, we utilized a GPS device to georeference several strategic locations (hunting area, villages, roads, and bridges), which were mapped and edited in ArcGis 9.3.2 software. The distance (km) of each hunting event was measured by straight line from the point of hunt event to the central point of the hunter's village.

5. *Wet and dry season*

We used rainfall and average temperature data from nearby (c. 100 km) weather stations to assess seasonality of the study area (INMET 2015). The dry season comprised some months with water deficit (Thornthwaite & Mather 1955). Therefore, we calculated of the water balance for the entire period using formulas according to Rolim et al. (1998).

The period which presented water deficit comprised the months of May to October 2014 (Supplementary Material Figure S1), with March and April 2014 classified as a dry period. There was no water deficit from November 2014 to February 2015 (Supplementary Material Figure S1), therefore was classified as the rainy season.

Data Analysis

We used a Redundancy analysis (RDA) to evaluate the effects of the explanatory variables village age and size, distance of village to town and season on the composition and occurrence of hunted species. First, we observed and removed the collinearity among explanatory variables. It was considered collinearity when two variables had Pearson correlation above 0.70. Only the distance of village to town and village size were correlated, and we maintain in the analysis only the distance of village for RDA. We used a logistic regression model to estimate the probability of hunting events related to distance. We consider the distance of 12 km a threshold of fauna depletion, indicating a long distance to hunt events. The distance of 12 km is in accordance with Read et al. (2010) in a study of the savannah environment in Guyana, as there are not yet any spatial parameters for hunting activities in the Brazilian Cerrado. Finally, we used a non-parametric Wilcoxon test to evaluate the effects of season on the number and biomass (kg) of hunted animals. All analyses were carried out using R statistical software (R Development Core Team 2014).

Results

1. *Preferred species*

The most preferred species were paca and nine-banded armadillo (Table 1). Within the assemblyrange of hunted species, capybara, lowland tapir, red brocket deer and white-lipped pecary were also considered preferred sepecies due to their larger biomass (Table 1).

Table 1. Food preference of Xerente hunters. For the scientific names of species see Methods section. Xerente Indegenous Land. Tocantins, Brazil – Mar. 2014 to Feb. 2015.

Species	Number of records for each category of food preference (%)			
	A	B	C	D
Agouti	-	2	8	18
Brazilian tapir*¥	-	16	30	8
Brown brocket deer	-	4	4	14
Capybara*#	-	-	2	6
Nine-banded armadillo	6	34	2	10
Paca	64	12	12	2
Pampas deer*	4	12	22	10
Red brocket deer*#	4	12	22	10
Collared peccary	-	-	-	16
White-lipped peccary*¥	10	12	2	12

* Species with large body mass (> 30 kg); # Average weight of individuals estimated in Participatory Monitoring of Hunting in Xerente Indigenous Land; ¥ Average body mass according Peres & Palacios (2007).

2. *Game composition*

Distance from village to town and season affected the composition of hunted species (Table 2). Preferred species were killed more frequently by hunters from villages near (8-13 km) to town, with capybara the only exception (Figure 2). During the dry season the predominant hunting events involved red brocket deer, paca, agouti, collared peccary and capybara. The lowland tapir was killed exclusively in this period (Figure 3). Hunting of the remaining species was almost evenly distributed between the two seasons.

Table 2. Results of Redundancy Analysis (RDA) for distance of villages to town and season (rainy and dry) in relation to game-species composition. Xerente Indigenous Land. Tocantins, Brazil – Mar. 2014 to Feb. 2015.

Source of variation	DF	SS	F	R^2	*P*
Distance	1	21,33	4,484	0,22	0,004
Season	1	13,788	2,898	0,14	0,033
Residue	13	61,845	-	-	-

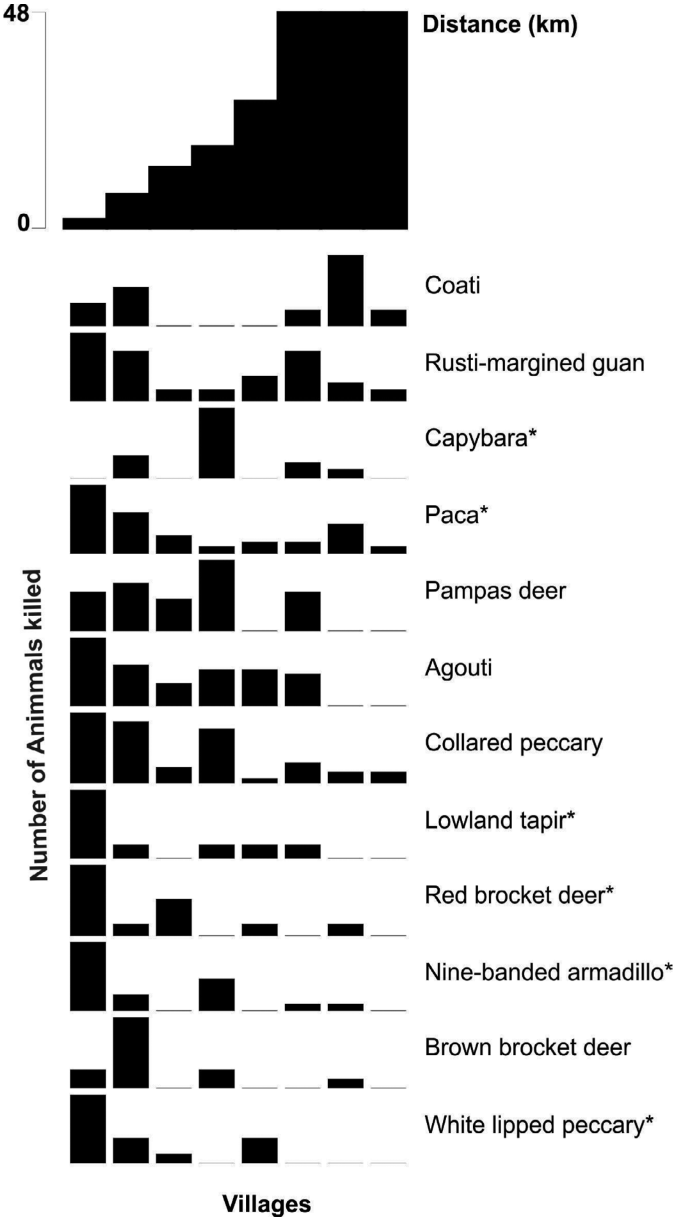

Figure 2. Relationship between the composition of game-species and distance of villages to town. *Preferred species. For the scientific names of species see Methods section.

3. *Distance of hunting events*

The probability of hunting events occurring father from villages (> 12 km) increased in villages closer to town (p < 0.001, Fig. 4). Salto and Porteira villages, which are near to town and are also the most populous (Supplementary Material Table S1), present the highest probability of hunting events occurring farther than 12 km, giving a strong indication of game-species depletion close to these villages. In these villages, 58% (n = 33) of the georeferenced events were in areas greater in distance than 12 km (Figure 1), with the greatest distance occurring 17.55 km from Salto village. For the remaining villages, georeferenced events (n = 75) were undertaken within 12 km, with 93% (n = 70) occurring from 0 to 6 km (Figure 1).

4. *Number and biomass killed*

Most hunting events occurred during the dry season (67%, n = 212). Season affected the number of killed animals (z = 2.52; p = 0.011) and biomass (z = 2.24; p = 0.025). Hunting events were more frequent in the dry season (35 ±28.67, n = 280), compared with the rainy season (17.37 ±15.61, n = 139) (Supplementary Fig. S2). Biomass was also greater in the dry season, with an average of 511.47 kg ±572.74 kg (n = 4,091.8 kg), more than twice that of the rainy period average of 186.5 kg ±172.01 kg (n = 1,492.05 kg) (Supplementary Material Figure S2).

Discussion

The difference in the composition of hunted species in relation to distance from village to town, with the highest availability of preferred species in villages nearest to town, may be an effect of the greater number of hunting events held by these villages (Supplementary Material Table S1), as non-preferred and smaller sized species were also more available. They are most populous and have more hunters, and consequently hold more hunting events. However, these villages present strong indications of game-species depletion within their proximity. Fauna depletion close to villages/settlements is indicative of low availability or absence of preferred species in hunted species assembly (Jerozolimski & Peres 2003, Constantino et al. 2008, Read et al. 2010, Parry & Peres 2015, Constantino, 2015, 2016). However, our results showed the opposite of these scenarios.

Currently, the Xerente hunters have great mobile capacity, through the use of bicycles, motorcycles or cars/tractors/trucks (motorcycles are predominant). This allows hunting to be conducted in remote areas (> 40 km, M. J. de Paula, personal observation), which otherwise could not be accessed by walking in a hunt where the individual returns home the same or next day. The use of these vehicles allows access to more suitable areas with higher concentrations of game-species (Read et al. 2010), and is facilitated by an extensive network of vicinal roads (most are state roads), thus eliminating the depletion effect of species close to the villages (Souza-Mazurek et al. 2000). However, this facility of locomotion may increase the depletion of an area (Benítez-López et al. 2017). One aggravating factor is illegal hunting by hunters that live around the XIL. This creates an undetectable impact which can place extensive pressure on fauna (Benítez-López et al. 2017).

As the number of killed animals and biomass was greater during the dry season, this indicates an intensification of hunting activities during this season. This increase in number of events is due to many Xerente hunters showing a preference for employing the waiting technique in this period (de Paula et al. 2017); called by many the “waiting period”. This happens because there is fruit drop, mainly of “mirimdiba” (*Buchenavia tomentosa* Eichler), whose fruits attract species such as paca and the nine-banded armadillo, in addition to large bodied species such as lowland tapir and red brocket deer. As red brocket deer, capybara, and

Figure 3. Relationship between the composition of game-species and the wet and dry season. For the scientific names of species see Methods section.

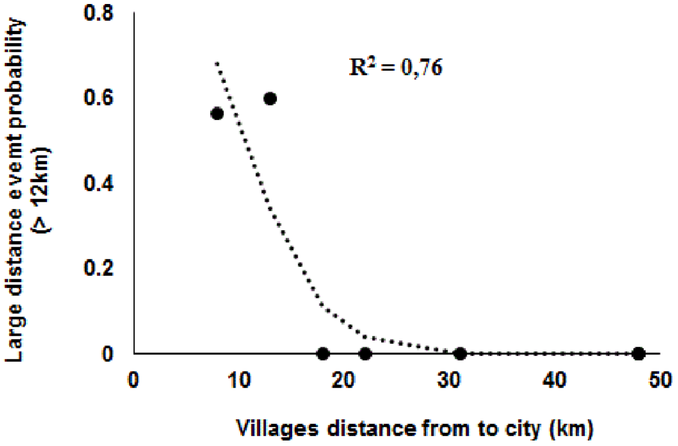

Figure 4. Effect of distance of village to town on the probability of hunting events occurring more than 12 km from the villages.

lowland tapir deaths are concentrated in the dry season, this helps to explain the notable difference in biomass averages between the two seasons, as the added biomass of these species represents 39% (n = 2171.5 kg) of the total killed biomass. The increase in hunting effort during the dry season was also observed in the Xavante (Leeuwenberg & Robinson 1999, Fragoso et al. 2000, Welch et al. 2013), who present great cultural affinities with the Xerente (Maybury-Lewis 1965) and also inhabit the Cerrado biome. However, this increase in hunting effort in the case of the Xavante is related to the use of fire in hunting, due to favorable conditions during this period (e.g. absence of rain).

The difference in game-species composition between seasons is reflective of the strategy to concentrate hunting activities in the dry season, which seems to be influenced by the phenological cycles of some plants. Even with strong indications of fauna depletion close to some villages, and the high mobility of the Xerente possibly increasing the depletion of an area, hunting pressure was different over time. The dynamics of natural phenomena such as season and flood pulse can have a direct effect on the exploitative pattern of fauna (Endo et al. 2016). The remarkable seasonality of the study area affected hunting activities and indicates an informal management practice that is seasonally regulated for some species, such as capybara, lowland tapir, paca and red brocket deer. This may contribute to the conservation of these species, and is more important in the case of the lowland tapir and red brocket deer, as these species are considered more sensitive to hunting and tend to be the first to suffer depletions due to overexploitation (Bodmer et al. 1997, Peres & Palacios 2007). Additionally, the impact of undetected hunting is a threat to informal management, and intensive law enforcement is necessary to decrease illegal hunting in XIL.

Today, the Xerente are not exclusively dependent on wild meat as many have jobs, receive scholarships or "Bolsa Família" from the government, and have relatively good access to cattle and chicken meat and others protein sources such as fishing. The uptake of occupations (jobs or studies) limits hunting actives (M.J. de Paula, personal observation), as the "Tupinambá de Olivença" indigenous (Pereira & Schiavetti 2010), and decreases the pressure on game-species. Hunting is an essential concept in the word-view and social organization of indigenous people (Shepard 2014). Even though economic factors are influencing a decrease in hunting, this activity still plays an essential role in maintaining the traditional culture of the Xerente people.

Our analysis shows that villages near the town present strong indications of game-species depletion close to these villages. However, the high mobility of hunters can eliminate this effect. This can explain the proportion of preferred game-species in these villages. Differences in game-species composition and hunting pressure between seasons can avoid the overexploitation of some sensitive species, such as low-land tapir. We argue that local management and conservation plans must take into consideration these issues beyond the cultural aspect involved in hunting. Enforcement of the law is also necessary to reduce the threat of illegal hunting, often present in XIL. The Xerente have access to other protein sources, however the practice of hunting is essential in maintaining their traditional culture. Finally, this study is also an attempt to better understand the wildlife exploitation mechanisms of the Cerrado biome, a priority area for conservation of global biodiversity (Myers et al. 2000), but as yet are not generally investigated in this aspect.

Supplementary material

The following online material is available for this article:

Table S1 - Villages of the study area with the respective numbers of age, distance to town, families, hunters and hunting events.

Figure S1 - Water balance of Xerente Indigenous Land for the study period (Mar. 2014 to Feb. 2015).

Figure S2 - Box plot between the mean number of animals killed and biomass killed in relation to the wet and dry season. A: animals killed; B: biomass killed.

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Author Contributions

Milton José de Paula: The main responsible for the elaboration and execution of research and main responsible for manuscript elaboration.

Valcir Sumekwa Xerente: Contributed to the data collection and discussion, mainly in relation to the cultural aspects of the Xerente.

Aion Angelu Ferraz Silva: Contributed to the analyzes space of hunting events and discussion.

Bruno Spacek Godoy: Contributed to the statistical analyzes and elaboration of the text.

Juarez Carlos Brito Pezzuti: Is the general advisor and supervised all research.

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

Bats of the Serra da Bocaina National Park, southeastern Brazil: an updated species list and a distribution extension for *Trinycteris nicefori* (Sanborn, 1949)

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Abstract: The Serra da Bocaina National Park (SBNP) is a large remnant of Atlantic Forest located within an endangered biodiversity hotspot, which contributes to the connectivity among protected areas in the region. Despite the ecological importance of the SBNP, its bat fauna is poorly-known, and no comprehensive inventory is available. The present study provides an updated list of the bat species found in the SBNP. The four-year study was based on a capture-mark-recapture approach at four sites within the park, in the municipality of Paraty, Rio de Janeiro state, Brazil. A total of 22 bat species were recorded, representing two families, Phyllostomidae (n = 19 species) and Vespertillionidae (n = 3). This added 14 species to the known bat fauna of the SBNP, which is hereby updated to 24 species, including *Dermanura cinerea* Gervais, 1856, *Platyrrhinus recifinus* (Thomas, 1901), and *Myotis ruber* (É. Geoffroy, 1806), which are listed as endangered in Rio de Janeiro state. The specimen of *Trinycteris nicefori* (Sanborn, 1949) captured in the present study is the first record of the occurrence of this species in Rio de Janeiro state. This record extends the geographic range of the species by approximately 650 km to the southeast. Additional inventories, in particular at poorly-studied Atlantic Forest sites, combined with environmental suitability analyses, and taxonomic and biogeographic data, are urgently required to elucidate the distribution of many Brazilian bat species, such as *T. nicefori*.
Keywords: *Atlantic Forest, Chiroptera, faunistic inventory, species richness.*

Morcegos do Parque Nacional da Serra da Bocaina, sudeste do Brasil: lista de espécies atualizada e expansão de distribuição para *Trinycteris nicefori* (Sanborn, 1949)

Resumo: O Parque Nacional da Serra da Bocaina (PNSB) é um grande remanescente de Mata Atlântica localizado em um *hotspot* de biodiversidade ameaçado, que contribui para a conectividade entre áreas protegidas na região. Apesar da importância ecológica do PNSB, a sua quiropterofauna é pobremente conhecida e nenhum inventário abrangente está disponível. O presente estudo fornece uma lista atualizada das espécies de morcegos do PNSB. O estudo de quatro anos foi baseado em uma abordagem de captura-marcação-e-recaptura em quatro localidades dentro do parque, no município de Paraty, estado do Rio de Janeiro, Brasil. Um total de 22 espécies de morcegos foi registrado, representando duas famílias, Phyllostomidae (n = 19 espécies) e Vespertillionidae (n = 3). Isso adiciona 14 espécies à quiropterofauna conhecida do PNSB, que é atualizada para 24 espécies, incluindo *Dermanura cinerea* Gervais, 1856, *Platyrrhinus recifinus* (Thomas, 1901) e *Myotis ruber* (É. Geoffroy, 1806), que são listadas como ameaçadas de extinção no estado do Rio de Janeiro. O espécime de *Trinycteris nicefori* (Sanborn, 1949) capturado no presente estudo representa o primeiro registro da espécie no estado do Rio de Janeiro. Esse registro expande a distribuição geográfica da espécie aproximadamente 650 km a sudeste. Inventários adicionais, particularmente em localidades de Mata Atlântica pobremente estudadas, combinados com análises de adequação, e estudos taxonômicos e biogeográficos, são urgentemente necessários para esclarecer a distribuição de muitas espécies de morcegos brasileiras, como *T. nicefori*.
Palavras-chave: *Chiroptera, inventário faunístico, Mata Atlântica, riqueza de espécies.*

Introduction

Bats play fundamentally important functional roles in ecosystems, including seed dispersal and the pollination of an enormous variety of plant species (e.g., Bolívar-Cimé et al. 2017). In this context, frugivorous bats may make a major contribute to the succession and regeneration of tropical forests (Muscarella & Fleming 2007). Bats also provide humans with a number of ecosystem services. Insectivorous bats may help to limit the populations of arthropods in agricultural landscapes, for example, and frugivores and nectarivores may contribute to the dispersal and reproduction of many crops (e.g., Williams-Guillén et al. 2016).

With *ca.* 180 species (Reis et al. 2017), Brazil has one of the most diverse bat faunas of any country in the world (Bernard et al. 2011). Most of these species (66.7%) occur in the Atlantic Forest biome (Graipel et al. 2017). In this biome, seasonal variation in temperature appears to be the principal factor influencing the distribution of bats species (Stevens 2013). Local species richness varies considerably along the latitudinal gradient of the Atlantic Forest, ranging from nine species in a seasonal forest in Rio Grande do Sul state (Weber et al. 2011) to 40 species in a regenerated forest in Rio de Janeiro state (Esbérard 2003).

The Serra da Bocaina National Park (SBNP) is located in southeastern Brazil, and encompasses approximately 104,000 ha of Atlantic Forest (http://www.icmbio.gov.br/parnaserradabocaina/). This large remnant of Atlantic Forest is part of the "Bocaina Mosaic", which contributes to the connectivity among the region's protected areas (http://www.icmbio.gov.br/portal/mosaicosecorredoresecologicos/moscaicos-reconhecidos-oficialmente/1869-mosaico-bocaina). Despite being located in an endangered biome recognized as a biodiversity hotspot (Galindo-Leal & Câmara 2003), the bat fauna of the SBNP is poorly-known, as no comprehensive inventory is available, although Delciellos et al. (2012) did record 10 species in the park, including the endangered *Lonchorhina aurita* Tomes, 1863, in a snapshot survey of its southern extremity. This is likely an underestimate of the bat species richness of the SBNP, given the enormous diversity of habitat types found along the park's altitudinal gradient (http://www.icmbio.gov.br/parnaserradabocaina/).

Data on the abundance, occurrence, and geographic distribution of species are of fundamental importance for the understanding of macroecological patterns (Rahbek 2005), the assessment the conservation status of species (Jenkins et al. 2015) and the management of conservation units (e.g., Silva et al. 2018). Six years on from Delciellos et al.'s (2012) survey of the mammalian fauna of the SBNP, which recorded 48 species belonging to nine orders, no additional studies of the park's mammals appear to have been published. During the four-year study presented here, an additional 14 bat species were recorded in the park, updating the total to 24 species, including *Trinycteris nicefori* (Sanborn, 1949), which was recorded in the Brazilian state of Rio de Janeiro for the first time.

Material and Methods

1. Study area

Twelve capture-mark-recapture sampling sessions were conducted between June 2013 and December 2016 at four sites distributed along the RJ-165 state highway, which traverses the SBNP in the municipality of Paraty, Rio de Janeiro state, Brazil (Figure 1). Site 1 (23°12′19″ S, 44°50′17″ W, Datum WGS84; 1193 m a.s.l.; Figure 1) is on an old hunting trail with secondary vegetation at an advanced stage of succession, including some *Pinus* trees and vegetation of low stature characteristic of flooded areas, adjacent to a small perennial watercourse. Site 2 (23°11′39″ S, 44°50′27″ W, Datum WGS84; 1122 m a.s.l.; Figure 1) is also located on an old trail, *ca.* 2 m wide, which starts at the margin of the RJ-165 highway. This trail is also surrounded by secondary vegetation at an advanced stage of succession, with a closed canopy. As at site 1, there is a small perennial watercourse. Site 3 (23°11′06″ S, 44°49′47″ W, Datum WGS84; 800 m a.s.l; Figure 1) also has secondary vegetation at an advanced stage of succession, with a much larger perennial watercourse, steep terrain, several rocky outcrops, and a small plantation of banana (Musaceae: *Musa paradisiaca* L.) located next to a house. Site 4 (23°11′28″ S, 44°50′39″ W, Datum WGS84; 1050 m a.s.l.; Figure 1) is located *ca.* 800 from site 2 on the same trail, which terminates at a river. The samples were collected on the trail near this river, which is surrounded by secondary vegetation at an advanced stage of succession, with an open canopy. The region's climate has two seasons, a super-humid rainy season between October and April, and a drier season from May to September, but with no months of water deficit (https://pt.climate-data.org/). The sampling sessions were divided equally between the two seasons, with six sessions being conducted during the rainy season and six in the dry season. The mean annual temperature in the region is 23.3ºC and mean annual precipitation 2284 mm (https://pt.climate-data.org/). The vegetation is classified as dense montane rainforest (IBGE 2012).

2. Bat sampling

Bats were sampled using mist-nets on one to three nights at each site during each sampling session. The sampling effort varied due to climatic conditions, with no mist-netting taking place under heavy rainfall. On each night of sampling, eight mist-nets (9.0 m x 2.5 m, with a 33 mm mesh) were set at ground level along trails and clearings within the forested habitat. The mist-nets remained open during the first six hours of the night, after sunset. Capture effort was calculated following Straube & Bianconi (2002). Trapping and handling conformed to the guidelines of the American Society of Mammalogists (Sikes & A.C.A.U.C. of the A. S. of Mammalogists 2016). This study was part of the Mammal Monitoring Program of the RJ-165 highway construction project (IBAMA/MMA process no. 02001.003937/2008-18, authorization numbers 248/2013 and 610/2015).

The specimens captured were identified from their external characters, such as length of the forearm, coloration pattern, the morphology of the ears, tragus and patagium, and dental traits, following field guides and identification keys (Simmons & Voss 1998, Gardner 2007, Reis et al. 2013). The sex and reproductive condition of all captured specimens were verified, and they were weighed (in grams) using a spring balance, measured (heady-body, tail and forearm lengths) with a digital calliper (0.01 mm precision), and marked with a numbered collar at first capture. The specimens that could not be identified in the field or died in mist-nets were retrieved, prepared, and conserved in 70° alcohol. The skull was extracted through the mouth opening and the specimens were deposited in the mammal collection of the National Museum (MN) at the Federal University of Rio de Janeiro (UFRJ). These specimens were identified through the detailed analysis of the external and cranial measurements, and the qualitative evaluation of

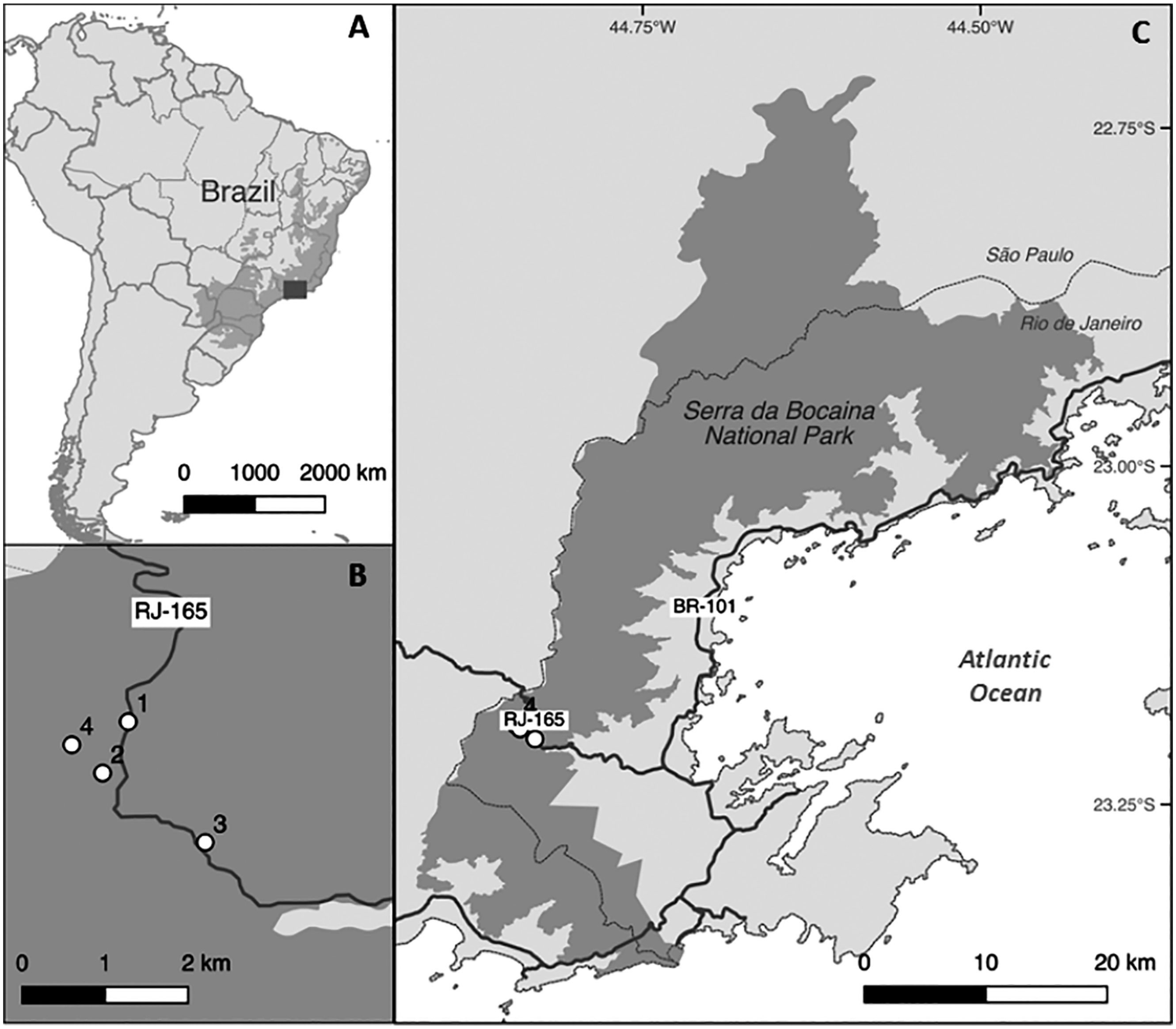

Figure 1. (A) South America, showing the Atlantic Forest in dark gray, with the study area in Brazil being indicated by the square; (B) The four study sites (circles) distributed along the RJ-165 state highway, which traverses the Serra da Bocaina National Park (C) in the municipality of Paraty, Rio de Janeiro state, Brazil.

diagnostic traits. Nomenclature followed Nogueira et al. (2014). Species richness was estimated using Chao2, an incidence-based nonparametric estimator (Colwell & Coddington 1994), using EstimateS 9.1 software (Colwell 2013).

In the specific case of the *Trinycteris nicefori* specimen, 22 cranial and external measurements were obtained, as in Vizotto & Taddei (1973), and compared with values found for the species in the literature (Sanborn 1949, Simmons & Voss 1998, Williams & Genoways 2007, Rocha et al. 2013). The geographic distribution of *T. nicefori* was defined as in the review of Rocha et al. (2013). Two new localities for *T. nicefori* have been published since Rocha et al. (2013), one in the Carboneras Reserve in the municipality of Livingston, Guatemala (Pérez et al. 2012) and the other from the Teles Pires River, in the municipalities of Nova Canaã do Norte and Itaúba, in the state of Mato Grosso, Brazil (Miranda et al. 2015). As this species has an ample geographic range in the Americas, but a disjunct distribution in Brazil, where it is found separately in the Amazon region and the northern half of the Atlantic Forest biome, on the eastern Brazilian coast (see Williams & Genoways 2007, Perez et al. 2012, Rocha et al. 2013, Miranda et al. 2015), the distribution map presented here focuses only on the Atlantic Forest, in which the present study was conducted.

Results

Over the four years of the study period, the total sampling effort was 129,600 m^2.h (Site 1 = 28,080 m^2.h; Site 2 = 33,480 m^2.h; Site 3 = 35,640 m^2.h; Site 4 = 32, 400 m^2.h), resulting in 557 captures of 505 different individuals (Table 1). These individuals represented 22 bat species belonging to two families, the Phyllostomidae (n = 19 species) and Vespertillionidae, n = 3 (Table 1; Figures 2 and 3). Three of the species are endangered in Rio de Janeiro state, based on the classification of Bergallo et al. (2000). Total abundance per site ranged from 103 to

Table 1. Bat species captured in the Serra da Bocaina National Park (SBNP) in Rio de Janeiro, Brazil. Abundance per site and total abundance of each species. Previous records in the SBNP obtained from Delciellos et al. (2012). Number of voucher specimens deposited at the mammal collection of the National Museum (MN) at the Federal University of Rio de Janeiro (UFRJ). † Endangered with extinction at national (ICMBio 2016) or state (Bergallo et al. 2000) level.

Species	**Site**				**Total abundance**	**Previous record**	**Voucher specimens**
	1	**2**	**3**	**4**			
FAMILY PHYLLOSTOMIDAE							
Subfamily Micronycterinae							
Micronycteris minuta (Gervais, 1856)	0	0	1	0	1		MN81509
Subfamily Desmodontinae							
Desmodus rotundus (É. Geoffroy, 1810)	0	1	0	4	5		MN81501
Diphylla ecaudata Spix, 1823	0	1	3	0	4		MN81506
Subfamily Lonchorhininae							
†*Lonchorhina aurita* Tomes, 1863	0	0	0	1	1	X	MN78128, MN78131
Subfamily Phyllostominae							
Chrotopterus auritus (Peters, 1856)	0	0	0	0	0	X	MN78127
Tonatia bidens (Spix, 1823)	0	0	0	0	0	X	MN77799
Subfamily Glossophaginae							
Anoura caudifer (É. Geoffroy, 1818)	2	1	3	3	9	X	MN78129
Anoura geoffroyi Gray, 1838	0	2	17	3	22	X	MN78132
Subfamily Carolliinae							
Carollia perspicillata (Linnaeus, 1758)	33	19	65	30	147	X	MN781130, MN78133, MN81515
Subfamily “Glyphonycterinae”							
Trinycteris nicefori (Sanborn, 1949)	0	0	1	0	1		MN81510
Subfamily Stenodermatinae							
Artibeus fimbriatus Gray, 1838	10	8	51	12	81	X	MN77796, MN81516, MN81517
Artibeus lituratus (Olfers, 1818)	9	1	3	3	16		MN81504, MN81511
Artibeus obscurus (Schinz, 1821)	0	0	3	1	4	X	MN77797
Artibeus planirostris (Spix, 1823)	0	1	0	0	1		
†*Dermanura cinerea* Gervais, 1856	0	0	1	2	3		MN81502
Platyrrhinus lineatus (E. Geoffroy, 1810)	0	0	1	0	1		
†*Platyrrhinus recifinus* (Thomas, 1901)	0	0	2	1	3		MN81507
Pygoderma bilabiatum (Wagner, 1843)	1	4		1	6		MN81518
Sturnira lilium (É. Geoffroy, 1810)	42	36	28	28	134	X	MN78134, MN78135
Sturnira tildae de la Torre, 1959	9	11	4	12	36	X	MN77799
Vampyressa pusilla (Wagner, 1843)	0	0	4	1	5		MN81505
FAMILY VESPERTILLIONIDAE							
Subfamily Myotinae							
Myotis nigricans (Schinz, 1821)	0	1	0	0	1		MN81513
Myotis riparius Handley, 1960	0	2	0	0	2		MN81508, MN81512
†*Myotis ruber* (É. Geoffroy, 1806)	0	16	5	1	22		MN81500
Total abundance	106	104	192	103	505		
Total richness	7	14	16	15	22		

Figure 2. Bat species recorded in the Serra da Bocaina National Park, in the municipality of Paraty, Rio de Janeiro state, Brazil. a) *Anoura caudifer*; b) *Anoura geoffroyi*; c) *Artibeus fimbriatus*; d) *Artibeus lituratus*; e) *Artibeus obscurus*; f) *Artibeus planirostris*; g) *Carollia perspicillata*; h) *Dermanura cinerea*; i) *Desmodus rotundus*; j) *Diphylla ecaudata*; k) *Lonchorhina aurita*; l) *Micronycteris minuta*. Photographs: Adarene Motta.

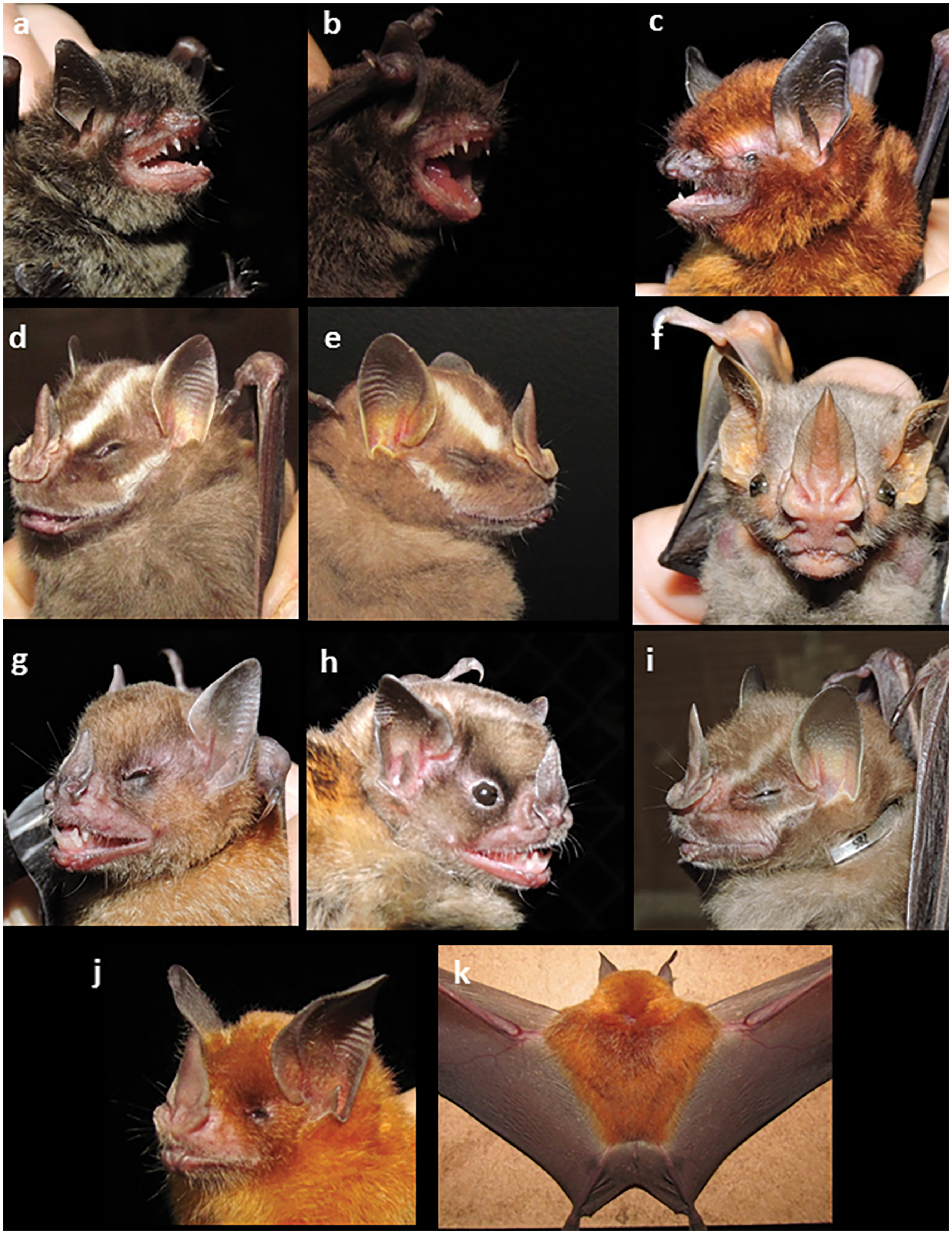

Figure 3. Bat species recorded in the Serra da Bocaina National Park, in the municipality of Paraty, Rio de Janeiro state, Brazil. a) *Myotis nigricans*; b) *Myotis riparius*; c) *Myotis ruber*; d) *Platyrrhinus lineatus*; e) *Platyrrhinus recifinus*; f) *Pygoderma bilabiatum*; g) *Sturnira lilium*; h) *Sturnira tildae*; i) *Vampyressa pusilla*; j) *Trinycteris nicefori*; k) *Trinycteris nicefori* (dorsal view). Photographs: Adarene Motta.

192 individuals captured, while between seven and 16 species were captured at each site (Table 1), with abundance and species richness being highest at site 3. *Carollia perspicillata* (Linnaeus, 1758) and *Sturnira lilium* (É. Geoffroy, 1910) were the most abundant species (Table 1). The species accumulation curve (observed richness) and the species richness estimated by Chao 2 both reached the asymptotes at the sixth trapping session (Figure 4).

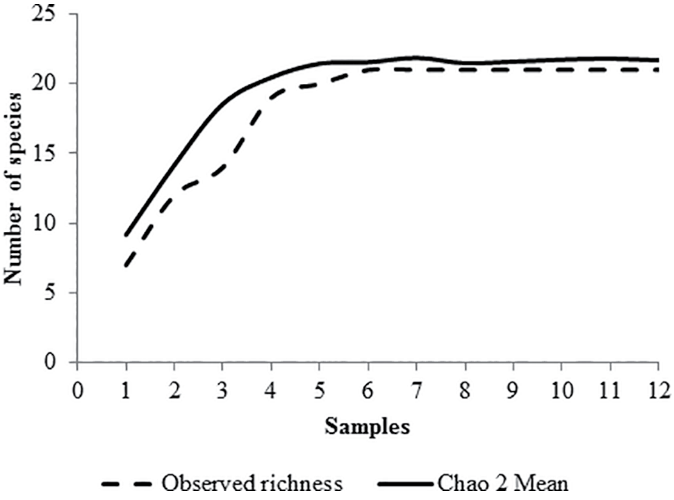

Figure 4. Species accumulation curve and estimated species richness based on the Chao 2 index for the bat species recorded in the Serra da Bocaina National Park in Rio de Janeiro, Brazil. Samples: 1 – June 2013, 2 – September 2013, 3 – December 2013, 4 – April 2014, 5 – June 2014, 6 – September 2014, 7 – December 2014, 8 – September 2015, 9 – November 2015, 10 – January 2016, 11 – July 2016, 12 – November 2016.

An adult male *Trinycteris nicefori* with scrotal testes (MN 81510) was captured at 18:30 h on April 12 2014, at site 3. This record extends the known geographic range of the species approximately 650 km to the southeast, and represents the first record of *T. nicefori* in the Brazilian state of Rio de Janeiro (Figure 5). The pelage of the *T. nicefori* specimen was reddish brown or in a "red phase", with four bands in dorsal pelage, a first (basal) band which was pale, narrow, and inconspicuous, a second band dark brown, a third band, broad and light reddish, and a fourth band (hair tip) darker brown. The ventral pelage was slightly paler and tricolored. No dorsal stripe was observed. Externally the specimen lacked an interauricular band, the ears were pointed with broadly concave outer margins; the noseleaf had a narrow, pointed tip and its lower margin was smoothly confluent with the upper lip; the chin had a pair of smooth tubercles divided by a median groove; the third metacarpal was longer than the other metacarpals (IV<V<III); the second phalanx of wing digits III and IV were longer than the first phalanx of the same digits; the calcar markedly shorter than the foot (less than half the length of the foot with claws). In the cranium, the specimen had the rostrum not inflated; deep basisphenoid pits separated by a high and narrow septum; zygomatic breadth larger than that of the mastoid; inner upper incisors not chisel-shaped; lower incisors trifid; and both P3 and p3 reduced in size with crown almost flat and small anterior cusp (Table 2; Figure 6). The specimen also had a small, supernumerary central lower incisor, totalling five lower incisors and 35 teeth (Figure 6).

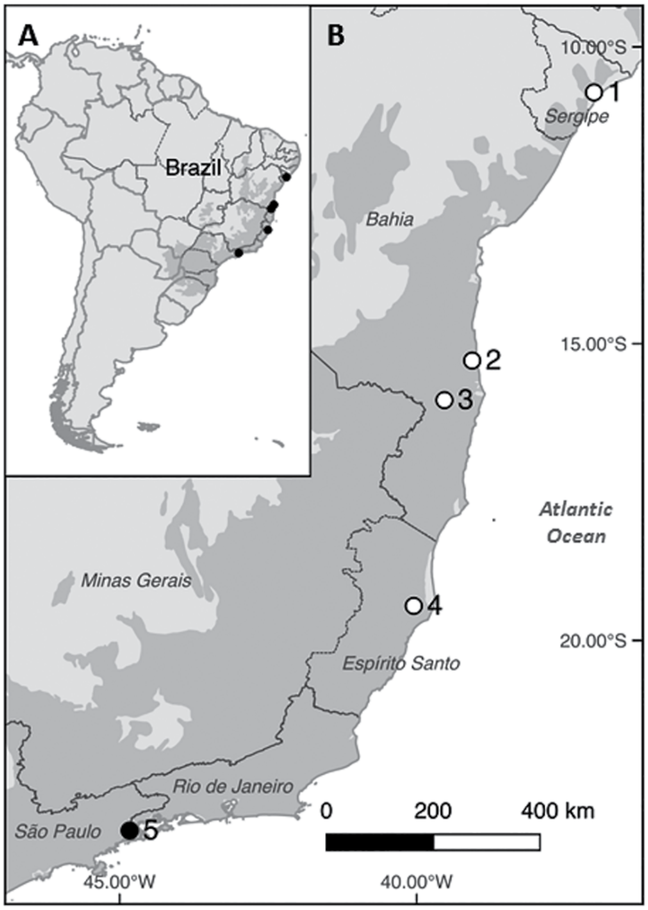

Figure 5. (A) South America, showing the Atlantic Forest in dark gray. (B) The portion of the geographical distribution of *Trinycteris nicefori* (Chiroptera, Phyllostomidae) located in the Brazilian Atlantic Forest. White circles: 1 = municipality of Capela, state of Sergipe (Brito & Bocchiglieri 2012); 2 = municipality of Una, Bahia (Faria et al. 2006); 3 = municipality of Itapebi, Bahia (Faria et al. 2006); 4 = municipality of Linhares, Espírito Santo (Peracchi & Albuquerque 1993). Black circle: 5 = Serra da Bocaina National Park, municipality of Paraty, Rio de Janeiro (present study). See Appendix 1 for details of the localities. For a review of the species' current distribution in the Americas, see Pérez et al. (2012), Rocha et al. (2013), and Miranda et al. (2015).

Discussion

The 24 bat species now known to occur in the SBNP correspond to 20.7% of the Atlantic Forest species that may potentially occur in the region (Graipel et al. 2017). As both the species accumulation curve and the estimated richness reached their asymptotes by the middle of the present study, however, additional species are unlikely to be recorded in the study area. Species richness was highest at site 3, which was the lowest in altitude, and had a small banana plantation. Banana is a resource rich in sugar (fruit) or nectar (flowers), and its availability can influence the presence and abundance of certain bat species, and consequently, their capture rates (Luz et al. 2015).

The bat species richness recorded in the SBNP in the present study (n = 22 species; sampling effort [SE] = 129,600 m^2.h) is consistent with the review of Costa (2014), who found a mean number of 20.84 bat species in the conservation units of the state of Rio de Janeiro. Specifically, the number of species recorded here in the SBNP was

Table 2. Selected measurements of the *Trinycteris nicefori* specimen (MN81510) captured in the Serra da Bocaina National Park in Rio de Janeiro, Brazil, and comparisons with the specimens collected at other localities within its geographic range. All measurements are given in millimeters (mm), except for body mass, given in grams (g). * Holotype.

	Present study	**Sanborn (1949)***	**Rocha et al. (2013)**	**Simmons & Voss (1998)**	**Genoways & Williams (1986)**
Locality	Serra da Bocaina, Paraty, RJ, Brazil	Cúcuta, Colômbia	Porto Velho, Rondônia State, Brazil	Paracou, French Guyana	Various localities
N/Sex	♂	♂	2♂, 2 ♀	3♂, 2 ♀	5♂, 4 ♀
Forearm length	37.00	37.90	–	36.00–41.00	35.30–38.60
Third metacarpal	34.50	35.70	–	–	–
First phalanx of wing digit III	12.50	11.80	–	–	–
Second phalanx of wing digit III	17.40	16.80	–	–	–
Third phalanx of wing digit III	8.90	8.10	–	–	–
Fourth metacarpal	32.90	34.30	–	–	–
First phalanx of wing digit IV	10.30	9.80	–	–	–
Second phalanx of wing digit IV	12.40	11.80	–	–	–
Fifth metacarpal	33.80	34.60	–	–	–
First phalanx of wing digit V	10.20	9.90	–	–	–
Second phalanx of wing digit V	11.10	8.60	–	–	–
Calcar length	5.00	4.70	–	–	–
Length of hind foot	11.70	12.00	–	12.00–14.00	12.00–14.00
Greatest length of the skull (excluding incisors)	19.40	20.50	19.48–20.05	19.54–20.49	20.10–20.80
Condyloincisive length	18.20	18.50	18.03–18.22	17.99–19.07	18.00–18.60
Upper toothrow length	7.10	7.30	5.69–7.40	6.99–7.56	7.10–7.50
Breadth across the cingula of upper canines	3.40	3.30	3.13–3.44	–	–
Postorbital breadth	4.20	–	4.12–4.36	3.92–4.21	3.90–4.50
Breadth across the upper molars	6.10	6.20	6.35–6.56	5.84–6.13	5.80–6.20
Braincase breadth	8.30	8.20	7.78–8.06	7.92–8.26	–
Zygomatic breadth	10.00	9.60	9.37–9.85	8.84–9.51	9.10–9.50
Mastoid breadth	9.30	8.90	8.35–8.78	8.43–9.05	8.50–8.80
Length of the mandible	12.80	–	12.81–13.52	–	–
Lower toothrow length	7.70	7.60	–	–	–

similar to that found in the Araras Biological Reserve (n = 23), in the municipalities of Petrópolis and Miguel Pereira (Costa 2014: 1100 m a.s.l.; SE = 66,297 h.m^2), and slightly higher than the numbers recorded in Desengano State Park (n = 16), in eastern Rio de Janeiro (Modesto et al. 2008: 1240 m a.s.l.; SE = 15,510 h.m^2), and in Itatiaia National Park (n = 15), in the municipality of Itatiaia (Martins et al. 2015: 500–1000 m a.s.l.; SE = 12,135 h.m^2). In Rio das Pedras Biological Reserve, which is near the SBNP in the municipality of Mangaratiba, however, Luz et al. (2011) recorded a much higher number of species (n = 30), with a sampling effort of 242,424 net.h. The species richness recorded in the present study was also higher than that recorded in non-protected areas at similar altitudes, such as Dores do Rio Preto municipality in Espírito Santo state (725-1422 m a.s.l.), where Lopes et al. (2017) recorded 14 species (SE = 1500 h.m^2), and in Rio Preto and Santa Bárbara do Monte Verde municipalities (800–1200 m a.s.l.) in Minas Gerais, where Nobre et al. (2009) recorded 15 species (SE = 22,140 h.m^2). It is important to note, however, that any such comparisons between sites are influenced by a series of factors, ranging from the type of site (protected versus non-protected areas) and total sampling effort to the type of habitat and forest strata sampled, as well as the climatic conditions and moon phases during the trapping sessions (e.g., Aguiar & Marinho-Filho 2004, Peracchi & Nogueira 2010, Mello et al. 2013).

The present study adds 14 species to the inventory of bats in the SBNP, including three species listed as endangered in Rio de Janeiro state by Bergallo et al. (2000): *Dermanura cinerea* Gervais, 1856, *Platyrrhinus recifinus* (Thomas, 1901), and *Myotis ruber* (É. Geoffroy, 1806). *Dermanura cinerea* is a common frugivore that occurs in several habitat types, such as primary and successional forests, and forest fragments (Zortéa 2007). *Platyrrhinus recifinus* is also a frugivore, and is found in primary and successional habitats in the Atlantic Forest, and in patches of humid forest in the Caatinga and Cerrado biomes (Tavares & Velazco 2010). *Myotis ruber* is an insectivore found in a range of habitat types, including well-preserved and secondary forests, and forest remnants in urban areas (Reis et al. 2017). The study also obtained the

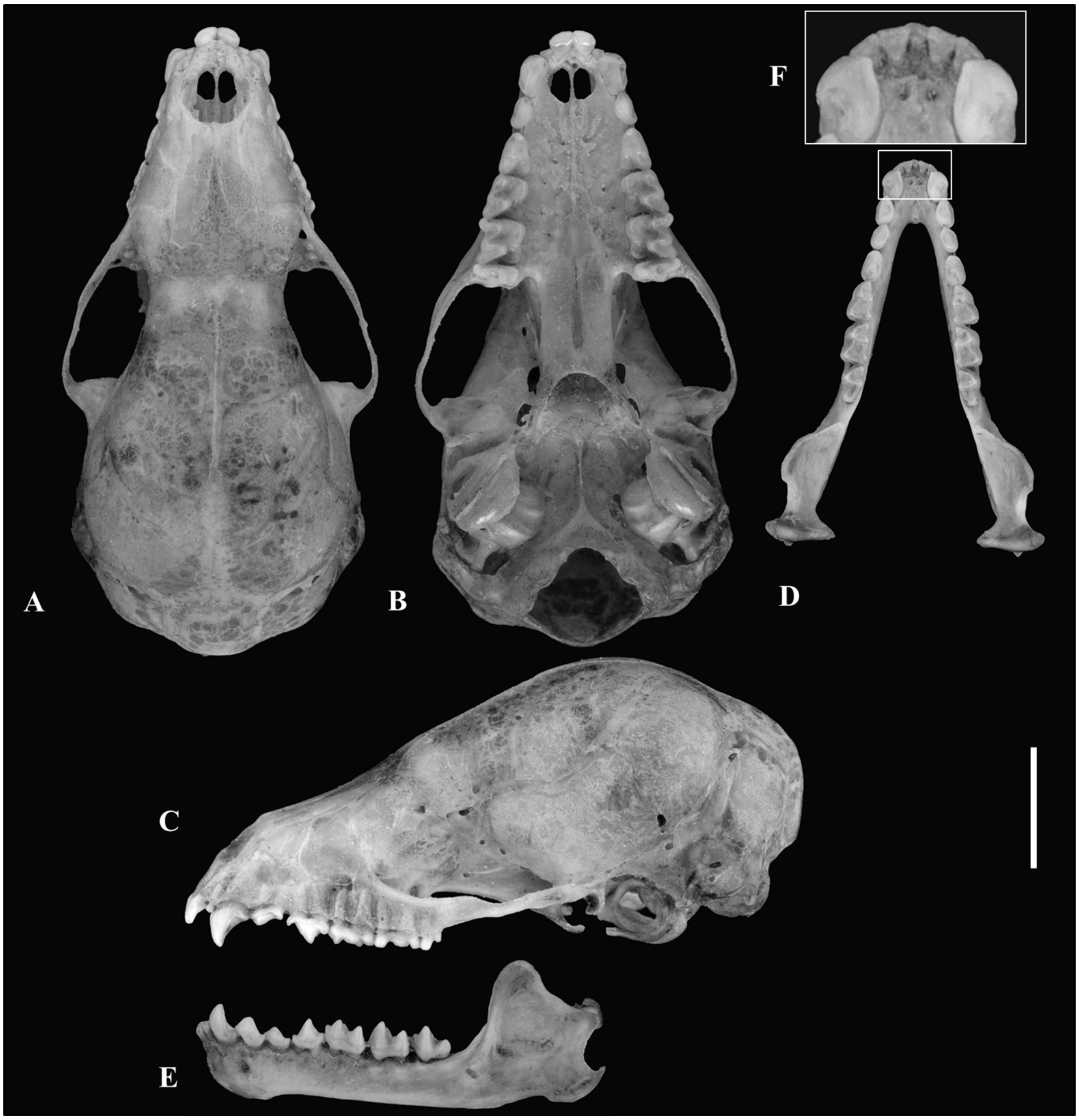

Figure 6. Dorsal (A), ventral (B) and lateral (C) views of the skull, and the dorsal (D) and lateral (E) views of the mandible of the *Trinycteris nicefori* specimen (MN81510) from the Serra da Bocaina National Park in Rio de Janeiro, Brazil. The detail (F) shows the small supernumerary central lower incisors. Scale bar: 5 mm.

first record of *T. nicefori* for the Brazilian state of Rio de Janeiro. This species is known to occur in several different habitat types, ranging from forests to farmland, but is normally captured at low rates in most faunal inventories (e.g., Genoways & Williams 1986, Simmons & Voss 1998, Rocha et al. 2013), as in the present study, given that only one individual was captured. All the species recorded in the previous study in the SBNP (Delciellos et al. 2012) were captured again, except by *Chrotopterus auritus* (Peters, 1856) and *Tonatia bidens* (Spix, 1823). Delciellos et al. (2012) recorded 10 species with a sampling effort of 2592 h.m^2 in a single session in May 2011 (dry season). As the same areas (sites 2 and 3) and habitat types were resampled in the present study, new records of the species recorded by Delciellos et al. (2012) were expected, although it is unclear why neither *C. auritus* nor *T. bidens* were captured in the present study. The absence of these species here may nevertheless be related to specific features of their population dynamics or distribution within the study area. In particular, *C. auritus* appears to be more common in undisturbed forest habitats (Gorresen & Willing 2004), rather than the types of environment sampled in the present study, given the proximity of the RJ-165 highway.

The most abundant species were *C. perspicillata* and *S. lilium*, which is consistent with the results of previous studies (e.g., Fleming et al. 1972, Bernard & Fenton 2002, Sampaio et al. 2003), that have found a predominance of phyllostomid species in the Neotropics, in particular frugivores, such as *C. perspicillata* and *S. lilium* (e.g., Baptista & Mello 2001, Mello & Schittini 2005, Moratelli & Peracchi 2007, Nobre et al. 2009, Costa 2014). The relative abundance of *S. lilium* in the SBNP may also be accounted for by the altitude of the sampled areas, given that this species tends to be more abundant at higher altitudes (e.g., Nobre et al. 2009, Costa 2014, Martins et al. 2015). As for species richness, total abundance (n = 505) recorded in the present study was relatively higher than that recorded in non-protected areas at similar altitudes (e.g., Nobre et al. 2009: n = 246), but lower than that recorded in the Rio das Pedras Biological Reserve, which is near the SBNP (Luz et al. 2011: n = 1228).

The characteristics of the *T. nicefori* specimen captured in the SBNP were highly consistent with the description of the species in previous studies (Sanborn 1949, Simmons 1996, Simmons & Voss 1998, Williams & Genoways 2007, Rocha et al. 2013), except for the lack of a lightly-colored dorsal stripe. This trait nevertheless varies considerably among individuals, and is barely discernible or even absent in some individuals, while it is highly conspicuous in others (Starret 1976, Simmons & Voss 1998, Williams & Genoways 2007). The specimen from the SBNP had reddish hair, which is consistent with the typical "red phase", rather than the rarer "gray phase" (Sanborn 1949, Starret 1976), and tetracolored dorsal fur, which is consistent with the description of Williams & Genoways (2007). Some studies mention that the dorsal pelage of *T. nicefori* is tricolored (Sanborn 1949, Goodwin & Greenhall 1961, Simmons 1996, Simmons & Voss 1998), but this is probably due to the presence of a narrow, inconspicuous pale basal band that may not be observed by all authors. All the cranial and external measurements are within the range of those reported previously for the species (Sanborn 1949, Simmons & Voss 1998, Williams & Genoways 2007, Rocha et al. 2013).

Trinycteris nicefori can be distinguished from other phyllostomid genera by a set of morphological traits (see descriptions in Sanborn 1949, Simmons & Voss 1998, Williams & Genoways 2007, Rocha et al. 2013). This species is most often confused with *Lampronycteris brachyotis* (Dobson, 1879) and *Glyphonycteris sylvestris* Thomas, 1896, which are similar species in size and morphology. However, in *L. brachyotis* (forearm length 38.3–42.5 mm), the fifth metacarpal is the shortest (V<IV<III), the calcar is similar in length or slightly shorter than the foot with claws, the inner upper incisors are markedly chisel-shaped and in line with canines, the basisphenoid pits are shallow, and the dorsal pelage is not banded (Simmons 1996, Simmons & Voss 1998, Wetterer et al. 2000). In *G. sylvestris* (forearm length 37.0–44.0 mm), the fifth metacarpal is the longest (IV<III<V), the rostrum is notably inflated, P3 is not reduced in size, as it is in *T. nicefori*, but is slightly larger than or is similar in height to P4, and both P3 and P4 have slightly curved anterior cusps (Sanborn 1949, Simmons & Voss 1998). Similar to *T. nicefori*, however, the dorsal hairs of *G. sylvestris* have three (Genoways & Williams 1986, Simmons 1996, Simmons & Voss 1998, Williams & Genoways 2007) or four (Sanborn 1949, Goodwin & Greenhall 1961) bands.

Trinycteris nicefori has an ample geographical distribution, ranging from Mexico to Brazil (see Williams & Genoways 2007, Perez et al. 2012, Rocha et al. 2013, Miranda et al. 2015). In Brazil, the species has a disjunct distribution, occurring in the north (Amazon) and on the east coast (Atlantic Forest) from Sergipe to Espírito Santo states (Rocha et al. 2013, Miranda et al. 2015). In addition to being the first record of *T. nicefori* from Rio de Janeiro, the specimen collected in the present study now represents the southernmost record of the species (Figure 5). The known distribution of *T. nicefori* indicates that it is totally absent from the Brazilian Caatinga and Cerrado biomes, indicating a preference for mesic environments, which may reflect an intolerance of arid habitats or, possibly, the reduced bat sampling effort in these environments (Rocha et al. 2013).

The present study has updated the list of bat species known to occur in the Serra da Bocaina National Park, which is part of one of the largest remaining continuous tracts of Brazilian Atlantic Forest. Additional inventories, especially at poorly-studied Atlantic Forest sites, combined with environmental suitability analyses, and taxonomic and biogeographic studies, are urgently needed to elucidate the distribution of many Brazilian bat species, such as *T. nicefori*.

Supplementary material

The following online material is available for this article:
Appendix 1

Author Contributions

Ana Cláudia Delciellos: 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.

Adarene Motta: Contribution to data collection; Contribution to manuscript preparation.

Brunna Almeida: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Daniela Dias: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Oscar Rocha-Barbosa: Substantial contribution in the concept and design of the study.

Conflicts of interest

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

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Environmental perception of fishermen: use and conservation of fisheries resources

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Abstract: The principal aim was to assess the environmental perception of the fishermen, and implement educational activities, on an island in Brazil. Twelve people between the ages of 23 and 63 were interviewed, of whom 75% were male, and 58.33% did not complete their elementary education. Regarding social benefits, 33.33% receive a family allowance and 16.66% receive the so-called 'green allowance' (financial assistance to extremely poor families who live in areas of environmental preservation). Fishing remains the main economic activity (58.73%). The most common types of fishing gear are the '*zangarias*' (41.66%) and '*muruadas*' (41.66%), used to catch white shrimp (*Litopenaeus schmitti*), the main resource traded on the island (83.33%). All those interviewed reported a change in the size and weight of the catch in recent years (100%), and 75% attribute this to the types of trap used. Talks, films and booklets, as environmental education activities, contributed to community awareness. The population is familiar with the effects of fishing traps, and shows an interest in changing the situation.
Keywords: *Fishery community, Environmental education, Neotropical Region, Marine environments.*

Percepção ambiental de pescadores: uso e conservação dos recursos pesqueiros

Resumo: O objetivo principal foi avaliar a percepção ambiental dos pescadores e implementar atividades educacionais, em uma ilha no Brasil. Doze pessoas entre as idades de 23 e 63 anos foram entrevistadas, das quais 75% eram do sexo masculino, e 58,33% não completou seu ensino fundamental. Em relação aos benefícios sociais, 33,33% recebem o Bolsa Família e 16,66% recebem o chamado "Bolsa Verde" (assistência financeira a famílias extremamente pobres que vivem em áreas de preservação ambiental). A pesca continua a ser a principal atividade econômica (58,73%). Os tipos mais comuns de artes de pesca são as '*zangarias*' (41,66%) e '*muruadas*' (41,66%), utilizadas para capturar camarão branco (*Litopenaeus schmitti*), o principal recurso comercializado na ilha (83,33%). Todos os entrevistados relataram uma mudança no tamanho e peso da captura nos últimos anos (100%) e 75% atribuem isso aos tipos de armadilhas utilizadas. Conversas, filmes e cartilhas, como atividades de educação ambiental, contribuíram para a conscientização da comunidade. A população está familiarizada com os efeitos das armadilhas de pesca e mostra interesse em mudar a situação.
Palavras-chave: *Comunidade pesqueira, Educação ambiental, Região Neotropical, Ambientes marinhos.*

Introduction

The vast expanse of the Maranhão coastline and its immense biological diversity are favourable to the occurrence of multiple fishery resources, which are exploited by a large variety of equipment and types of fishing, from small-scale fishing without boats, or the use of skiffs or sailing boats, to large vessels (Isaac et al., 2006).

In recent decades, artisanal fishing has been growing due to the setting up of housing in coastal areas, free access to resources, and reduced control and supervision, but mainly due to the lack of other employment opportunities for the riverine population (Haimovici, 2011). According to Almeida et al. (2006), in the State of Maranhão, 15% of the active population is engaged in fishing, and this has led to conflicts between conservation of the resources and the environment, and the economic development of the population (Haimovici, 2011). However, in order for fishing in Maranhão and in other regions of Brazil not to collapse, institutional management and conservation measures have been implemented, including the creation of Environmental Protection Areas (EPA) and Extractive Reserves (EXRES), with a view to maintaining the natural resources and to integrating man and the environment. It is therefore not enough to consider only the biological and ecological characteristics of these resources, it is also necessary to consider the social and economic aspects involved in exploiting the resources. Through multidisciplinary and integrated studies, it is possible to better understand the economic role of fishing and its impact on the environment (Almeida, 2008).

One of the most common environmental impacts of fishing activities comes from discarding non-target species (Alarcon et al., 2009). This type of practice poses a threat, both to the stocks of target species and to the populations of discarded species that have no attractive commercial value (Zhou et al., 2015). Environmental education, directed towards the resolution of environmental problems as an added dimension to the content and practice of education, can contribute to the minimisation of such impacts.

Environmental perception is a tool to measure and evaluate the environment in which an individual works, in addition to directing their activities and way of life (Evangelista-Barreto, 2014), allowing to evaluate how the community visualizes local aspects related to the exploration of the natural resources, effects of human actions on the environment, benefits derived from biodiversity conservation, and others. According to UNESCO (1973), the different perceptions of culturally distinct individuals, or socioeconomic groups that perform different social roles, are one of the barriers to the protection of natural environments. Therefore, to evaluate the environmental perception to implement environmental education activities is the most effective way of implementing public policies related to the environment and, consequently, to communities that have natural resources for their subsistence (Oliveira and Corona, 2008).

Therefore, using this knowledge to develop community awareness measures and providing the information needed to implement sustainable fisheries and care for the environment, whether for adults or children, is the most effective way to practice environmental education in traditional communities, because it will be based on the causes and motives described by the social actors themselves and in the constructive thinking of suitable methodologies for satisfactory results (Cunha & Leite, 2009).

Based on these ideas, the aim with this work was to assess the environmental perception of fishermen concerning the impact of '*zangaria*' (fixed-trap) fishing, as an aid to the implementation of educational activities for fisheries management in the EXRES of Cururupu.

Material and Methods

1. *Study area*

The municipality of Cururupu is located on the western coastline of the State of Maranhão in Brazil, and has an area of 495 km². It comes under the Brazilian System of Conservation Units, with the region included and authorised as an Environmental Protection Area (The so-called *Reentrâncias Maranhenses*) and Extractive Marine Reserve (Costa et al., 2006).

Created by Presidential Decree on 2 June 2004, the Marine EXRES of Cururupu contains around 185,046 ha, and is part of the Brazilian marine biome, comprising a diverse fauna with almost 1,300 species of fish, 19 of which are endangered and 32 in a state of decline (ICMBIO, 2016). It is made up of 15 islands with an area which is used by traditional extractive populations: Mangunça, Caçacueira, São Lucas, Valha-me-Deus, Guajerutiua, Lençóis, Ponta Seca, Porto Alegre, Retreat, Bate-Vento, Porto do Meio, Mirinzal, Iguará, Beiradão and Peru, the last serving as the study area for this work (Figure 1).

2. *Field activities*

The researchers initially presented the project to the deliberation council of the EXRES to obtain authorisation to carry out the work, and later gave a talk to the residents of Peru Island (Cururupu, in the State of Maranhão) to present the project and to propose a partnership with the residents in carrying out each of the activities, so as to seek solutions for sustainable fishing and to generate information for fisheries management. The talk was also aimed at clarifying technical terms and the results which would be obtained with a scientific project (the breeding of economically important species and the determination of fish diversity in the region) carried out in parallel with this environmental education project under SISBIO license No 47170-1, and with the permission of the Committee for Ethics and Animal Experimentation of the Maranhão State University (Protocol No 016/2014).

In February and March of 2015, to analyse environmental perception in the community, qualitative-quantitative approach was used (Lefèvre and Lefèvre, 2005, Kirschbaum, 2013), seeking a perspective of the social and economic situation, as well as assessing the way the community viewed environmental impacts related to fishing such as wastage and bycatch, solutions for reducing the size of the catch, and the use of selective fishing gear. At this stage, semi-structured questionnaires (Diniz et al., 2011) were used with a sample size of 12 individuals, considered by the community as "key informants", since they demonstrated knowledge acquired locally, either by observation, use or exploration (Sousa et al., 2015). To select the informants, the snow-ball technique was used, in which a first key informant indicates the subsequent one, and so on (Atkinson & Flint, 2001). The inclusion criteria included people of both sexes that worked with fisheries.

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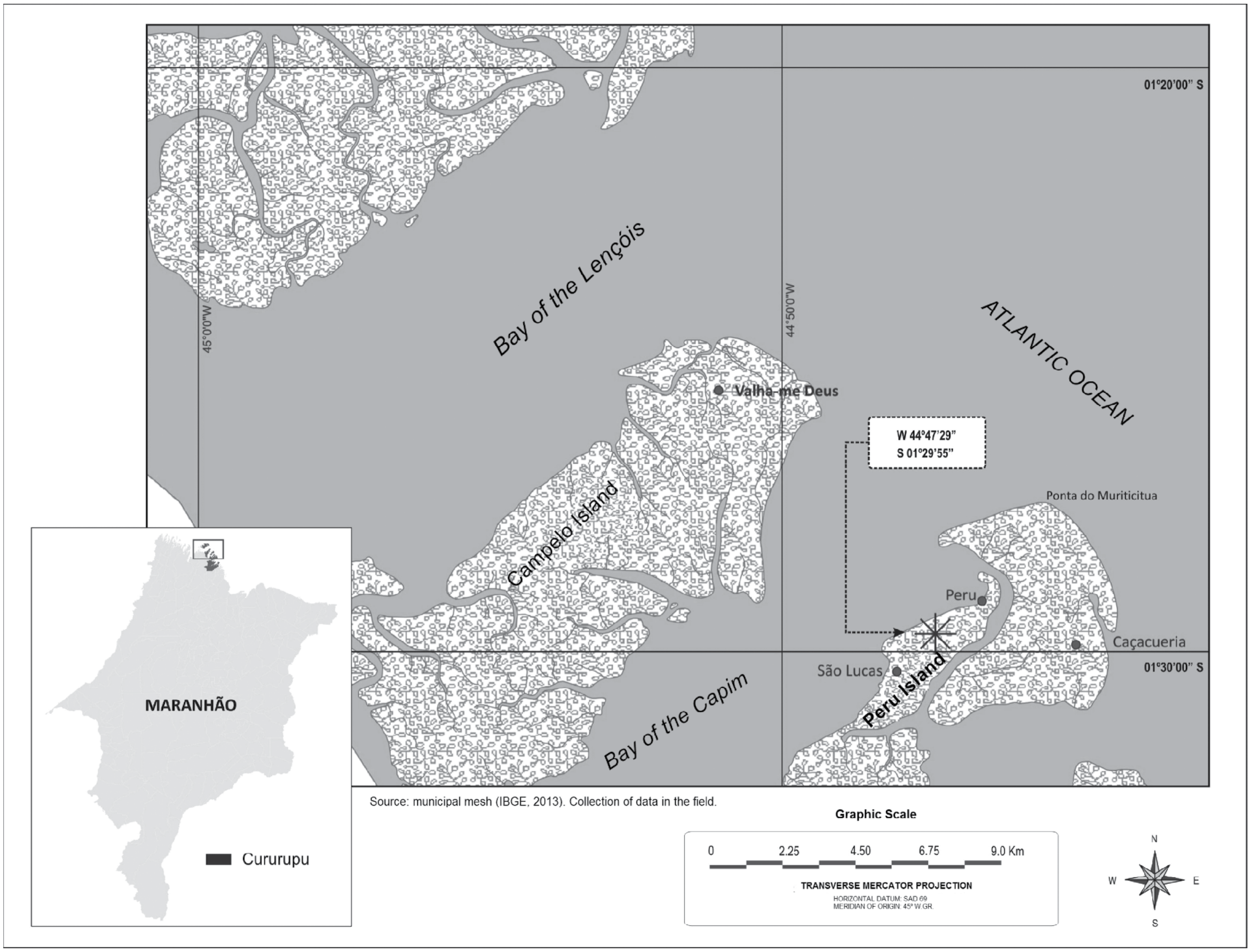

Source: municipal mesh (IBGE, 2013). Collection of data in the field.

Figure 1. Location of the study area: Peru Island, Cururupu, Maranhão, Brazil.

The sample number represents 25% of the island's total population and the chosen informants were nominated by the community itself, including island councilors, as the most appropriate people to discuss and report on the local situation.

For the data analysis, a database was created in Microsoft Excel software, where frequency tables that were generated indicated the representativeness of the informants' opinion. Besides, the interviews were recorded and analyzed later, and the most representative discourses were transcribed in original language.

After obtaining the results, an educational booklet was produced, in the form of comics and in accessible language, in which the information obtained was made available in order to keep the population aware of their role in conserving resources. Moreover, the film "Mônica's Gang - A Plan to Save the Planet" (Sousa, 2011) for children, which portrays the conservation of natural resources in a didactic and accessible way, was broadcast. Although the interviews were not conducted with the children, this activity was included based on what was concluded about the knowledge that is transmitted from the parents to the children in the community.

The identification of participants was kept secret, with their names not included in the databases, ensuring the anonymity and confidentiality of the information. We obtained the participation and consent of those involved after clarifying the purpose of the research, their agreement expressed by Informed Consent Form.

Results

1. *Establishing the initial aims of the research*

At the initial talk, was counted on the participation of the residents, who raised important questions, such as the return of results to the community, benefits of the scientific research (concerning fish diversity in the region and determination of the reproductive period of four economically important species) and the position of the organs responsible (Figure 2).

2. *Profile of the community under investigation*

The age of the informants ranged from 23 to 63, with a predominance of individuals in the 35-45 age group, and those >55 (33.33%) who had lived on the island for over 20 years. As seen in several surveys related to fishing, the male sex predominated, representing 75% of those interviewed.

Figure 2. Initial talk to the Peru Island community, Cururupu EXRES, Maranhão, Brazil.

The lack of representation of the female sex can be verified by the testimony of one resident when asked about women's involvement in fishing activities:

> *"[...] When he gets back from the sea, I help him lay the shrimp in the sun, take them out of the sun... actual fishing, no. Sometimes as I said, I go down to the water's edge."*

(Female resident, Peru Island, 51)

In relation to schooling, 58.33% of those interviewed had not completed elementary education. Most of those interviewed receive no kind of social benefit, and among those who do, the Family Allowance was the most represented, at 33.33% (Table 1).

We noted a great appreciation of social benefit by the respondents, who said that the benefit came in useful when helping to pay expenses, since fishing is an unstable source of income, where some periods are more productive than others are.

Almost 60% of those interviewed work in fishing, an indication that this is the principal economic activity on Peru Island.

Among the types of fishing gear used by the fishermen are the so-called '*zangaria*'[1], '*tarrafa*'[2], '*curral*'[3], '*muruada*'[4] e and '*puçá*'[5]. The '*zangarias*' (41.66%) and the '*mouaries*' (41.66%) are the most common types of fishing gear. We noticed that the fish are used mainly for personal consumption or sold at a lower value, and that shrimp is the most commercially valued product (83.33%), explaining the results found for the fishing gear.

The most traded species according to the fishermen are White shrimp (*Litopenaeus schmitti*), King Weakfish (*Macrodon ancylodon*), Coco sea catfish (*Bagre bagre*), Whitemouth Croaker (*Micropogonias furnieri*), Crucifix sea catfish (*Sciades proops*), Tripletail (*Lobotes surinamensis*), Parassi mullet (*Mugil incilis*) and Common Snook (*Centropomus undecimalis*).

Family income, for the most part, does not exceed the minimum wage (41.67%), but a large number explained that there was no way to state that with conviction (Table 2) since the monthly income from fishing activities is highly variable due to fluctuations in the product, ranging from the minimum wage (BRL 788.00 - USD 291.85 [in 2015]) to four minimum wages (BRL 3,152.00 - USD 1,167.40 [in 2015]).

Regarding perceived changes in the size and weight of the fish, 100% of the respondents said that these aspects have changed over the years. A total of 69.2% related this to the intense exploitation of resources due to the use of selective fishing gear and the removal of juvenile individuals that have not yet seen an increase in weight or length, and are therefore considered unsuitable for consumption.

> *"The change is because they are catching very small fish... all kinds of fish ... there is no time to grow, they catch them early to sell. As I say, there is a lot of fish."*

(Resident, 37)

Around 75% of those interviewed said that the traps used by the community cause damage, and that the weighted nets[6] used by fishermen from neighbouring communities end up hampering fishing in the Peru Island community.

On the other hand, some respondents also consider the trawl net and high trap (*zangaria alta*) to be harmful types of fishing gear.

> *"Man, it causes damage, right, because actually the fixed trap... it always kills both the big and small fish, right?! And other types of fishing, the high trap ... that's a really high net. Whatever is there is caught. We don't have it here, but they come from another places to fish here, understand?! They put it right at the edge of the mangrove".*

(Resident, 33)

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[1] A fixed trap placed at the ebb tide, when stakes are set up to mark out the maximum points of a semicircle. Stakes marking the minimum points of the semicircle are only set up at low tide, when the expanse is completely exposed (Almeida, 2008).

[2] A small circular net with lead weights on the lower part called the bag, where the fish are held (Almeida, 2008).

[3] An enclosure of pieces of fabric and sticks, tied to stakes with liana. It is divided into two sections, known as the 'room' and the 'pen'. The room is where the fish lose orientation, and due to the current, are no longer able to get out, entering the pen where they are trapped (Almeida, 2008).

Table 1. Socioeconomic data of the fishing community of Peru Island, Cururupu EXRES, Maranhão, Brazil.

Aspects	Response	Percentage (number of individuals/total)
Age	15 to 25 years	8.33% (1/12)
	25 to 35 years	16.67% (2/12)
	35 to 45 years	33.33 (4/12)
	45 to 55 years	8.33 (1/12)
	> 55 years	33.33 (4/12)
Sex	Male	75% (9/12)
	Female	25% (3/12)
Education	No education	8.33% (1/12)
	Elementary education (incomplete)	58.33% (7/12)
	Elementary education (complete)	8.33% (1/12)
	Secondary education (incomplete)	0.00% (0/12)
	Secondary education (complete)	16.67% (2/12)
	Higher education (incomplete)	8.33% (1/12)
	Secondary education (complete)	0.00% (0/12)
Social benefit	No benefit	66.66% (8/12)
	Family allowance	33.33% (4/12)
	Green allowance	16.66% (2/12)
	Closed-season Insurance	0.00% (0/12)

Table 2. Fishing economic data of the fishing community of Peru Island, Cururupu EXRES, Maranhão, Brazil.

Aspects	Response	Percentage (number of individuals/total)
Working in fishing	Yes	58.33% (7/12)
	No	25.00% (3/12)
	Occasionally	16.67% (2/12)
Fishing gear	Zangaria	41.66% (5/12)
	Tarrafa	25% (3/12)
	Curral	8.33% (1/12)
	Muruada	41.66% (5/12)
	Puçá	8.33% (1/12)
Principal species caught for sale	Shrimp	83.33% (10/12)
	King Weakfish	33.33% (4/12)
	Coco sea catfish	25% (3/12)
	Whitemouth Croaker	8.33% (1/12)
	Crucifix sea catfish	16.66% (2/12)
	Tripletail	16.66% (2/12)
	Parassi mullet	8.33% (1/12)
	Common Snook	8.33% (1/12)
	Whitefish	8.33% (1/12)
Family income	< 1 minimum salary	41.67% (5/12)
	1 minimum salary	25.00% (3/12)
	Between 1 and 4 minimum salaries	8.33% (1/12)
	5 or more minimum salaries	0.00% (0/12)
	Variable	25.00% (3/12)

[4] A semi-fixed trap that is placed against the current in holes and small streams, with stakes set up about 1.5 m apart, in the apparent form of a wall (Almeida, 2008).

[5] Funnel-shaped nets for shrimp fishing. The net is tied to two pieces of wood so that it can be dragged (Almeida, 2008).

[6] Type of holding net, where many weights are used to fix the net to the sides of channels to prevent free circulation of the water (BRASIL, 2014).

> *"It's bad. Fishing with nets, they're doing away with the fish there in the river. Trawling ... causes a lot of damage here. They trawl the river, there are times we go and don't even catch enough to eat. They're "squeezing the fish out", as we say here".*
>
> (Resident, 39)

Regarding a possible solution to the visible reduction in fish, 25% of respondents pointed to the closed season, and another 25% stated that the problem has no solution. Some of those interviewed pointed to a reduction in the number of nets and a change in the current closed season as possible solutions.

Currently, the closed season in the Cururupu EXRES is from June to August; this corresponds to the migration of the sardines and not to the reproductive period of the fish.

The residents themselves stated during the interviews that June to August is the wrong period as that is when the fish are of a good size for fishing.

> *"They should have come to do the research, because the one time that is good for fishing, they are prohibiting it; it's the wrong time."*
>
> (Female resident, 31)

One resident also drew attention to better enforcement of the regulations with punishment for those who disagree:

> *"Besides having the ban when you can't fish, we believe there has to be control anyway, and punishment for those people who 'may disagree' with the management agreement in this notice that arrived. Because if I call a meeting of the community to get rid of a fisherman's net from here, very soon we are going to have trouble, and at the moment not even the law gives supports us, it doesn't punish that fisherman, it won't help at all. So we need control and punishment for anyone who disagrees with the management agreement."*
>
> (Resident, 23)

A total of 81.8% of those interviewed said that they did not notice any type of pollution on the island; this result being directly related to receipt of the Green Allowance benefit (financial assistance to extremely poor families who live in areas of environmental preservation). Regarding guidance on the minimum catch size for the most traded fish, around 50% said that there had been a meeting where this information was passed on, and 50% say that there is no regulation for a minimum catch size (Table 3).

In an attempt to get them to reflect on the environmental problems that may affect or are affecting the island, we asked how they imagined the environment in which they lived 20 years from now. The majority answered that the situation would be more critical than it is today and that resources would be more scarce.

> *"That's right ... if no one is worried and there is not even the law to take the necessary measures, I think we probably won't have as much as we had before; even today I don't see so much anymore, we only have something now through divine help. But if you say it's because of our own awareness, that it's improving because of us, because we are taking care of the reservation, then it's a lie because that's not happening. It's the old question 'only the smartest survive'".*
>
> (Resident, 23)

3. *Didactic tools used with the community*

At the end, we gave the children and adults an educational booklet with information obtained scientifically and from actions carried out throughout the year, which serves as a basis for current and future fishing activities, and to reflect on how each one can contribute to the sustainability of Peru Island. The contents include fish diversity in the region, information on the reproductive biology of four economically important species, the final destination of the specimens taken to the laboratory, photographs, reflections of the residents themselves during the interviews, and finally, a look at the local situation 10 years from now with the collaboration of the community (Figure 3).

After the screening of the film "Monica's Gang - A Plan to Save the Planet", it was noticed that the children had learned important lessons regarding the disposal of solid wastes and contaminants in the aquatic environment and the consequences of that for fishing. To evaluate this learning, one wondered what would be done with plastic cups and bags used for food and beverage consumption during the film. They replied that they would apply the logic of the three R's that was taught during the film: reduce and reuse, using only a glass; and recycle, to produce some kind of garnish or handicraft.

Discussion

Throughout the research, it was noticed that the population is aware of the effects caused by the fishing traps and show interest in transforming this reality. This perception of the fishing community was the initial step so that the actions of environmental education could collaborate in the process of knowledge construction, conservation of fishing resources, and sensitization of local social agents.

The longer the time lived in an area, the greater the level of knowledge and familiarity with that area, therefore, people of more advanced age are best suited to talk about local activities and the environment as a whole. According to Silvano and Begossi (2012), there is an accumulation of knowledge with the passing years, so that older people tend to know more about matters of interest to the community, and are considered the most suitable because of that knowledge.

Because fishing is still a traditionally male activity, we saw a greater representation of men. Similar results were reported by Santos et al. (2011) in the fishing community in the town of Raposa, Maranhão, stating that this pattern is the result of the need for great physical effort in fishing activities and of the danger, both of which end up being limiting for women. According to Costa (2009), only now are women beginning to show interest in the activity together with their husbands, sometimes

Table 3. Environmental perception data of the fishing community of Peru Island, Cururupu EXRES, Maranhão, Brazil.

Aspects/Questions	Response	Percentage (number of individuals/total)
Are the traps used by the community harmful?	Yes	75.00% (9/12)
	No	8.33% (1/12)
	Don’t know	25.00% (3/12)
Pollution	Yes	8.33% (1/12)
	No	75.00% (9/12)
	Don’t know	16.67% (2/12)
Solution for the reduction in catch size	Closed season	25.00% (3/12)
	Reduce the number of nets	8.33% (1/12)
	Change the period of the closed season	8.33% (1/12)
	There is no solution	25.00% (3/12)
	Don’t know	33.33% (4/12)
Is there any kind of guidance on minimum catch size?	Yes	50.00% (6/12)
	No	50.00% (6/12)

Cartilha Educativa

AÇÕES PARA O FORTALECIMENTO DO ORDENAMENTO PESQUEIRO DO SISTEMA DE PRODUÇÃO DA REDE DE ZANGARIA NA RESEX DE CURURUPU, MARANHÃO, BRASIL

São Luís
2015

Olá! Eu sou Lorrane e estas são Polliana e Luane. Nós somos pesquisadoras do Laboratório de Pesca e Ecologia Aquática da Universidade Estadual do Maranhão e por um ano realizamos pesquisas científicas e ações de educação ambiental aqui na Ilha de Peru. Através desta cartilha educativa nós trouxemos os resultados para vocês!

Todos os meses eu e meus parceiros de equipe vinhamos acompanhar a pescaria nas zangarias e coletar peixes, camarões e siris para nossa pesquisa.

Diversidade de peixes na Ilha do Peru-MA

Figure 3. Part of the educational booklet produced from results obtained during the project. Graphic design and illustration: Yuri Almeida.

leaving off other activities such as handicrafts or even household chores. However, their main function is still to clean the fish, organise the gear and prepare the food (Knox and Firme, 2016).

The results found for level of schooling may be related to the geographic isolation imposed on the inhabitants of an island, or to the economic conditions, where children and grandchildren end up starting fishing activities early, either to help or to support the family. The results found by Santos et al. (2011) in the town of Raposa, support this, since the author found a higher level of schooling among the younger fishermen, and related this to the proximity of the state capital, São Luís, where transportation is regular and more schools are available.

The fact that fishing is still the most predominant activity in the community can be explained by the richness and abundance of the resources occurring at the site, and by the conditions, which are favourable to fishing. However, fishing is considered an activity of unstable return, and fishermen and their families therefore supplement their income with financial aid provided by the government and with other types of activities, such as tourist transport and the sale of handicrafts. This way of working in fishing communities is common in other regions of the Amazon (Ceregato and Petrere Jr., 2003, Cardoso et al., 2004, Ruffino, 2005, Lima et al., 2012). Furtado (1993) states that artisanal fishermen are known for being multitalented, due to the extensive range of work they carry out daily, where they mix several activities so as to guarantee the sale of various products and generate a minimum income.

Shrimp as the target product is directly related to the types of fishing gear used and the size of the net opening. This practice may have future impact on the region, since fish of all sizes are caught, including juveniles, which end up not being used for sale or consumption, and are discarded on a large scale (Alarcon et al., 2009, Leitão et al., 2014, Klautau et al., 2016).

As the "*zangaria*" (fixed trap) is already prohibited in the country, with Maranhão being one of the only states where it is still allowed (with activities suspended from June to August), the fear of a possible ban may have influenced the questions on harmful fishing gear. According to Pereira (2008) and Golden et al. (2016), the daily need for food is the main reason why fishing is increasingly more intense. The shortage of resources leads to the use of nets of a finer-mesh with the aim of capturing smaller species to achieve results similar to earlier scenarios. As a result, there is greater damage to the environment, compromising stocks and jeopardising future catches.

The closed-season insurance benefit, implemented by the Ministry of Labour and Employment, where fish, shrimp and lobster fishermen receive a minimum wage not to fish during the reproductive period of the species (Cavalcante et al., 2013), is not received by any of those interviewed. Difficulties in delivering and checking documents are the main reasons for the delay in implementing this aid.

Adequate cleaning of the environment is related to the Green Allowance benefit received by the residents, encouraging them to carry out collective clean-ups and meetings to organise the disposal of garbage. The Green Allowance Environmental Conservation Support Program (*Programa de Apoio à Conservação Ambiental Bolsa Verde*), instituted by Law No 12,512 of 14 October 2011, and regulated by Decree No 7,572 of 28 September 2011, grants a benefit of BRL 300 every quarter to families in situations of extreme poverty that live in areas considered a priority for environmental conservation. Around 47% of the 16.2 million people living in extreme poverty are in rural areas, so the specific aim is to reconcile an increase in the income of this population to the preservation of ecosystems and the sustainable use of natural resources (Brazil, 2011). This new benefit, part of the Brazil Without Poverty Program (*Programa Brasil Sem Miséria*), is intended for those who develop activities for the sustainable use of natural resources in Extractive Reserves, National Forests, Federal Sustainable Development Reserves and the Environmentally Differentiated Settlements of the Agrarian Reform (Brazil, 2011, Pereira et al., 2016).

Regarding the local knowledge about the impacts of activities on fish resources and on the environment, it was possible to perceive that fishermen are aware that the fish stocks are decreasing and that affects the standard of living of those who depend solely on the activity to survive. However, most do not consider themselves responsible for this and do not consider the fishing gear used to pose any risk, except for those used by neighboring communities. It was also noticed that the community does not know how to react to the problem of fish reduction and, moreover, there are those who consider that the reduction of fish is a momentary phenomenon, making clear the need for lectures and informative courses about the consequences of unsustainable fishing. It was noted that there is already some empirical knowledge about the need to allow the reproduction and growth of the animals, since the community indicates the closure as a solution for the reduction of fishes and crustaceans.

Our environmental education activities included giving talks, films for children and delivering educational booklets, and they proved to be good tools for raising awareness in the community, since besides being informative, they led the residents to reflect on their present attitudes and the implications for the future. The booklet, produced in the form of comic strips and in simple language, also covered the child audience, who are the future fishermen of the region. Van Bressem et al. (2006) pointed out that the use of educational booklets is an extremely valuable tool in education, as biological information is presented in the form of a story that is easy to understand and remember. In addition, they can be read at any time and lent to other people. The cinema and the production of images gain strength in the contemporary world, appearing as a powerful tool of socialization of information and being able to function as special sources of education and knowledge construction (Oliveira, 2006, Fischer, 2009). According to Otto and Pensini (2017), in childhood the motivation to be ecologically friendly is formed and probably the effect lasts a lifetime (Evans et al., 2007).

Finally, the scenario 20 years from now as described by the residents themselves, shows a more critical situation than the current one, with scarcer resources and the search for new forms of livelihood. In this way, we can see a clear need for intervention by both the authorities and residents, as well as educational activities, and activities to raise awareness promoted by educational institutions and by governmental and non-governmental agencies.

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Authors' Contributions

Lorrane Gabrielle Cantanhêde: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to critical revision, adding intelectual content.

Luane Raisa De Moraes Pereira: Substantial contribution in the concept and design of the study; contribution to data collection; contribution to critical revision, adding intelectual content.

Polliana Farias Veras: Substantial contribution in the concept and design of the study; contribution to data collection.

Wallacy Borges Teixeira Silva: Contribution to data collection.

Raimunda Nonata Fortes Carvalho-Neta: Contribution to critical revision, adding intelectual content.

Zafira Da Silva de Almeida: Substantial contribution in the concept and design of the study; 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

Endemic angiosperms in Bahia Coastal Forests, Brazil: an update using a newly delimited area

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Abstract: The forest along the coast of Bahia state, in northeastern Brazil, has been acknowledged as an area of endemism for several groups of organisms. The first study concerning endemic angiosperm species in this region, through which 395 taxa were registered, was conducted 15 years ago. However, this number is probably underrated. In this article, we present an updated checklist of angiosperm taxa in Bahia Coastal Forests (BCF) based on geographic distribution data available from the Brazilian Plant List (Flora do Brasil 2020). The records were checked in herbaria, recent taxonomic literature, and, when necessary, reviewed by experts. The final checklist consists of 547 taxa endemic to BCF, distributed in 69 families, with seven endemic genera: *Bahiella* (Apocynaceae), *Santosia* (Asteraceae), *Harleyodendron* (Fabaceae), *Cubitanthus* (Linderniaceae), *Anomochloa* and *Parianella* (Poaceae), and *Andreadoxa* (Rutaceae). The families with highest richness in endemic taxa were Bromeliaceae (108 spp.), Fabaceae (41 spp.) and Myrtaceae (32 spp.). This updated checklist shares only 143 taxa with a previous list published about 15 years ago. Those two lists differ mostly due to slightly different area limits, and to the large number of additional endemic species described since then. We hope our list will be used as a resource in future studies and contributes to the conservation of the highly diverse Bahia Coastal Forest.
Keywords: *Atlantic Forest, checklist, conservation, endemism, hotspot.*

Angiospermas endêmicas da Floresta Costeira da Bahia, Brasil: uma atualização utilizando uma nova delimitação de área

Resumo: A floresta costeira do estado da Bahia, no nordeste do Brasil, tem sido apontada como área de endemismo para diversos grupos de organismos. O primeiro levantamento de espécies de angiospermas endêmicas dessa região foi realizado há cerca de 15 anos e revelou a ocorrência de 395 táxons. Entretanto, esse número provavelmente está subestimado. Neste trabalho apresentamos uma lista atualizada de táxons de angiospermas endêmicas da Floresta Costeira da Bahia (FCB), tendo como base as informações de distribuição geográfica disponíveis na lista da Flora do Brasil. Estas informações foram posteriormente checadas em registros de herbários, estudos taxonômicos recentes e, quando necessário, revisadas por especialistas. A lista final de espécies é constituída por 547 táxons endêmicos da Floresta Costeira da Bahia, distribuídos em 69 famílias e incluindo sete gêneros endêmicos: *Bahiella* (Apocynaceae), *Santosia* (Asteraceae), *Harleyodendron* (Fabaceae), *Cubitanthus* (Linderniaceae), *Anomochloa* e *Parianella* (Poaceae), and *Andreadoxa* (Rutaceae). As famílias mais ricas em táxons endêmicos são Bromeliaceae (108 spp.), Fabaceae (41 spp.) e Myrtaceae (32 spp.). Apenas 143 táxons são compartilhados por esta lista atualizada e uma lista preliminar publicada há cerca de 15 anos. As duas listas diferem especialmente por ligeiras diferenças nos limites da área de estudo e também pelo acréscimo de novas espécies endêmicas descritas desde então. Espera-se que a lista seja usada como ferramenta para estudos futuros e para a conservação da rica flora costeira da Bahia.
Palavras-chave: *Checklist, conservação, endemismo, hotspot, Mata Atlântica.*

Introduction

The Atlantic Forest is considered a biodiversity hotspot due to the occurrence of a high number of endemic and threatened species (Myers et al. 2000). The natural distribution of the Atlantic Forest was originally continuous along the Brazilian coast and extended to some areas in Argentina and Paraguay (Fundação SOS Mata Atlântica 2013). Nowadays, it is one of the eight most critically endangered hotspots, as only 12,5% of the original forest remains (Fundação SOS Mata Atlântica 2014). Nevertheless, it is the richest Brazilian phytogeographic domain in plant diversity, with 8,728 endemic species of angiosperms (Flora do Brasil 2020, under construction).

Some studies have emphasized the division of the Atlantic Forest in northern and southern regions (Cracraft 1985, Colombi et al. 2010, Martins 2011) with a limit along the Doce river, in the north of Espírito Santo state. The floristic differences between these two regions were highlighted by Oliveira-Filho & Fontes (2000) and Oliveira-Filho et al. (2005), who observed several taxa with Amazonian affinities occuring in the northern region, while taxa with subtropical Andean affinities are mostly found in the southern region (Berry et al. 2004, Santos et al. 2007, Menini Neto et al. 2016). Other authors recognize three main centers of endemism in the Atlantic Forest: northern (Pernambuco and Alagoas states), southern (from Rio de Janeiro to Santa Catarina states), and central (southern Bahia and Espírito Santo states) (Murray-Smith et al. 2009, Thomas et al. 1998).

Recently, Saiter et al. (2016a) divided the central region of the Atlantic Forest in three subregions based on the composition of tree species, indicating the most important abiotic factors affecting that division. Contrary to previous knowledge, the rivers did not play an important role in this division, which lies about 100 km north of the Doce river and is best explained by climatic factors, such as moisture, elevation and temperature (Saiter et al. 2016a). One of the regions, referred to as Bahia Coastal Forests (BCF) by Saiter et al. (2016a), encompasses the northern extreme of Espírito Santo and most of the coast of the state of Bahia. Due to high biodiversity and the incidence of endemic and threatened species, this region has been designated as a hot-point within the Atlantic Forest hotspot (Martini et al. 2007). Many studies to this date on different groups of organisms support this claim, as the region is considered an area of endemism for frogs (Carnaval et al. 2009), birds (Silva et al. 2004), Myrtaceae (Murray-Smith et al. 2009) and trees in general (Thomas et al. 2003).

An estimate of endemic vascular plant species present in two protected areas in southern Bahia showed that the distribution of 59% of the species is restricted to Bahia and Espírito Santo states (Thomas et al. 1998). These results motivated the compilation of a preliminary list of endemic angiosperm species in the region comprising Espírito Santo to the north of the Doce river and the south of Bahia, including inland Seasonal Dry Forests (Thomas et al. 2003) (Figure 1a). This list consisted of 395 endemic species of angiosperms in the region (Thomas et al. 2003), among which several are known to occur only in dry forests, such as *Chrysophyllum subspinosum* Monach. (Sapotaceae) and *Colicodendron bahianum* Cornejo & Ilts (Capparaceae). That study reported eight endemic genera in this area: *Arapatiella* R.S.Cowan, *Brodriguesia* R.S.Cowan and *Harleyodendron* R.S.Cowan (Fabaceae), *Atractantha* McClure, *Anomochloa* Brogn., *Alvimia* Calderón ex Soderstr. & Londoño, and *Sucrea* Soderstr. (Poaceae), and *Trigoniodendron* E.F.Guim & Miguel (Trigoniaceae) (Thomas et al, 2003).

Checklists of endemic taxa are critical to overcome issues that hinder conservation strategies in highly biodiverse regions, such as: 1) basic taxonomic information (Linnean shortfall, or “which species are there?”), and 2) lack of geographic information (Wallacean shortfall, or “where do these species occur?”) (Whittaker et al. 2005). Both shortfalls are part of reality in Bahia Coastal Forests, world-renowned as one of the highest diversity areas in tree species (Thomas et al. 1998, Martini et al. 2007, Murray-Smith et al. 2009).

We aimed to provide a list of endemic species of angiosperms in Bahia Coastal Forest following Saiter et al. (2016a) to verify whether the current list agrees with the preliminary checklist by Thomas et al. (2003). We expect this updated list to further highlight the importance of BCF as a critical area for biodiversity conservation in the Brazilian Atlantic forest.

Material and Methods

1. *Study area*

To delimit the study area we used the BCF *sensu* Saiter et al. (2016a) (Figure 1b) with a slight modification to include the municipalities whose territory was at least 50% inside that perimeter. Municipalities were used as a base for the retrieval of taxonomic information from online datasets (see data collection). To remove the arbitrary northern limit from Saiter et al. (2016a) (Figure 1b), the study area was expanded to include seven municipalities complying with the 50% territory criterion (Figure 1c). Municipal limits were defined based on data from the Brazilian Institute for Geography and Statistics (IBGE 1993) (Figure 1c).

The resulting area comprised 156 municipalities, which account for approximately 99,000 km^2 (Figure 1c) at southern Bahia state, and a small fraction from northern Espírito Santo state and northeastern Minas Gerais state, contrasting with an area of about 119,000 km^2 at southern Bahia which included more inland areas, and northen Espírito Santo with a southern limit at the Doce River (Thomas et al. 2003) (Figure 1a).

2. *Data collection*

To compile the checklist of endemic species of angiosperms in BCF, we started by performing a data search in the Brazilian Plant List (Flora do Brasil 2020) on March 9, 2017, using the following filters simultaneously: “group: angiosperms”, “endemism: only endemic to Brazil”, “origin: native”, “state: Bahia”, and “phytogeographic domain: Atlantic Forest”. We decided not to search only for species endemic to the Atlantic Forest in Bahia to avoid excluding species that could also occur in the north of Espírito Santo and in a small area in northeastern Minas Gerais (Figure 1c). The initial search resulted in 3,338 species, 59 subspecies and 154 varieties (3,551 taxa). These records were then checked in the SpeciesLink database (splink.cria.org.br). Each taxon map generated by the SpeciesLink website was visually checked, first to remove taxa that were clearly not endemic to BCF, i.e. those with several records outside the study area. Each of the 1,336 remaining taxa were then mapped with QGis 2.14 (Quantum GIS Development Team 2016) and we verified whether the points of occurrence were enclosed in the limits of the study area, which resulted in the further removal of 701 taxa.

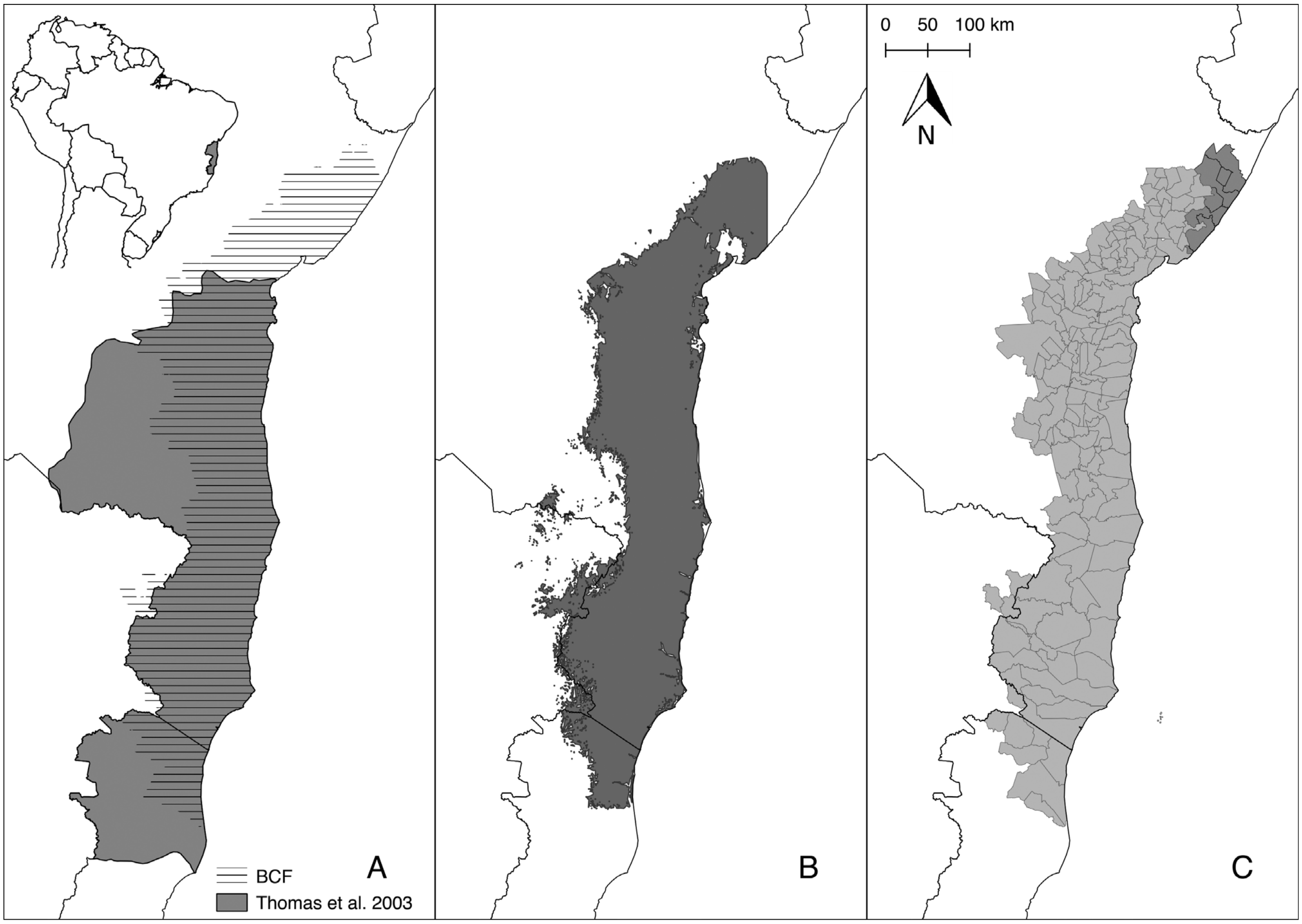

Figure 1. a) Study area in which Thomas et al. (2003) based their list of endemic species. b) Bahia Coastal Forest (BCF) according to Saiter et al. (2016a). c) Municipalities with at least 50% of its area within the BCF (light grey), and those added to the study area based on IBGE (1993) (dark grey). Coordinates: UTM. Datum WGS 84.

From the 635 taxa left, the records with incongruent data were verified by experts or checked using recent taxonomic studies (Appendix 1). This step resulted in 30 additional non-endemic species discarded from the list. The taxa for which no scientific records were found in Google Scholar (scholar.google.com), Biodiversity Heritage Library (biodiversitylibrary.com), SpeciesLink (splink.cria.org.br), and the Brazilian Plant List (Flora do Brasil 2020) were complemented by a search for location records in the CEPEC herbarium. Once this verification was completed, 53 species from the list had to be removed because no records were available and a further five species were removed due to lack of precise geographic information.

Results

The final checklist consisted of 547 angiosperm taxa endemic to BCF (535 species, six subspecies, and six varieties) in 69 families (none endemic) and 230 genera (Table 1). Seven genera are endemic to BCF: *Bahiella* J.F.Morales (Apocynaceae), *Santosia* R.M.King & H.Rob. (Asteraceae), *Harleyodendron* (Fabaceae, Figure 2f), *Cubitanthus* Barringer (Linderniaceae), *Anomochloa* and *Parianella* Hollowell, F.M.Ferreira & R.P.Oliveira (Poaceae), and *Andreadoxa* Kallunki (Rutaceae). The genera with the highest number of endemic species in the BCF were *Aechmea* Ruiz & Pav. (Bromeliaceae) (33 spp.), *Pavonia* Cav. (Malvaceae) and *Myrcia* DC. (Myrtaceae), each with 16 species. The three families with the highest numbers of endemic taxa (species + infraspecific taxa) were Bromeliaceae (108), Fabaceae (41) and Myrtaceae (32).

Discussion

The families with the highest numbers of endemic taxa in BCF are also among the ten richest angiosperm families in Brazil and in the Atlantic Forest (BFG 2015). Thomas et al. (2003) reported Fabaceae (53 spp.) as the richest family of a total of 65 families in the previous list. Compared with the BFG (2015), it is remarkable that Orchidaceae, one of the three richest families in Brazil and in the Atlantic Forest, was not among the richest families in this study, with only 25 taxa. This may be due to the lack of geographic records for 14 species in this family, which had to be discarded.

Among the 20 richest angiosperm genera in Brazil (BFG 2015), *Aechmea* and *Myrcia* are also among the genera with the highest number of endemic species in the BCF. In a preliminary list of the endemic angiosperm species from southern Bahia and northern Espírito Santo, Thomas et al. (2003) cited *Conchocarpus* J.C.Mikan (Rutaceae) (19 spp.) as the richest genus, followed by *Pavonia* Cav. (Malvaceae) (13 spp.), *Couepia* Aubl., and *Erythroxylum* P.Browne (seven spp. each).

Table 1. Checklist of angiosperms endemic to Bahia Coastal Forest *sensu* Saiter et al. (2016a). * Taxon also in Thomas et al. (2003).

Acanthaceae

Aphelandra ignea Nees ex Steud.
Clistax bahiensis Profice & Leitman
Harpochilus phaeocarpus Nees
Herpetacanthus magnobracteolatus Indriunas & Kameyama
Herpetacanthus strongyloides Indriunas & Kameyama
Herpetacanthus tetrandrus (Nees & Mart.) Herter
Justicia antirrhina Nees & Mart.
Justicia physogaster Lindau
Lepidagathis cuneiformis Kameyama
Mendoncia bahiensis Profice
Mendoncia blanchetiana Profice
Pseuderanthemum albiflorum (Hook.) Radlk.
Pseuderanthemum verbenaceum (Nees & Mart.) Radlk.
Ruellia sessilifolia (Nees) Lindau
Schaueria gonyostachya (Nees & Mart.) Nees
Schaueria hirsuta Nees
Schaueria marginata Nees
Schaueria pyramidalis A.L.A.Côrtes

Achariaceae

Kuhlmanniodendron macrocarpum Groppo, Favaretto & Fiaschi

Amaranthaceae

Lecosia formicarum Pedersen

Amaryllidaceae

Griffinia arifolia Ravenna
Griffinia parviflora Ker Gawl.
Griffinia paubrasilica Ravenna

Annonaceae

Annona bahiensis (Maas & Westra) H.Rainer *
Duguetia magnolioidea Maas *
Duguetia restingae Maas *
Duguetia reticulata Maas *
Guatteria stenocarpa Lobão, Maas & Mello-Silva
Hornschuchia cauliflora Maas & Setten *
Hornschuchia leptandra D.M.Johnson *
Hornschuchia obliqua Maas & Setten *
Hornschuchia polyantha Maas *
Hornschuchia santosii D.M.Johnson *
Malmea obovata R.E.Fr. *
Pseudoxandra bahiensis Maas *
Unonopsis bahiensis Maas & Orava
Xylopia involucrata M.C.Dias & Kinoshita *

Apocynaceae

Aspidosperma thomasii Marc.-Ferr. *
Bahiella blanchetii (A.DC.) J.F.Morales
Bahiella infundibuliflora J.F.Morales

Continuation Table 1.

Lacmellea bahiensis J.F.Morales
Marsdenia carvalhoi Morillo & Carnevali
Matelea riparia Morillo
Matelea santosii Morillo & Fontella
Oxypetalum laciniatum Rapini & Farinaccio
Rauvolfia atlantica Emygdio

Araceae

Anthurium bromelicola subsp. *bahiense* Mayo et al. *
Anthurium illepidum Schott
Anthurium molle E.G.Gonç. & J.G.Jardim
Anthurium teimosoanum E.G.Gonç. & J.G.Jardim
Asterostigma riedelianum (Schott) Kuntze
Dracontioides salvianii E.G.Gonç.
Philodendron aemulum Schott
Zomicarpa steigeriana Maxim. ex Schott

Araliaceae

Schefflera aurata Fiaschi

Arecaceae

Bactris soeiroana Noblick ex A.J.Hend.
Geonoma pohliana subsp. *rubescens* (Wendland ex Drude) Henderson
Geonoma pohliana subsp. *unaensis* Henderson
Syagrus × *camposportoana* (Bondar) Glassman
Syagrus itapebiensis (Noblick & Lorenzi) Noblick & Meerow
Syagrus santosii K. Soares & C.A.Guim.

Aristolochiaceae

Aristolochia brunneomaculata I.Abreu & Giul.
Aristolochia longispathulata F.González *

Asparagaceae

Hagenbachia brasiliensis Nees & Mart.

Asteraceae

Acmella paniculata (Wall. ex DC.) R.K.Jansen
Austroeupatorium morii R.M.King & H.Rob.
Barrosoa atlantica R.M.King & H.Rob. *
Diacranthera hebeclinia H.Rob.
Litothamnus ellipticus R.M.King & H.Rob. *
Mikania amorimii Borges & Forzza
Mikania kubitzkii R.M.King & H.Rob. *
Mikania mattos-silvae R.M.King & H.Rob. *
Mikania santosii R.M.King & H.Rob. *
Piptocarpha riedelii (Sch.Bip.) Baker
Santosia talmonii R.M.King & H.Rob. *
Vernonanthura vinhae (H.Rob.) H.Rob. *

Begoniaceae

Begonia delicata Gregório & J.A.S.Costa
Begonia elianeae Gregório & J.A.S.Costa
Begonia epibaterium Mart. ex A.DC.

Continuation Table 1.

Begonia goldingiana L.Kollmann & A.P.Fontana
Begonia mattos-silvae L.B.Sm. ex S.F.Sm. & Wassh. *
Begonia pinheironis L.B.Sm. ex S.F.Sm. & Wassh. *
Begonia russelliana L.B.Sm. ex S.F.Sm. & Wassh. *
Begonia saxifraga A.DC.
Begonia subacida Irmsch.
Begonia sylvatica Meisn. ex A.DC.
Bignoniaceae
Handroanthus parviflorus Espírito-Santo & M.M. Silva-Castro
Bromeliaceae
Aechmea amicorum B.R.Silva & H.Luther
Aechmea amorimii Leme *
Aechmea ampla L.B.Sm.
Aechmea andersoniana Leme & H.Luther
Aechmea andersonii H.Luther & Leme
Aechmea carvalhoi E.Pereira & Leme
Aechmea correia-araujoi E.Pereira & Moutinho
Aechmea curranii (L.B.Sm.) L.B.Sm. & M.A.Spencer
Aechmea digitata L.B.Sm. & R.W.Read
Aechmea discordiae Leme
Aechmea disjuncta (L.B.Sm.) Leme & J.A.Siqueira
Aechmea echinata (Leme) Leme *
Aechmea farinosa (Regel) L.B.Sm.
Aechmea glandulosa Leme
Aechmea gregaria Leme & L.Kollmann
Aechmea guaratingensis Leme & L.Kollmann
Aechmea heterosepala Leme
Aechmea incompta Leme & H.Luther
Aechmea ituberaensis Leme & L.Kollmann
Aechmea laevigata Leme
Aechmea lanata (L.B.Sm.) L.B.Sm. & M.A.Spencer
Aechmea limae Leme
Aechmea lymanii W.Weber
Aechmea miniata Beer ex Baker
Aechmea mira Leme & H.Luther
Aechmea mollis L.B.Sm.
Aechmea pendulispica Leme & L.Kollmann
Aechmea ramusculosa Leme
Aechmea tentaculifera Leme, Amorim & J.A. Siqueira
Aechmea turbinocalyx Mez
Aechmea viridipetala A.F.Costa & Amorim
Aechmea viridostigma Leme & H.Luther
Aechmea weberi (E.Pereira & Leme) Leme
Alcantarea cerosa Leme, A.P.Fontana & O.A.B.Ribeiro
Alcantarea pataxoana Versieux
Araeococcus montanus Leme

Continuation Table 1.

Araeococcus nigropurpureus Leme & J.A.Siqueira
Araeococcus sessiliflorus Leme & J.A.Siqueira
Billbergia fosteriana L.B.Sm.
Billbergia macrocalyx Hook.
Canistrum fosterianum L.B.Sm.
Canistrum guzmanioides Leme
Canistrum lanigerum H.Luther & Leme
Canistrum sandrae Leme
Canistrum seidelianum W.Weber
Cryptanthus colnagoi Rauh & Leme
Cryptanthus coriaceus Leme
Cryptanthus ilhanus Leme
Cryptanthus lyman-smithii Leme
Cryptanthus pseudopetiolatus Philcox *
Cryptanthus ruthiae Philcox
Cryptanthus ubairensis I.Ramírez
Cryptanthus vexatus Leme
Cryptanthus viridovinosus Leme
Cryptanthus walkerianus Leme & L.Kollmann
Hohenbergia barbarespina Leme & Fraga
Hohenbergia brachycephala L.B.Sm.
Hohenbergia burle-marxii Leme & W.Till
Hohenbergia capitata Schult. & Schult.f.
Hohenbergia castellanosii L.B.Sm. & Read
Hohenbergia correia-araujoi E.Pereira & Moutinho
Hohenbergia flava Leme & C.C.Paula
Hohenbergia hatschbachii Leme *
Hohenbergia itamarajuensis Leme & Baracho
Hohenbergia littoralis L.B.Sm.
Hohenbergia pabstii L.B.Sm. & Read
Hohenbergia reconcavensis Leme & Fraga
Hohenbergia sandrae Leme
Lymania alvimii (L.B.Sm. & R.W.Read) R.W.Read *
Lymania azurea Leme
Lymania brachycaulis (E.Morren ex Baker) L.F.Sousa
Lymania corallina (Brong. ex Beer) R.W.Read
Lymania globosa Leme
Lymania languida Leme
Lymania marantoides (L.B.Sm.) R.W.Read
Lymania spiculata Leme & Forzza *
Neoregelia azevedoi Leme
Neoregelia crispata Leme *
Neoregelia longisepala E.Pereira & I.A.Penna *
Neoregelia rothinessa Leme, H.Luther & W.Till
Neoregelia silvomontana Leme & J.A.Siqueira
Neoregelia viridolineata Leme

Continuation Table 1.

Neoregelia wilsoniana M.B.Foster
Orthophytum buranhense Leme & A.P.Fontana
Orthophytum guaratingense Leme & L.Kollmann
Orthophytum rubrum L.B.Sm.
Portea alatisepala Philcox *
Portea filifera L.B.Sm.
Portea grandiflora Philcox *
Portea kermesina K.Koch
Portea nana Leme & H.Luther
Quesnelia alborosea A.F.Costa & T.Fontoura
Quesnelia clavata Amorim & Leme
Quesnelia dubia Leme
Quesnelia koltesii Amorim & Leme
Ronnbergia brasiliensis E.Pereira & I.A.Penna *
Ronnbergia carvalhoi Martinelli & Leme *
Ronnbergia neoregelioides Leme
Ronnbergia silvana Leme
Vriesea dictyographa Leme
Vriesea graciliscapa W.Weber
Vriesea longisepala A.F.Costa
Vriesea minuta Leme
Vriesea minutiflora Leme
Vriesea roberto-seidelii W.Weber
Vriesea ruschii L.B.Sm. subsp. ruschii
Vriesea sandrae Leme
Vriesea silvana Leme
Burseraceae
Protium icicariba var. *talmonii* Daly *
Trattinnickia lorenziana Daly & M.F.F.Melo
Cactaceae
Rhipsalis hileiabaiana (N.P.Taylor & Barthlott) N.Korotkova & Barthlott *
Calophyllaceae
Kielmeyera itacarensis Saddi
Kielmeyera marauensis Saddi
Capparaceae
Colicodendron martianum Cornejo
Caryocaraceae
Caryocar edule Casar.
Chrysobalanaceae
Couepia bondarii Prance *
Couepia coarctata Prance *
Couepia impressa subsp. *cabraliae* Prance *
Couepia longipetiolata Prance *
Licania bahiensis Prance
Licania lamentanda Prance *

Continuation Table 1.

Licania littoralis Warm. var. *littoralis*
Licania santosii Prance *
Licania turbinata Benth.
Parinari alvimii Prance *
Clusiaceae
Tovomita iaspidis L.Marinho & Amorim
Tovomita megantha L.Marinho & Amorim
Commelinaceae
Dichorisandra jardimii Aona & M.C.E.Amaral
Dichorisandra leucophthalmos Hook.
Dichorisandra leucosepala Aona & M.C.E.Amaral
Dichorisandra marantoides Aona & Faden
Dichorisandra ordinatiflora Aona & Faden
Dichorisandra radicalis Nees & Mart.
Dichorisandra subtilis Aona & M.C.E.Amaral
Connaraceae
Connarus blanchetii var. *laurifolius* (Baker) Forero
Connarus cuneifolius Baker
Connarus portosegurensis Forero
Rourea bahiensis Forero *
Rourea carvalhoi Forero et al. *
Rourea discolor Baker
Rourea macrocalyx Carbonó et al. *
Rourea tenuis G.Schellenb.
Cucurbitaceae
Cayaponia nitida Gomes-Klein & Pirani
Fevillea bahiensis G.Rob. & Wunderlin
Gurania wawrei Cogn.
Cyclanthaceae
Asplundia maximiliani Harling
Cyperaceae
Becquerelia discolor Kunth
Hypolytrum bahiense M.Alves & W.W.Thomas *
Hypolytrum jardimii M.Alves & W.W.Thomas *
Hypolytrum lucennoi M.Alves & W.W.Thomas
Dichapetalaceae
Stephanopodium magnifolium Prance *
Tapura martiniae Amorim & D.Lisboa
Tapura zei-limae Amorim & Fiaschi
Dilleniaceae
Davilla bahiana Aymard
Davilla macrocarpa Eichler
Dioscoreaceae
Dioscorea macrothyrsa Uline
Ebenaceae
Diospyros amabi B.Walln.

Continuation Table 1.

Diospyros riedelii (Hiern) B.Walln.
Diospyros scottmorii B.Walln.
Eriocaulaceae
Actinocephalus ochrocephalus (Körn.) Sano
Erythroxylaceae
Erythroxylum compressum Peyr.
Erythroxylum leal-costae Plowman
Erythroxylum martii Peyr.
Erythroxylum mattos-silvae Plowman *
Erythroxylum membranaceum Plowman *
Erythroxylum santosii Plowman *
Erythroxylum splendidum Plowman *
Euphorbiaceae
Actinostemon lasiocarpus (Müll.Arg.) Baill.
Algernonia bahiensis (Emmerich) G.L.Webster *
Bernardia gambosa Müll.Arg.
Bernardia micrantha Pax & K.Hoffm.
Croton sapiifolius Müll.Arg.
Croton thomasii Riina & P.E.Berry
Dalechampia armbrusteri G.L.Webster
Dalechampia viridissima G.L.Webster *
Gymnanthes gaudichaudii Müll.Arg.
Ophthalmoblapton pedunculare Müll.Arg.
Fabaceae
Andira carvalhoi R.T.Penn. & H.C.Lima *
Andira marauensis N.F.Mattos *
Arapatiella psilophylla (Harms) R.S.Cowan *
Canavalia cassidea G.P.Lewis *
Canavalia dolichothyrsa G.P.Lewis *
Chamaecrista amabilis H.S.Irwin & Barneby *
Chamaecrista amorimii Barneby *
Chamaecrista onusta H.S.Irwin & Barneby *
Chamaecrista salvatoris (H.S.Irwin & Barneby) H.S.Irwin & Barneby
Copaifera majorina Dwyer
Dahlstedtia bahiana (A.M.G.Azevedo) M.J.Silva & A.M.G.Azevedo
Harleyodendron unifoliolatum R.S.Cowan *
Inga aptera (Vinha) T.D.Penn. *
Inga conchifolia L.P.Queiroz
Inga pedunculata (Vinha) T.D.Penn. *
Inga pleiogyna T.D.Penn.
Machaerium aureum Filardi & H.C.Lima
Moldenhawera blanchetiana var. *multijuga* L.P.Queiroz et al.
Moldenhawera intermedia G.P.Lewis & L.P.Queiroz
Moldenhawera luschnathiana Yakovlev
Moldenhawera nutans L.P.Queiroz et al.

Continuation Table 1.

Muellera longiunguiculata (MJ.Silva & AMG.Azevedo) MJ.Silva & AMG.Azevedo
Ormosia lewisii D.B.O.S.Cardoso, C.H.Stirt. & Torke *
Ormosia limae D.B.O.S.Cardoso & L.P.Queiroz
Ormosia timboensis D.B.O.S.Cardoso, Meireles & H.C.Lima
Parapiptadenia ilheusana G.P.Lewis *
Phanera carvalhoi (Vaz) Vaz
Piptadenia killipii var. *cacaophila* G.P.Lewis *
Piptadenia ramosissima Benth.
Piptadenia santosii Barneby ex G.P.Lewis *
Schnella lilacina (Wunderlin & Eilers) Wunderlin
Senegalia amorimii M.J.F.Barros & M.P.Morim
Senegalia olivensana (G.P.Lewis) Seigler & Ebinger
Senegalia piptadenioides (G.P.Lewis) Seigler & Ebinger
Swartzia alternifoliolata Mansano
Swartzia arenophila R.B.Pinto, Torke & Mansano
Swartzia curranii R.S.Cowan
Swartzia pinheiroana R.S.Cowan *
Swartzia riedelii R.S.Cowan
Swartzia thomasii R.B.Pinto, Torke & Mansano
Zollernia magnifica A.M.Carvalho & Barneby *
Gentianaceae
Macrocarpaea atlantica J.R.Grant & V.Trunz
Macrocarpaea dolichophylla J.R.Grant & V.Trunz
Macrocarpaea orbiculata J.R.Grant & V.Trunz
Icacinaceae
Pleurisanthes brasiliensis (Val.) Tiegh.
Iridaceae
Neomarica brachypus (Baker) Sprague
Neomarica floscella A.Gil & M.C.E.Amaral
Neomarica portosecurensis (Ravenna) Chukr *
Neomarica unca (Ravenna) A.Gil
Lauraceae
Nectandra micranthera Rohwer
Ocotea montana (Meisn.) Mez
Ocotea ramosissima L.C.S.Assis e Mello-Silva
Ocotea sperata P.L.R.Moraes et van der Werff
Ocotea thinicola van der Werff et P.L.R.Moraes
Lecythidaceae
Eschweilera complanata S.A.Mori
Eschweilera mattos-silvae S.A.Mori *
Eschweilera sphaerocarpa M.Ribeiro & S.A.Mori
Linderniaceae
Cubitanthus alatus (Cham. & Schltdl.) Barringer
Loganiaceae
Spigelia genuflexa Popovkin & Struwe
Strychnos alvimiana Krukoff & Barneby *

Continuation Table 1.

Strychnos setosa Krukoff & Barneby
Loranthaceae
Psittacanthus excrenulatus Rizzini
Psittacanthus salvadorensis Kuijt
Struthanthus longiflorus Rizzini
Malpighiaceae
Bunchosia itacarensis W.R.Anderson *
Heteropterys sanctorum W.R.Anderson *
Peixotoa sericea C.E.Anderson *
Stigmaphyllon hispidum C.E.Anderson
Stigmaphyllon macropodum A.Juss.
Malvaceae
Byttneria cristobaliana Dorr
Pavonia cauliflora (Nees) Fryxell ex G.L.Esteves
Pavonia ciliata G.L.Esteves & Krapov. *
Pavonia crispa Krapov. *
Pavonia gerleniae Gonçalez & M.C.Duarte
Pavonia goetheoides (Hassl.) Fryxell ex G.L.Esteves
Pavonia latibracteolata Krapov. *
Pavonia longifolia A.St.-Hil.
Pavonia macrobracteolata Gonçalez & M.C.Duarte
Pavonia ovaliphylla G.L.Esteves & Krapov. *
Pavonia paucidentata Fryxell
Pavonia pilifera Krapov.
Pavonia rubriphylla G.L.Esteves
Pavonia sancti Krapov.
Pavonia spectabilis Krapov. *
Pavonia spiciformis Krapov. *
Pavonia stipularis Krapov. *
Marantaceae
Goeppertia fasciata (Linden ex K.Koch) Borchs. & S.Suárez
Goeppertia rufibarba (Fenzl) Borchs. & S.Suárez
Ischnosiphon bahiensis L.Andersson *
Monotagma grallatum Hagberg & R. Erikss. *
Stromanthe bahiensis Yosh.-Arns, Mayo & J.M.A. Braga
Marcgraviaceae
Schwartzia geniculatiflora Gir.-Cañas & Fiaschi
Melastomataceae
Bertolonia alternifolia Baumgratz, Amorim & A.B.Jardim
Bertolonia bullata Baumgratz, Amorim & A.B.Jardim
Huberia carvalhoi Baumgratz
Huberia sessilifolia R. Godenberg & Michelangelo
Meriania inflata Michelangeli & R.Goldenb.
Miconia lurida Cogn.
Ossaea loligomorpha R.Goldenb. & Reginato
Ossaea sulbahiensis D´El Rei Souza

Continuation Table 1.

Physeterostemon aonae Amorim, Michelangeli & R.Goldenb.
Physeterostemon fiaschii R.Goldenb. & Amorim
Physeterostemon jardimii R.Goldenb. & Amorim
Physeterostemon thomasii Amorim, Michelangeli & R.Goldenb.
Pleiochiton amorimii Reginato & R.Goldenb.
Tibouchina bahiensis Wurdack *
Tibouchina bradeana Renner
Tibouchina paulo-alvinii Guimarães da Vinha *
Tibouchina stipulacea Vinha *
Tibouchina taperoensis Wurdack *
Tibouchina tomentulosa Wurdack
Meliaceae
Guarea anomala T.D.Penn
Trichilia florbranca T.D.Penn. *
Trichilia magnifoliola T.D.Penn. *
Menispermaceae
Curarea crassa Barneby *
Moraceae
Dorstenia setosa Moric.
Myrtaceae
Calyptranthes blanchetiana O.Berg
Eugenia barrana Sobral
Eugenia beruttii (Mattos) Mattos
Eugenia fissurata Mattos
Eugenia itacarensis Mattos *
Eugenia longifolia DC.
Eugenia serraegrandis Sobral
Eugenia sessilifolia DC.
Eugenia unana Sobral
Marlierea lealcostae G.M.Barroso & Peixoto
Marlierea verticillaris O.Berg
Myrcia abrantea (O.Berg) E.Lucas & Sobral
Myrcia cataphyllata M.F.Santos
Myrcia felisbertii (DC.) O.Berg
Myrcia gigantea (O.Berg) Nied.
Myrcia grazielae NicLugh.
Myrcia marianae Staggemeier & E.Lucas
Myrcia monoclada Sobral
Myrcia pendula Sobral
Myrcia pseudomarlierea Sobral
Myrcia raminfinita L.Marinho & E.Lucas
Myrcia spathulifolia Proença
Myrcia stigmatosa O.Berg
Myrcia teimosa Sobral
Myrcia tetraphylla Sobral
Myrcia thomasii B.S.Amorim & A.R.Lourenço

Continuation Table 1.

Myrcia truncata Sobral
Plinia callosa Sobral *
Plinia longiacuminata Sobral
Plinia muricata Sobral *
Plinia rara Sobral *
Plinia spiciflora (Nees & Mart.) Sobral
Nyctaginaceae
Neea alumnorum M.Pignal, Soares Filho & Romaniuc
Neea duckei (Huber) Furlan
Ochnaceae
Ouratea bahiensis Sastre *
Ouratea gigantophylla (Erhard) Engl.
Ouratea longipes Sastre *
Ouratea papulosa Sastre
Ouratea platicaulis Sastre *
Orchidaceae
Anathallis velvetina Luer & Toscano
Aspasia silvana F.Barros *
Bifrenaria silvana V.P.Castro *
Brassavola reginae Pabst
Bulbophyllum teimosense E.C.Smidt & Borba
Cattleya alaorii (Brieger & Bicalho) Van den Berg
Cattleya grandis (Lindl. & Paxton) A.A.Chadwick
Cattleya kerrii Brieger & Bicalho *
Cirrhaea silvana V.P.Castro & Campacci *
Coryanthes bahiensis Marçal & Chiron
Coryanthes buerarensis Campacci & Bohnke
Elleanthus hymenophorus (Rchb.f.) Rchb.f.
Encyclia fimbriata C.A.Bastos, Van den Berg & Meneguzzo
Epidendrum garciae Pabst
Gomesa silvana (V.P.Castro & Campacci) M.W.Chase & N.H.Williams
Gongora meneziana V.P.Castro & G.Gerlach
Koellensteinia abaetana L.P.Queiroz
Leptotes bohnkiana Campacci
Masdevallia sururuana Campacci
Pabstiella dasilvae Chiron & Xim.Bols.
Promenaea silvana F.Barros & Cath. *
Prosthechea bohnkiana V.P.Castro & G.F.Carr
Prosthechea buerarensis (Campacci) Campacci
Stanhopea buerarensis Campacci & Marçal
Stenia bohnkiana V.P.Castro & G.F.Carr
Oxalidaceae
Oxalis alata var. *hirta* Lourteig *
Oxalis bela-vitoriae Lourteig *
Oxalis kuhlmannii var. *adpressipila* Lourteig

Continuation Table 1.

Passifloraceae
Passiflora igrapiunensis T.S.Nunes & L.P.Queiroz
Phyllanthaceae
Discocarpus pedicellatus Fiaschi & Cordeiro
Phyllanthus carvalhoi G.L.Webster
Picramniaceae
Picramnia coccinea W.W.Thomas *
Piperaceae
Peperomia epipremnifolia D.Monteiro & Leitman
Peperomia riparia Yunck.
Peperomia serpentarioides Miq.
Peperomia sulbahiensis D.Monteiro & M.Coelho
Piper bahianum Yunck.
Piper robustipedunculum Yunck.
Piper vellosoi Yunck.
Poaceae
Alvimia auriculata Soderstr. & Londoño *
Alvimia gracilis Soderstr. & Londoño *
Anomochloa marantoidea Brongn. *
Arberella bahiensis Soderstr. & Zuloaga *
Atractantha cardinalis Judz. *
Atractantha radiata McClure *
Chusquea clemirae A.C.Mota, R.P.Oliveira & L.G.Clark
Diandrolyra pygmaea Soderstr. & Zuloaga ex R.P.Oliveira & L.G.Clark
Digitaria doellii Mez *
Eremetis robusta Hollowell, F.M.Ferreira & R.P.Oliveira
Eremocaulon aureofimbriatum Soderstr. & Londoño *
Ichnanthus longhi-wagnerae A.C.Mota & R.P.Oliveira
Merostachys annulifera Send.
Merostachys argentea Send.
Merostachys lanata Send.
Merostachys magnispicula Send.
Merostachys medullosa Send.
Merostachys ramosissima Send.
Olyra bahiensis R.P.Oliveira & Longhi-Wagner
Olyra filiformis Trin.
Olyra latispicula Soderstr. & Zuloaga *
Parianella carvalhoi (R.P.Oliveira & Longhi-Wagner) F.M.Ferreira & R.P. Oliveira
Parianella lanceolata (Trin.) F.M.Ferreira & R.P.Oliveira
Paspalum restingense Renvoize *
Paspalum strigosum Döll
Piresia palmula M.L.S.Carvalho & R.P.Oliveira
Raddia distichophylla (Schrad. ex Nees) Chase *
Raddia stolonifera R.P.Oliveira & Longhi-Wagner

Continuation Table 1.

Polygalaceae
Caamembeca martinelli (Marques & E.F.Guim.) J.F.B.Pastore *
Caamembeca martinelli var. *carnosa* (Marques & E.F.Guim.) J.F.B.Pastore
Securidaca revoluta (A.W.Benn.) Marques
Primulaceae
Cybianthus nemoralis (Mez) G.Agostini
Rubiaceae
Chomelia bahiae J.H.Kirkbr.
Denscantia andrei (E.L.Cabral & Bacigalupo) E.L.Cabral & Bacigalupo *
Denscantia macrobracteata (E.L.Cabral & Bacigalupo) E.L.Cabral & Bacigalupo *
Faramea bicolor J.G.Jardim & Zappi
Faramea nocturna J.G.Jardim & Zappi
Ixora bahiensis Benth.
Ixora cabraliensis Di Maio et Peixoto
Malanea harleyi J.H.Kirkbr. *
Mitracarpus anthospermoides K.Schum.
Posoqueria bahiensis Macias & Kin.-Gouv.
Psychotria martiusii Müll.Arg.
Psychotria megalocalyx Müll.Arg.
Psychotria salzmanniana Müll.Arg.
Psychotria silvicola Müll.Arg.
Psychotria strigosa Müll.Arg.
Psychotria wawrana Müll.Arg.
Rudgea hileiabaiana Zappi & Bruniera
Rudgea ilheotica Müll.Arg.
Rudgea involucrata Müll.Arg.
Rudgea malpighiacea Standl.
Rudgea mouririoides Standl.
Salzmannia arborea J.G. Jardim
Sphinctanthus insignis Steyerm.
Rutaceae
Andreadoxa flava Kallunki *
Conchocarpus concinnus Kallunki *
Conchocarpus dasyanthus Kallunki *
Conchocarpus fissicalyx Pirani *
Conchocarpus gaudichaudianus subsp. *bahiensis* Kallunki *
Conchocarpus hirsutus Pirani *
Conchocarpus inopinatus Pirani *
Conchocarpus longipes Kallunki *
Conchocarpus mastigophorus Kallunki *
Conchocarpus modestus Kallunki *
Conchocarpus punctatus Kallunki *
Conchocarpus racemosus (Nees & Mart.) Kallunki & Pirani

Continuation Table 1.

Conchocarpus santosii Pirani & Kallunki *
Ertela bahiensis (Engl.) Kuntze
Galipea revoluta Pirani
Neoraputia calliantha Kallunki
Neoraputia micrantha Kallunki
Zanthoxylum nemorale Mart.
Zanthoxylum retusum (Albuq.) P.G.Waterman
Sapindaceae
Cardiospermum integerrimum Radlk. *
Paullinia livescens Radlk.
Paullinia unifoliolata Perdiz & Ferrucci
Serjania morii Acev.-Rodr.
Serjania scopulifera Radlk.
Sapotaceae
Chromolucuma apiculata Alves-Araújo & M.Alves
Pouteria atlantica Alves-Araújo & M.Alves
Pouteria glauca T.D.Penn.
Pouteria synsepala Popovkin & A.D.Faria
Pouteria trifida Alves-Araújo & M.Alves
Pradosia longipedicellata Alves-Araújo & M.Alves
Verbenaceae
Citharexylum obtusifolium Kuhlm.
Stachytarpheta hirsutissima Link
Vochysiaceae
Vochysia talmonii M.C.Vianna et al.

The occurrence of only 12 endemic species of *Conchocarpus* in the present checklist probably indicates that several endemic species of this genus are exclusively found at more inland seasonally dry forests (Kallunki & Pirani 1998).

Among the endemic genera from southern Bahia and northern Espírito Santo reported by Thomas et al. (2003), such as *Arapatiella* and *Brodriguesia* (Fabaceae), *Atractantha*, *Alvimia* and *Sucrea* (Poaceae), and *Trigoniodendron* (Trigoniaceae), several are not endemic to BCF. For example, *Brodriguesia*, *Atractantha* and *Trigoniodendron* have been recorded outside our study area, respectively, in Sergipe, Amazonas, and near the Doce River. Similarly, *Physeterostemon* R.Goldenb. & Amorim (Melastomataceae), a recently described endemic genus from the Atlantic Forest of Bahia state (Amorim et al. 2014, Goldenberg et al. 2016, Goldenberg & Amorim 2006), was not considered endemic to BCF in this checklist, as among its species *P. gomesii* Amorim & R.Goldenb. occurs in the municipality of Boa Nova (Amorim et al. 2014), which lies slightly outside our study area.

In comparison with the preliminary list of Thomas et al. (2003), which included 395 endemic angiosperm taxa from an area that covered a larger part of northern Espírito Santo state, as far as the Doce River, and the southern Bahia Atlantic Forest, including more inland areas, and almost reaching the municipality of Salvador (i.e., an area ca. 20% larger than ours) (Figure 1a), the list presented here surprisingly

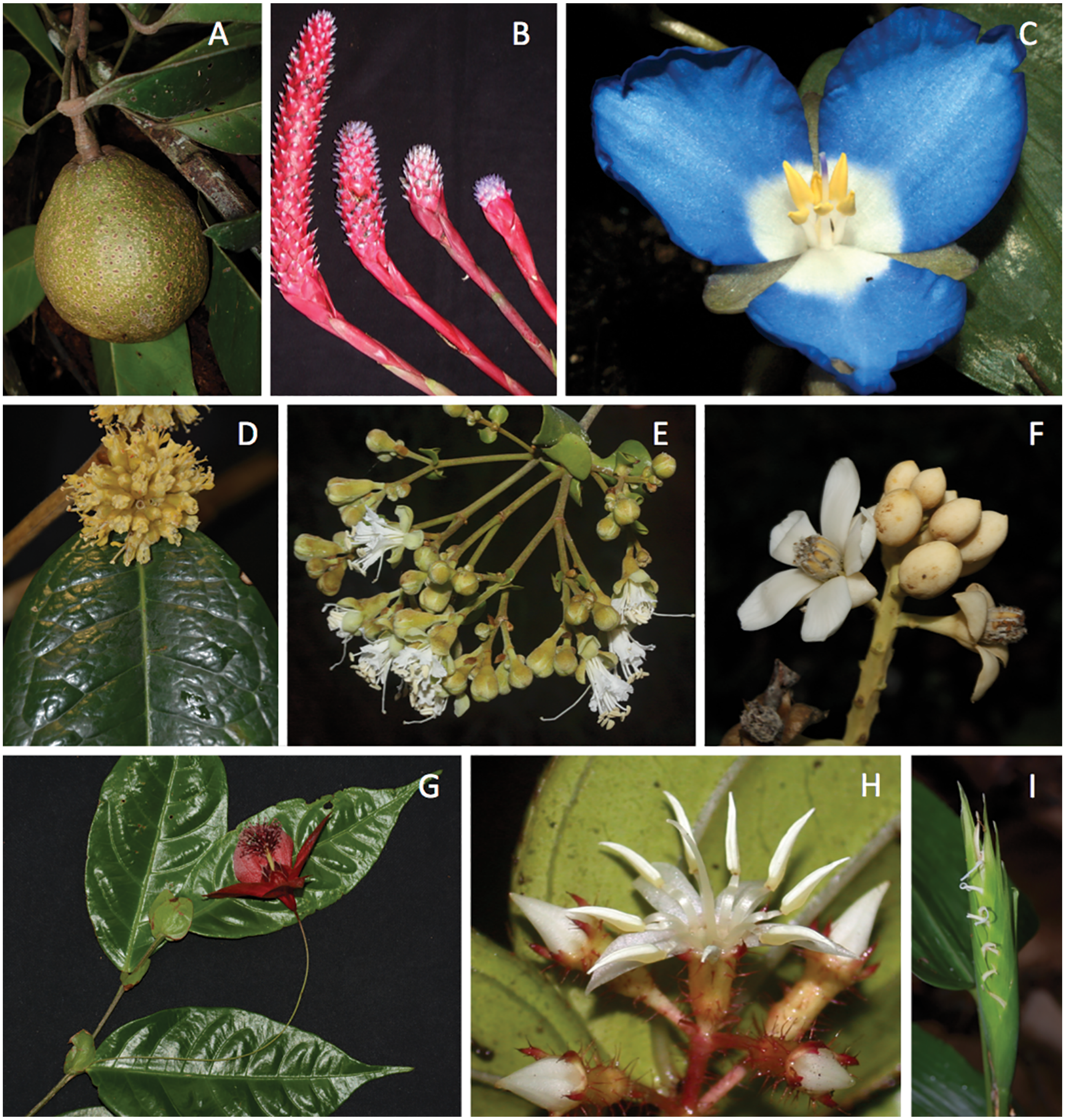

Figure 2. Some species found in the checklist of endemic angiosperms of BCF: a) *Kuhlmanniodendron macrocarpum* (Achariaceae). b) *Quesnelia koltesii* (Bromeliaceae). c) *Dichorisandra leucophtalmos* (Commelinaceae). d) *Tapura zei-limae* (Dichapetalaceae). e) *Arapatiella psilophylla* (Fabaceae). f) *Harleyodendron unifoliolatum* (Fabaceae). g) *Pavonia goetheoides* (Malvaceae). h) *Pleiochiton amorimii* (Melastomataceae). i) *Anomochloa marantoidea* (Poaceae). Photos: a, b, d, g, h) A.Amorim; c) L.Aona; e, f) D.Cardoso; i) J.Jardim.

shares only 143 taxa (Table 1). If we had used the Doce River as our area southern limit, it would have included at least 45 additional species restricted to the Linhares region, such as *Cryptanthus beuckeri* E.Morren (Bromeliaceae), *Rourea luizalbertoi* Forero, L.A.Vidal & Carbonó (Connaraceae), *Simira eliezeriana* Peixoto (Rubiaceae) and the endemic genus *Riodocea* Delprete (Thomas et al. 2003, Rolim et al. 2016). Despite being geographically close and having several species in common (Saiter et al. 2016b), the vegetation of Linhares and the Doce River floodplain are ecologically different from the BCF (Rolim et al. 2005, 2006). In floristic terms, the Linhares region may be more similar to northern Rio de Janeiro, southern Espírito Santo (Silva & Nascimento 2001, Saiter et al. 2016b) and eastern Minas Gerais states (see Krenák-Waitaká Forests *sensu* Saiter et al. 2016a).

Another difference between the two checklists is due to the time gap of about 15 years between studies. A total of 174 species in our checklist were described after 2003, probably due to increased efforts in collecting and studying plants in the Atlantic Forest in Bahia. For instance, about 41.5% of all new angiosperms in the Brazilian flora described between 1990 and 2006 are endemic to the Atlantic Forest (Sobral & Stehmann 2009). Moreover, 23 species of angiosperms endemic to BCF were described between 2015 and 2017 (e.g., Araújo et al. 2015, Santos et al. 2015, Abreu & Giulietti 2016, Amorim et al. 2016, Côrtes et al. 2016, Ferreira et al. 2016, Marinho & Lucas 2016, Popovkin et al. 2016, Ribeiro et al. 2016, Terra-Araujo et al. 2016, Daly & Melo 2017, Gonçalez et al. 2017).

Compared to the results of a search in the Brazilian Plant List (Flora do Brasil 2020, under construction) using the following filters: "group: angiosperms", "endemism: only endemic to Brazil", "occurrence: only occurs in", "origin: native", "state: Bahia", and "phytogeographic domain: Atlantic Forest", the number of species in our checklist (547 spp.) was much smaller than the 795 species identified by the search engine. Among the reasons for this large difference (248 spp.) it is worth mentioning that a) more than 50 taxa from our list had to be removed due to lack of precise geospatial data, such as the municipality name, which precluded us from citing them as endemic to BCF; b) among the taxa exclusive to the Brazilian Plant List (Flora do Brasil 2020, under construction) that are not restricted to BCF, several occur in more inland seasonally dry forests in the Atlantic Forest, such as *Chrysophyllum subspinosum* Monach. (Sapotaceae) and *Colicodendron bahianum* Cornejo & Iltis (Capparaceae); and c) at least 29 species exclusive to the Brazilian Plant List search occur close to, but outside the study area, such as *Canistrum camacaense* Martinelli & Leme (Bromeliaceae), *Inga grazielae* (Vinha) T.D.Penn. (Fabaceae), and *Bertolonia carmoi* Baumgratz (Melastomataceae), all from the municipality of Boa Nova, and *Aechmea bicolor* L.B.Sm. (Bromeliaceae), *Heteropterys jardimii* Amorim (Malpighiaceae), and *Passiflora timboensis* T.S.Nunes & L.P.Queiroz (Passifloraceae), from the municipality of Santa Teresinha.

There are additional reasons to anticipate that the difference in species numbers between the Brazilian Plant List and our list could be even greater, as the list presented here includes 84 taxa that also occur in northern Espírito Santo and in a small area in northeastern Minas Gerais (Figure 1c). Moreover, the Brazilian Plant List (Flora do Brasil 2020, under construction) is an ongoing project on which new taxa can be continuously added and identified as endemic, what could bring the total number of endemic angiosperm species using the same filters even larger. Alternatively, if the native distribution of several taxa is shown to extend beyond the Atlantic Forest domain in Bahia, the difference between the lists could decrease, as fewer species from the Brazilian Plant List would appear as endemic while applying the same search filters.

Developing knowledge on patterns of endemism for plant species is extremely important and must be taken into account in conservation strategies. This information is relevant for the establishment of new protected areas (Kerr 1997), restoration policies (Chazdon 2008) and in directing additional studies (Francisco-Ortega et al. 2010). Considering the key role of endemic species in the design of conservation policies (Myers et al. 2000, Van Der Werff & Consiglio 2004, Moraes et al. 2005, Lamoreux et al. 2006, Martinelli et al. 2008, Essl et al. 2009, Nowak & Nobis 2010), we expect this checklist could guide future studies and conservation strategies, as well as emphasize the importance of the BCF region as a key area for plant conservation.

Supplementary material

The following online material is available for this article:
Appendix 1 – Taxonomic studies and specialists consulted by family.

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Author Contributions

Piera Ostroski: substantial contribution in the concept and design of the study; contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Felipe Saiter: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

André Amorim: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Pedro Fiaschi: substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision augmenting intellectual content.

Conflicts of interest

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

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Inventory

Inventory of ferns and lycophytes within forest fragments of Araucaria in southern Brazil

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Abstract: The Araucaria Forest is a unique plant formation, practically restricted to the Atlantic Forest biome, in the South and Southeast of Brazil. It is immensely fragmented and its area is reduced to a minimum fraction, from 13 to 2% of the original area, due to intense anthropic exploitation. Our study evaluated the richness and floristic composition of ferns and lycophytes within three Araucaria forest fragments, in Rio Grande do Sul, Brazil. For the floristic inventory, a parcel of 1 ha (100x100m) was demarcated inside three fragments, which were classified by size: large (246 ha - H1LF), medium (57 ha - H2MF) and small (5.2 ha - H3SF). Occurring species life forms and preferential substrates up to 4 meters above ground were recorded. In total, 55 species were catalogued, only two of which were lycophytes, distributed in 32 genera and 17 families. Considering the area of fragments, the recorded species richness has decreased, presenting greater numbers in H1LF (48), followed by H2MF (33), and H3SF (29). In the three sites, terrestrial substrate and the hemicryptophyte species life form predominated. We observed that one hectare of a larger forest fragment presented 65% more species than that of a smaller one, considering that the smaller fragment total area represents only 2% of the larger forest's area. On the other hand, one hectare of the smaller fragment contributed 17% to fern and lycophyte species conservation occurring in Brazilian Araucaria Forests, and 8% to the state of Rio Grande do Sul's total richness. The diverse heterogeneity and species composition grants floristic identity to each of the studied forest interiors. Our results highlight the need to include the botanical parameters here analyzed in management, conservation degree evaluation, and maintenance of Araucaria Forests plant biodiversity.

Keywords: *Mixed Ombrophilous Forest, fragmentation, seedless vascular plants, floristic inventory.*

Inventário de samambaias e licófitas em interiores de fragmentos de Floresta com Araucária no Sul do Brasil

Resumo: A Floresta com Araucária é uma formação vegetal única do planeta com ocorrência praticamente restrita ao bioma Mata Atlântica, no Sul e Sudeste brasileiro. Ela está imensamente fragmentada e sua área reduzida a uma fração mínima, de 2 a 13% da cobertura original, devido à intensa exploração antrópica. Nosso estudo avaliou a riqueza e a composição florística de samambaias e licófitas, no interior de três fragmentos de Floresta com Araucária, no Rio Grande do Sul, Brasil. Para o inventário florístico, foi demarcada uma parcela de 1 ha (100x100m) no interior de três fragmentos, os quais, por sua vez, foram classificados por tamanho: grande (246 ha - H1LF), médio (57 ha - H2MF) e pequeno (5,2 ha - H3SF). Foram registradas a forma de vida e o substrato preferencial das espécies ocorrentes até 4 m de altura do solo. Ao total, foram inventariadas 55 espécies, das quais apenas duas licófitas, distribuídas em 32 gêneros e 17 famílias. Considerando a área dos fragmentos, a riqueza registrada de espécies foi decrescente, sendo maior no H1LF (48), seguida do H2MF (33) e H3SF (29). Nos três sítios predominaram as espécies no substrato terrícola e a forma de vida hemicriptófita. Observamos que um hectare do fragmento florestal grande apresenta 65% mais espécies que o de um pequeno, considerando que a área total do fragmento menor representa apenas 2% da área do maior. Por outro lado, o hectare do fragmento pequeno contribui para a conservação de 17% das espécies de samambaias e licófitas ocorrentes em Floresta com Araucária no Brasil e 8% da riqueza total para o Estado do Rio Grande do Sul. A heterogeneidade na riqueza e na composição de espécies confere identidade florística própria a cada um dos interiores florestais estudados. Os nossos resultados destacam a necessidade da inclusão dos parâmetros botânicos analisados no manejo, na avaliação do grau de conservação e na manutenção biodiversidade vegetal da Floresta com Araucária.

Palavras-chave: *Floresta Ombrófila Mista, fragmentação, plantas vasculares sem sementes, inventário florístico.*

Introduction

Araucaria Forest (AF) or Mixed Ombrophilous Forest (MOF) is a unique vegetation formation on the planet (Koch 2002), practically restricted to the Atlantic Forest biome in South and Southeast Brazil (Backes, 2009). It is estimated that, originally, this forest covered an extension of 200 thousand km², immensely fragmented due to the intense exploration occurred from the 19th century onwards. Araucaria Forest territory is reduced to a minimal fraction (2 to 13% of the original area) of which only 3% are within conservation units (Ribeiro et al. 2009). *Araucaria angustifolia* (Bertol.) Kuntze is an arboreal species that stands out in the upper stratum and confers typical and unique characteristics to the forest structure composition (Kozera et al. 2006). Another typical species of MOF is the *Dicksonia sellowiana* Hook., which is a tree fern found more frequently in areas with high araucaria density (Fernandes 2000). In addition to the fact that they harbor characteristic species and unique biological attributes (Oliveira-Filho et al. 2013), Araucaria Forest remaining areas have been considered an environmental conservation priority (Duran & Peixe 2008).

Habitat fragmentation is a global process and possibly the most intense man-made change to the environment, especially in tropical regions (Harris 1984). The Atlantic Forest biome presents 83% of the remaining fragments with less than 50 ha total area (Ribeiro et al. 2009), expressively disturbed, isolated, poorly protected (Viana 1995), and poorly studied (Safford 2007). The impacts are a consequence of this biome's use and occupation history, which houses the largest Brazilian industrial cities and centers (MMA 2000). According to Rands e Whitney (2010), fragmentation of forests is threatening the sustainability of their interior environment, along with their inherent ecological attributes and functions. Consequently, maintaining many forest ecological values requires the maintenance of forest interiors.

In Brazil, the most abundant and endemic species in the Atlantic Forest biome, in the South and Southeastern regions, especially in the Dense and Mixed Ombrophilous Forests (Sehnem 1979), are seedless vascular plants (Smith et al. 2006). Considering ferns and lycophytes, these regions are among the most diverse and endemic on the planet (Brummitt et al. 2016). A total of 1,313 are described for Brazil, being 1,143 ferns species and 170 lycophytes, out of which 38% are endemic (Prado & Sylvestre 2016). These two groups are Brazilian flora's most threatened plants (Martinelli & Moraes 2013).

Ferns and lycophytes have developed a number of adaptations throughout their evolution in relation to the substrate (Windisch 1992), and this biological diversity demonstrates these plants' ability to inhabit different environments (Senna & Waechter 1997). Among ferns and lycophytes floristic inventories in Araucaria Forests in Rio Grande do Sul, we highlight the study by Silva & Schmitt (2015), who verified a significantly greater richness in the interior environment. Blume et al. (2010) collected a total of 42 ferns and lycophytes samples in a unit of 1ha inside the forest interior. These are one of the most important forest components (Costa 2004), representing up to 80% of the herbaceous stratum vegetative cover in Seasonal Forests (Inácio & Jarenkow 2008).

The objectives of the present study were: (1) to evaluate ferns and lycophytes richness and floristic composition within three Araucaria Forest fragments in Rio Grande do Sul; (2) to analyze life forms and preferential substrates of the species, and (3) to compare, floristically, these sites with other inventories of the same sample size.

Material and Methods

1. *Study Area*

The study was conducted in Aparados da Serra National Park (ASNP) in Cambará do Sul (29°07'58.53"S and 50°06'18.89"W, 1024m altitude), Rio Grande do Sul, Brazil. The Park, created in 1959, has an area of 13,141.05ha, and is bordered by the Serra Geral National Park both to the south and north, and the two units are managed by Ministry of the Environment's Chico Mendes Institute for Biodiversity Conservation (ICMBio). According to the Köppen-Geiger classification, regional climate is Cfb, that is, humid continental (C), with rains distributed during all months of the year (f), with average temperature of the warmest month being inferior to 22ºC (b) (Peel et al. 2007). The soil of the region is shallow to deep, classified as Cambissolo Húmico Alumínico, associated with a litolical neosol. Because an area of high rainfall and low temperatures, organic matter accumulation is favored (Streck et al. 2008).

Three plots of 1 ha (100x100m) were demarcated for the inventory, within three Araucaria Forest fragments of different sizes, being the matrix habitat composed of natural fields. The large fragment (LF) has a total area of 246 ha (29°08'04.09"S and 50°07'06.86"W), the medium-sized fragment (MF) has a total area of 57 ha (29°07'10.43"S and 50°06'54.53"W), and the small fragment (SF) has 5.2 ha (29°07'58.70"S and 50°06'17.89"W), referred to as H1LF, H2MF and H3SF, respectively (Figure 1).

2. *Collection and Identification of Botanical Material*

During one year, periodic expeditions to the sample units were carried out in order to perform the floristic inventory. This survey was made on the hectare demarcated through observation of ferns and lycophytes occurring in the herbaceous and epiphytic strata (up to 4m in height). Fertile specimens were collected according to techniques proposed by Windisch (1992). The samples were deposited at *Herbarium Anchieta* (PACA), São Leopoldo, Rio Grande do Sul. The taxonomic identification was made through specialized bibliography, comparisons with material determined in herbarium and by specialist consultation. Species classification in families and botanical genera adopted the system suggested by Schuettpelz et al. (2016). The plants were evaluated for life forms following Raunkiaer (1934), and adaptations of Mueller-Dombois & Ellenberg (1974), and Senna & Waechter (1997). As to their preferential substrate, they were classified as terrestrial (species that occur exclusively in the soil), corticicolous (species that occur in tree bark) or hemicorticicolous (species that establish themselves in the soil and grow in the phorophyte) as proposed by Mynssen (2000), modified by Athayde Filho & Windisch (2006).

3. *Statistical analysis and comparison with other studies*

For the floristic composition similarity analysis of the three sites studied, a binary matrix was constructed, representing the species' presence or absence in each sample unit. A grouping analysis was carried out using the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) and the Sorensen index (BRAY-CURTIS) with a cophenetic correlation of 0,99 (Gotelli & Ellison 2001), in the Paleontological Statistical Program Statistics - PAST (Hammer et al. 2001) version 3.0. Five other sites with the same sample area were

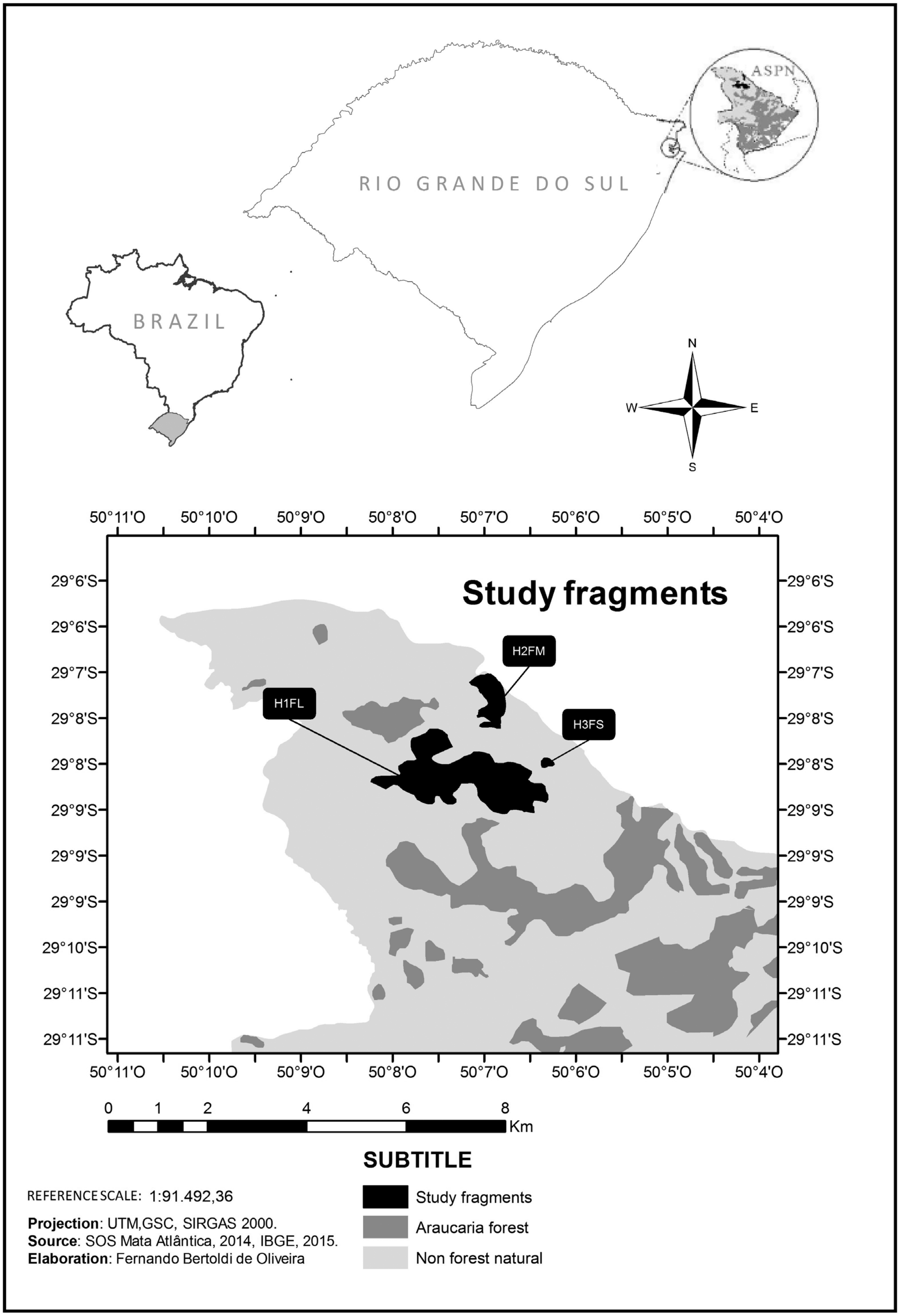

Figure 1. Location of the Araucaria forest fragments showing H1LF, H2MF and H3SF sites in the Aparados da Serra National Park (ASNP), State of Rio Grande do Sul, Brazil.

included for comparison purposes: Blume et al. (2010), in Araucaria Forest; Burmeister & Schmitt (2016) and Athayde-Filho & Windisch (2006), both on the coastal plain, in Rio Grande do Sul; the inventory of Dittrich et al. (2005), carried out in Dense Ombrophilous Forest (DOF), in Paraná, also in southern Brazil; and the Poulsen & Nielsen (1995) study, in an Ecuadorian Tropical Rainforest.

Results

The total floristic survey indicated the occurrence of 55 species, of which 53 are ferns and two lycophytes, represented 38 genera and 17 families (Table 1). Among the sites studied, H1LF had the highest number of species (48). H2MF had similar richness to H3SF, with 33 and 29 species, respectively. The three fragments registered 20 species in common, and in H1LF, 14 species were exclusive (not found in the other two sites), whereas in H3SF and H2MF there were only one and four exclusive species, respectively.

The most represented families in the three fragments were Polypodiaceae (10 species), followed by Hymenophyllaceae (seven), Aspleniaceae (seven) and Thelypteridaceae (six). Together these four families accounted for 56.36% of the total surveyed species. Seven families were represented by only one species (Table 1). The richest genera were *Asplenium* (seven species), *Amauropelta* (five species), *Hymenophyllum*, *Pecluma* and *Pleopeltis* (three species).

Regarding the type of preferential substrate, considering the three fragments, the terrestrial rate predominated with 29 species registered (approximately 52.72%), followed by corticicolous, 25 species (45.45%), and one hemicorticicolous species (1.81%). Separately, the terrestrial species (H1LF: 54.16%, H2MF: 57.57% and H3SF: 51.72%) also predominated.

Among the 24 hemicryptophyte species, 16 presented rosulate growth and eight reptant growth. From the corticicolous species, 21 were reptant and three rosulate. The two phanerophyte species were of rosulate growth, as were the two camephyte species.

The analysis of floristic similarity (Table 2) evidenced the formation of two groups (A and B) (Fig. 2). The demarcated hectare's floristic composition in the Ecuadorian rain forest presented the lowest similarity, forming an independent group (A) and sharing only *Vittaria lineata* (L.) Sm. with the other studies. Group B integrated all inventories made in Brazil and shared *Pleopeltis hirsutissima* (Raddi) de la Sota and *V. lineata*. The Ca subgroup was composed by all the inventories made in Araucaria Forests, and the present study's three sites formed subgroup (Db), which presented the highest similarity sharing 20 species. The survey by Blume et al. (2010), also in Araucaria Forest (Da), shared 21 species with H1LF, whereas 18 species were shared with H2MF and H3SF. Both inventoried hectares in the coastal plain of Rio Grande do Sul remained in the same subgroup (Cb).

Discussion

Ferns and lycophytes richness demonstrated the importance of studying AF interiors for spore-producing vascular plant biodiversity conservation. Our results corresponded to 40% of the species cited for Araucaria Forests in Rio Grande do Sul, as well as 14.62% of the total species recorded in this state. The number of fern and lycophyte families sampled represented half of the 34 occurrences in Rio Grande do Sul (Flora do Brasil 2020). The fact that lycophytes are less representative when compared to ferns is related to this group's low richness, which currently represents less than 1% of all vascular plants (Smith et al. 2006).

H1LF richness is 45% greater than that of H2MF, and the forest area in which this second hectare is inserted corresponds to 23% of the first's area. This comparison indicates that fragments with greater forest interior areas have a tendency to present higher seedless vascular plant species richness. Other ferns and lycophytes studies show negative impacts on the composition, richness, diversity and abundance of these species in the fragmentation process and habitat loss (Paciencia & Prado 2005, Barros et al. 2006, Silva & Schmitt 2015). In general, ferns and lycophytes are known to inhabit wet and shaded sites in a variety of microenvironments (Kessler et al. 2011). Favorable environmental conditions may depend on both forest interior area size and natural environment preservation degree, since fragmentation tends to homogenize this type of ecosystem in its most impacted areas (Fahrig 2003; Cagnolo et al. 2006; Lôbo et al. 2011).

Comparing the present study's results with other surveys performed in a sample area of 1ha, it is evident that H1LF presented similar richness to that observed by Poulsen & Nielsen (1995) (S=50) in tropical forests in Ecuador. It was also close to the richness verified by Blume et al. (2010) (S = 42) in AF, Rio Grande do Sul. Only in the Dittrich et al. (2005) study in Paraná's Dense Ombrophilous Forest, species richness was higher (S=81) than that recorded in this study's sites, due to the high number of epiphytes (49 species), corroborating with Waechter (1998), who states that the richness of epiphytic flora decreases in more southern latitudes.

In H3SF, richness (S=29) was similar to that recorded in Rio Grande do Sul's coastal region by Burmeister & Schmitt (2016) (S=28), and by Athayde-Filho & Windisch (2006) (S=26) in fragments with a total area similar to the ones presented here: 6 ha and 4 ha, respectively. These three sites shared only four species (*Cyathea atrovirens, Pleopeltis hirsutissima, Serpocaulon catharinae* (Langsd. and Fisch.) A.R.Sm. (Fig. 3) and *Vittaria lineata*). However, H3SF shared seven species with the other surveys. The three other sites are more floristically heterogeneous among themselves when compared to the three hectares inventoried in this study, which in turn shared 13 more species. In general, the fragment's spatial proximity increases the likelihood of propagule movements between them (Guevara & Laborde, 1993); therefore, the present study's three hectares were more floristically homogeneous out of all the compared sites.

AF and coastal sites that have formed distinct subgroups have different climatic conditions. In Campos de Cima da Serra, where our AF hectares were located, the average monthly temperature is lower than the average in the coastal plain (Peel et al. 2007). Thus, it is evident that, besides forest type, climate was also important for floristic differentiation. It is important to evaluate that in spite of these vegetative and climatic differences between mountain and coastal plain, some species can occupy different niches due to their functional characteristics, which makes them generalist, plastic, of wider distribution, and, therefore, resilient to the fragmentation processes. Morphological properties such as small surface and coriaceous leaf texture, thick rhizome with water reserve, and nutrients

Table 1. Fern and lycophyte species distribution in the three Araucaria Forest sites of Rio Grande do Sul, grouped by family, with respective life form and preferential substrate.

FAMILY/SPECIES	LIVE FORM	SUBSTRATE	H1LF	H2MF	H3SF
FERNS					
ANEMIACEAE					
Anemia phyllitidis (L.) Sw.	HCR/ROS	TER		X	
ASPLENIACEAE					
Asplenium claussenii Hieron.	HCR/ROS	TER		X	
Asplenium gastonis Fée	EPI/ROS	COR		X	X
Asplenium harpeodes Kunze	HCR/ROS	TER	X	X	X
Asplenium incurvatum Fée	EPI/REP	COR	X		
Asplenium martianum C.Chr.	HCR/ROS	COR	X	X	
Asplenium serra Langsd. & Fisch.	HCR/REP	COR	X		X
Asplenium ulbrichtii Rosenst.	EPI/ROS	COR		X	
ATHYRIACEAE					
Athyrium dombeyi Desv.	HCR/REP	TER	X		
Deparia petersenii (Kunze) M.Kato	HCR/REP	TER	X	X	X
BLECHNACEAE					
Blechnum auriculatum Cav.	HCR/ROS	TER	X	X	
Blechnum austrobrasilianum de la Sota	HCR/ROS	TER		X	X
Lomaridium acutum (Desv.) Gasper & V.A.O. Dittrich	HCR/REP	HCO	X		X
Lomaria spannagelii Rosenst. Gasper & V.A.O. Dittrich	CAM/ROS	TER	X		
Neoblechnum brasiliense (Desv.) Gasper & V.A.O. Dittrich	CAM/ROS	TER	X		
CYATHEACEAE					
Cyathea atrovirens (Langsd. & Fisch.) Domin	FAN/ROS	TER	X	X	X
DENNSTAEDTIACEAE					
Dennstaedtia globulifera (Poir.) Hieron.	GEO/RIZ	TER	X	X	X
Histiopteris incisa (Thunb.) J.Sm.	HCR/REP	TER	X		X
DICKSONIACEAE					
Dicksonia sellowiana Hook.	FAN/ROS	TER	X	X	X
DRYOPTERIDACEAE					
Elaphoglossum sellowianum (Klotzsch ex Kuhn) T. Moore	EPI/REP	COR	X	X	X
Lastreopsis amplissima (C.Presl) Tindale	HCR/REP	TER	X	X	X
Polystichum platylepis Fée	HCR/ROS	TER	X		X
Rumohra adiantiformis (G.Forst.) Ching	HCR/REP	COR	X	X	X
HYMENOPHYLLACEAE					
Polyphlebium pyxidiferum (L.) Ebihara & Dubuisson	EPI/REP	COR	X	X	
Didymoglossum ovale E.Fourn.	EPI/REP	COR	X		
Hymenophyllum caudiculatum Mart.	EPI/REP	COR	X	X	
Hymenophyllum polyanthos (Sw.) Sw.	EPI/REP	COR			X
Hymenophyllum rufum Fée	EPI/REP	COR	X		
Polyphlebium angustatum (Carmich.) Ebihara & Dubuisson	EPI/REP	COR	X	X	
Trichomanes anadromum Rosenst.	EPI/REP	COR	X		
LINDSAEACEAE					
Lindsaea botrychioides A.St.-Hil.	GEO/RIZ	TER	X	X	X

Continuation Table 1.

FAMILY/SPECIES	**LIVE FORM**	**SUBSTRATE**	**H1LF**	**H2MF**	**H3SF**
MARATTIACEAE					
Eupodium kaulfussii (J.Sm.) J.Sm.	HCR/ROS	TER	X		
OPHIOGLOSSACEAE					
Botrypus virginianus (L.) Michx.	GEO/RIZ	TER	X	X	
Campyloneurum aglaolepis (Alston) de la Sota	EPI/REP	COR	X		
POLYPODIACEAE					
Campyloneurum austrobrasilianum (Alston) de la Sota	EPI/REP	COR	X	X	X
Microgramma squamulosa (Kaulf.) de la Sota	EPI/REP	COR	X	X	X
Niphidium crassifolium (L.) Lellinger	EPI/REP	COR	X		
Pecluma pectinatiformis (Lindm.) M.G.Price	EPI/REP	COR	X	X	X
Pecluma recurvata (Kaulf.) M.G.Price	EPI/REP	COR	X		X
Pecluma sicca (Lindm.) M.G.Price	EPI/REP	COR	X	X	X
Pleopeltis hirsutissima (Raddi) de la Sota	EPI/REP	COR	X	X	X
Pleopeltis macrocarpa (Bory ex Willd.) Kaulf.	EPI/REP	COR	X		
Pleopeltis pleopeltidis (Fée) de la Sota	EPI/REP	COR	X	X	X
Serpocaulon catharinae (Langsd. & Fisch.) A.R.Sm.	EPI/REP	COR	X	X	X
PTERIDACEAE					
Adiantum raddianum C.Presl	HCR/ROS	TER	X		
Anogramma leptophylla Link	HCR/ROS	TER	X	X	
Vittaria lineata (L.) Sm.	EPI/REP	COR	X	X	X
THELYPTERIDACEAE					
Amauropelta amambayensis (Christ) Ponce	HCR/ROS	TER	X	X	X
Amauropelta decurtata (Link) de la Sota	HCR/ROS	TER	X		
Amauropelta recumbens (Rosenst.) Salino & T.E.Almeida	HCR/ROS	TER	X		X
Amauropelta retusa (Sw.) Pic.Serm.	HCR/ROS	TER	X	X	X
Amauropelta stierii (Rosenst.) Salino & T.E.Almeida	HCR/ROS	TER	X		
Goniopteris riograndensis (Lindm.) Ching	HCR/ROS	TER		X	
LYCOPHYTES					
LYCOPODIACEAE					
Phlegmariurus quadrifariatus (Bory) B.Øllg.	EPI/ROS	COR	X		
SELAGINELLACEAE					
Selaginella muscosa Spring	HCR/REP	TER	X	X	X
TOTAL SPECIES 55			48	33	29

Life forms: HCR/ROS: rosulate/hemicryptophyte, EPI/ROS: rosulate/epiphyte, EPI/REP: reptant/epiphyte; HCR/REP: reptant/hemicryptophyte; CAM/ROS: rosulate/camephyte; FAN/ROS: rosulate/phanerophyte; GEO/RIZ: rhizomatous/geophyte. Preferential substrate: TER= terrestrial; COR= corticicolous; HCO = hemicorticicolous. Scientific names were confirmed using the List of Species of Flora of Brazil (Flora do Brasil 2020).

Table 2. Ferns and lycophytes inventories carried out in a sample area of one hectare in different forest types

Acronyms	**Forest type**	**Number of species**	**Reference**
(AF-RS6)	Araucaria Forest	48	Present study (H1LF)
(AF-RS8)	Araucaria Forest	33	Present study (H2MF)
(AF-RS7)	Araucaria Forest	29	Present study (H3SF)
(AF-RS5)	Araucaria Forest	42	Blume et al. (2010)
(SF-RS4)	Swamp Forest	28	Burmeister & Schmitt (2016)
(RF-RS3)	Rain Forest	26	Athayde-Filho & Windisch (2006)
(RF-PR2)	Rain Forest - Paraná	81	Dittrich et al. (2005)
(RF-EC1)	Rain Forest - Ecuador	50	Poulsen & Nielsen (1995)

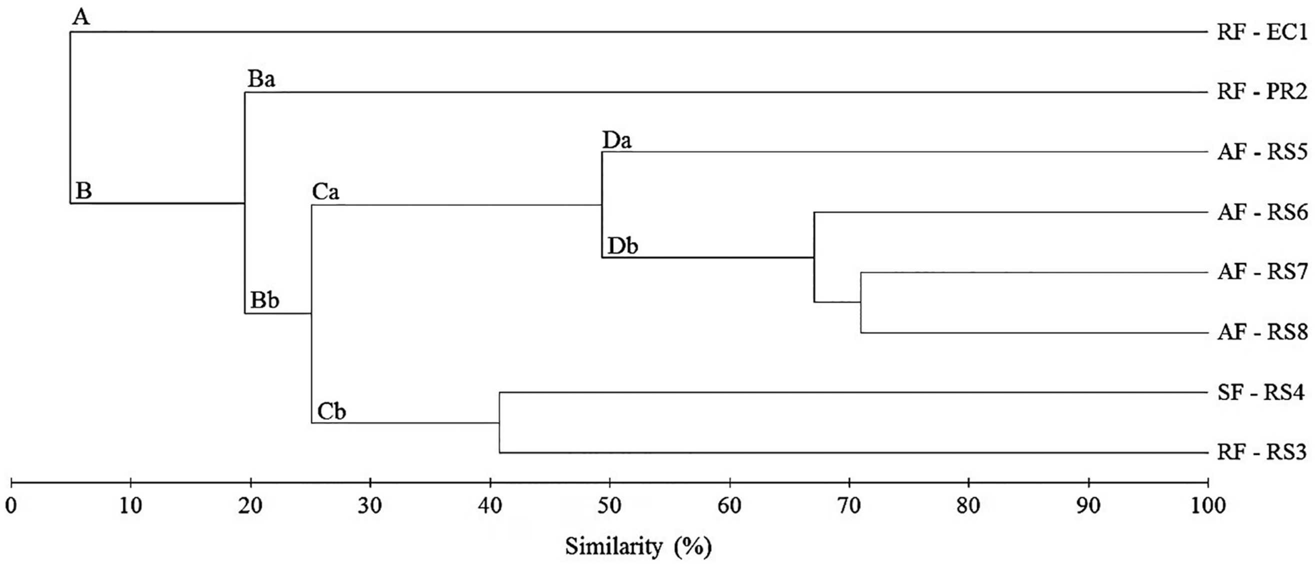

Figure 2. Dendrogram of floristic similarity between eight ferns and lycophytes inventories carried out in a sample area of one forest hectare. Araucaria Forest (AF), Rain Forest (RF), Swamp Forest (SF), Ecuador (EC), Paraná (PR), Rio Grande do Sul (RS). [1]Poulsen e Nielsen (1995), [2]Dittrich et al (2005), [5]Blume et al. (2010), [6,7,8]Present study, [4]Burmeister e Schmitt (2016) and [3]Athayde-Filho e Windisch (2006). Cophenetic correlation: 0.99.

in *Vittaria lineata, Serpocaulon catharinae* and *Pleopeltis hirsutissima* (Ranal 1993), may have favored the establishment of these species in those sites. Studies indicate that *P. hirsutissima* is a generalist species and *S. catharinae* occurs both in forest interior and in altered areas (Prado et al. 2010). The same is true for *C. atrovirens*, which was found in a variety of places, from locations conditioned to full sunlight to places with moderate shade, in forest areas, ravines and trenches along roads and highways (Lehn & Leuchtenberger 2008).

Analysis of fern and lycophyte communities' composition and their ecological aspects expresses the environmental quality of the studied fragments and the disturbance variation levels of these sites. Ferns and lycophytes are considered important indicators of environmental quality because they are directly affected by alterations (habitat loss and fragmentation) and are considered important ecological indicators because they are susceptible to environmental changes (Silva et al. 2018; Silva & Schmitt 2015; Mallmann et al. 2016). During field work, we observed that *Dicksonia sellowiana* officially threatened with extinction according to Ordinance No. 443 of December 17, 2014 (MMA 2014), presented only young individuals in H2MF, whereas in H1LF and H3SF they presented as adults. Considering that a 5m adult individual is approximately 90 years old, since the slow growth of that species (5.6cm year-[1]), according to estimation by Schmitt et al. (2009), the highest degree of conservation and succession is evident in the two last fragments. Corroborating with field observations, previous documented records indicate that only in 2001 the H2MF fragment was effectively incorporated into the Park Unit.

The removal of *D. sellowiana* individuals, one of the most typical AF species, not only uncharacterizes this forest formation (Fernandes 2000), but also reduces microhabitats availability for species that use their caudice as a preferential substrate, as is the case of *Polyphlebium angustatum* (Carmich.) Ebihara and Dubuisson and *Trichomanes anadromum* Rosenst. (Schmitt et al. 2005), recorded in the H1LF. In the absence of *D. sellowiana, P. angustataum* only occurred in H2MF because it used *Cyathea atrovirens*, another species of arborescent fern, as mechanical support.

H2MF also recorded the lowest epiphytic richness (42.42%) among the three forest interiors sampled, which refers to the shortest time period in which the fragment was legally protected. Epiphytic plants are considered to be excellent environmental quality indicators due to their physiological and nutritional characteristics (Bataghin et al. 2010), affecting the local preservation degree (Johansson 1989, Wolf 2005). In the comparison, including two sites, the largest number of species was shared between H2MF and H1LF.

H3SF recorded only one Hymenophyllaceae species, while H1LF presented six of the seven species inventoried for this family. Hymenophyllaceae comprises a group of 10% of fern species with green spores, which, due to their chlorophyll content, have limited viability of only a few weeks (Lloyd & Klekowski 1970), and are strongly dependent on microenvironments with constant humidity, making them more vulnerable to forest disturbances. However, because they have small spores, their chances of establishing themselves in favorable microhabitats increases, making them locally abundant and forming large colonies (Mehltreter 2010), as observed in H1LF.

Similarly to the present study, hemicryptophyte species prevalence was also observed by Blume et al. (2010) in fern and lycophyte communities inventoried in one AF hectare. Hemicryptophytes have a widespread occurrence in different environments (Schmitt & Goetz 2010) and can survive well under unfavorable climatic conditions since their buds are located at ground level and protected by scales or previous season's dry leaves (Caiafa & Silva 2005), which favors the this life form's establishment in low temperature environments (Raunkiaer 1934). However, this observation is not standard in inventories of seedless vascular plants, so much so that Poulsen e Nielsen (1995) recorded 50% epiphytes. Dittrich et al. (2005) found that this life form predominated in rain forests, totaling 60% of the richness. This last vegetation type is considered the most diverse in epiphytes in South-Brazilian forests, followed by AF (Roderjan et al. 2002).

Plant communities and species responses to fragmentation are known to vary in response to several factors (Laurance 1990, Ouborg 1993) and that forest area alone is an incomplete indicator of the forests'

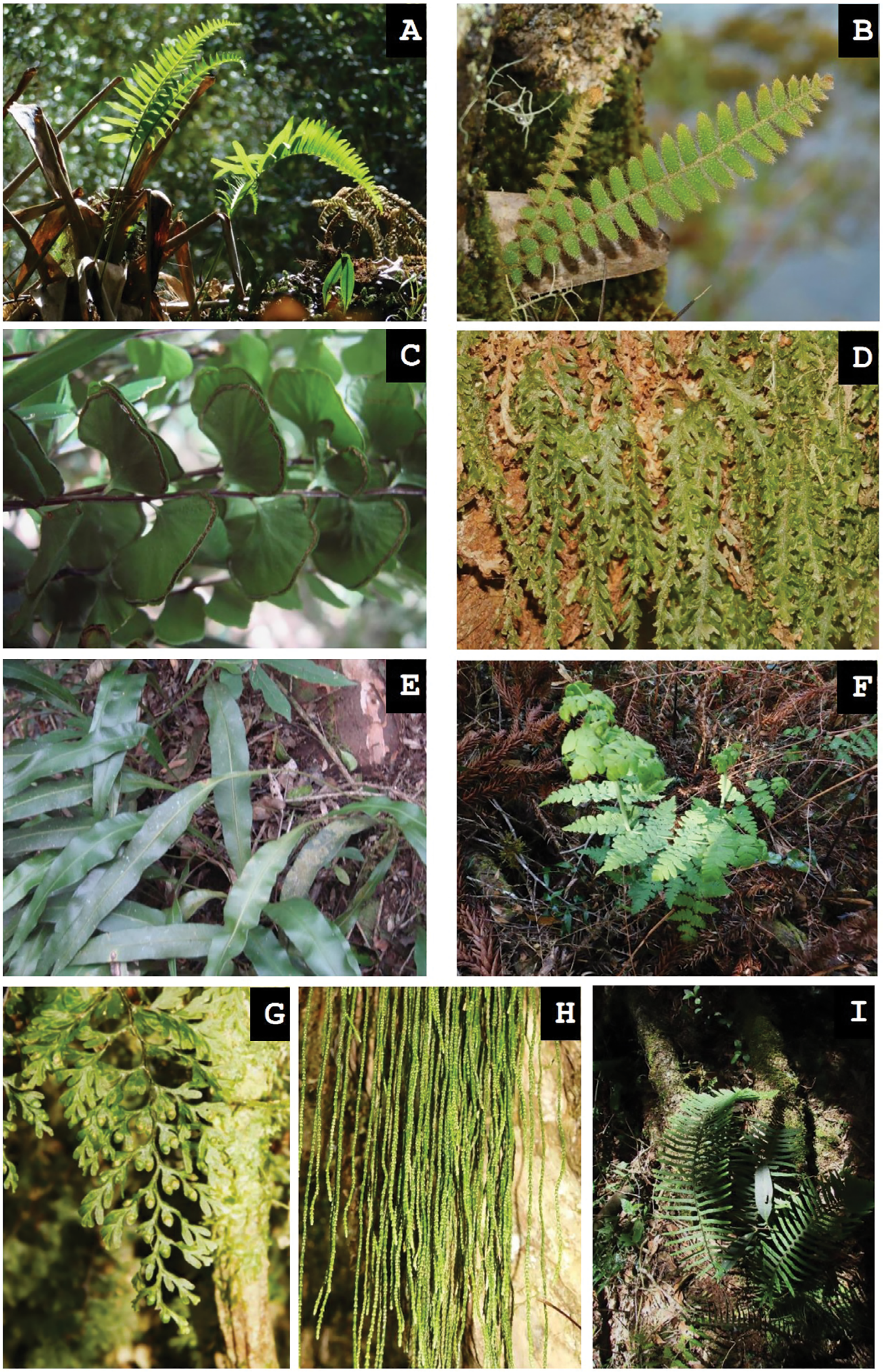

Figure 3. Fern and lycophyte species occurrences in the three Araucaria Forest sites of Rio Grande do Sul: A) *Serpocaulon catharinae*, B) *Pleopeltis hirsutissima*, C) *Lindsaea botrychioides*, D) *Trichomanes anadromum*, E) *Elaphoglossum sellowianum*, F) *Histiopteris incisa*, G) *Hymenophyllum polyanthos*, H) *Phlegmariurus quadrifariatus*, I) *Pecluma recurvata*.

ability to maintain ecological services (Chazdon 2008). Long-term experiments are needed to elucidate and predict long-term fragmentation effects (Haddad et al. 2015).

Based on the floristic survey, our study showed that H1LF was the site that presented the most favorable conditions for fern and lycophyte communities' development. We observed that a large forest fragment supports 65% more species than a small one, considering that the smaller fragment area represents only 2% of the LF area. Although its area is reduced, the SF presents a greater degree of conservation and succession than the MF. It also presents the less exposed soil, due to the greater coverage of the herbaceous stratum. This remnant represents a stepping stone in the displacement and the dispersion of species by the landscape, reducing the isolation between the larger fragments. Even a small fragment can contribute to fern and lycophyte conservation, since it sustains 17% of fern and lycophyte richness for this type of forest in Brazil, and 8% of total richness in Rio Grande do Sul, including one threatened species (*Dicksonia sellowiana*). Ferns and lycophytes species richness at local scale is determined by habitat heterogeneity, since even if there is no restriction for dispersion; the spores need a favorable microhabitat for germination (Mehltreter 2010). As in the present study, AF fragments floristic heterogeneity was also observed in surveys conducted in the State of São Paulo by Polisel et al. (2014), who analyzed the subsurface community of four fragments, and found significant differences in richness and diversity.

The set of the three forest fragments should be considered a priority for preservation and conservation. Richness and heterogeneity in species composition that confer their own floristic characteristics to each of the forest interiors studied, along with the critical conservation status of AF, confirm that sites such as these are particularly important for plant biodiversity maintenance. The floristic data obtained support the determination of the zoning of the Park area by the managers of this Conservation Unit. In addition, they support the importance and necessity of including the botanical parameters analyzed in the management and evaluation of the Araucaria Forest conservation degree.

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Author Contributions

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

Vinícius Leão da Silva: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Jairo Lizandro Schmitt: 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

Moenkhausia lepidura (Kner, 1858) (Characiformes, Characidae): osteology and relationships

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Abstract: Cladistic analysis of fishes are mostly based on osteological studies. Phylogenetic relationships within the family Characidae are poorly known in part due to the lack of anatomical studies of its members, including osteology. The present contribution aims to offer a detailed description of all bony complexes of *Moenkhausia lepidura*. Two remarkable morphological conditions present in the species are discussed: a bony lamella on the proximal portion of the ribs and a basal expansion of the gill rakers. A morphological survey of several species of Characidae along with available phylogenetic information of the family indicates the putative relationships of *Moenkhausia lepidura* with other small characids presenting bony lamella on ribs and a dark mark on the caudal fin.
Keywords: *Osteology,* Moenkhausia lepidura*-group, ribs, gill rakers.*

Moenkhausia lepidura (Kner, 1858) (Characiformes, Characidae): osteologia e relações de parentesco

Resumo: Análises cladísticas morfológicas de peixes são, em grande parte, baseadas em estudos osteológicos. As relações filogenéticas em Characidae são pouco conhecidas, em parte devido à falta de estudos anatômicos de seus representantes, incluindo a osteologia. O presente estudo tem por objetivo oferecer uma descrição detalhada dos complexos ósseos de *Moenkhausia lepidura*. Duas condições morfológicas notáveis presentes na espécie são discutidas: uma lamela óssea na porção proximal das costelas e uma expansão basal dos rastros branquiais. Um estudo morfológico de diversas espécies de Characidae, juntamente com informações filogenéticas disponíveis na família, indica a possível relação de *M. lepidura* com pequenos caracídeos que apresentam lamelas ósseas nas costelas e marcas escuras na nadadeira caudal.
Palavras-chave: *Osteologia, grupo* Moenkhausia lepidura*, costelas, rastros branquiais.*

Introduction

Moenkhausia Eigenmann is one of the species-richest genus in the Characidae, represented by currently 90 valid species (Soares et al. 2017, Eschmeyer, et al. 2018) widespread throughout South American drainages (Lima et al. 2003). Nearly a century ago (Eigenmann, 1917), a combination of morphological characters was proposed and it is still used to diagnose the genus: series of scales on the lateral line completely pored, premaxilla with two tooth rows, the inner row with five teeth and caudal-fin lobe partially covered by small scales. These characters, however, are not unique for *Moenkhausia*, genus that has long been considered polyphyletic (Fink, 1979, Costa, 1994, Weitzman & Palmer, 1997, Lucena & Lucena, 1999, Lima & Toledo-Piza, 2001, Malabarba & Weitzman, 2003, Benine et al. 2004, Bertaco & Lucinda, 2006, Lima & Birindelli, 2006, Lima et al. 2007, Mirande 2009, 2010, Mariguela et al. 2013). In the phylogenetic analysis of Characidae undertaken by Mirande (2010), the included species of *Moenkhausia* were not recovered as monophyletic. This was confirmed by the molecular study by Mariguela et al. (2013), which obtained the genus distributed into five distinct clades along with species of other genera.

Moenkhausia lepidura is one of the oldest name in the genus, originally described in *Tetragonopterus* in 1858. Based on the presence of a black mark on the upper caudal-fin lobe Eigenmann (1908, 1910, 1917) recognized several subspecies of *M. lepidura* that were later raised to the species level and grouped by Géry (1977, 1992) into the "*Moenkhausia lepidura* group". Marinho & Langeani (2016) considered *Gymnotichthys hildae* Fernández-Yépez, 1950 synonymous with *M. lepidura*. The osteology of the species of *Moenkhausia*, and in fact most characids, is poorly known given the morphological and taxonomic diversity of the group. The only osteological study within the genus is that of Walter (2013), in which the author performed a developmental study of the neurocranium of *Moenkhausia sanctafilomenae* Steindachner, 1907. However, no complete description of all bony complexes is available.

Given these scarce osteological information, the availability of a generic name associated with *M. lepidura*, the polyphyletic nature of *Moenkhausia* and the fact that *M. lepidura* bears a group name of probable related species, the osteological study and comments about its relationships are welcome. In this paper, the description of the skeleton of *Moenkhausia lepidura* is presented, and the relationships of the species are discussed.

Material and Methods

The specimens were cleared and stained (c&s) following the method proposed by Taylor & Van Dyke (1985) and photographed with a ZEISS Discovery V20 stereomicroscope with ZEISS Axiocam ERc 5s digital camera attached. Standard length (SL) is given in millimeters. Dissection follows Weitzman (1974) and the models from figures of Weitzman (1962) with some adaptations: the infraorbital series, mandibular, hyoid, hyopalatine and branchial were removed from the skull; neurocranium was kept linked with vertebral column; pectoral and pelvic girdles were dissected from the body. Additionally, the third ribs from *Hemigrammus ulreyi*, *Moenkhausia lepidura*, *Moenkhausia pirauba*, and *Parecbasis cyclolepis* (see Material examined) were removed from body to photograph. Vertebrae of the Weberian ossicles were counted as four elements and the vertebrae of the compound caudal centra (PU1+U1) as a single element. Precaudal vertebrae include the Weberian ossicles and the vertebrae associated with ribs or haemal arches without haemal spine. The last two branched anal-fin rays fused on the base and supported by the last pterygiophore were counted as one single ray. The sclerotic bones, pelvic-fin radials and ear otoliths were not included in the description.

Osteological observations of *Moenkhausia lepidura* were taken from seven cleared and stained specimens from several river basins in Brazil (see Material examined). In the description, bony counts are followed by the number of specimens observed in parenthesis. Osteological terminology follows Weitzman (1962) with the following modifications suggested by subsequent authors (e.g., Vari 1979, 1995, Fink & Fink, 1981, 1996, Zanata & Vari, 2005, Carvalho et al., 2013): mesethmoid instead of ethmoid, vomer instead prevomer, epioccipital instead of epiotic, endopterygoid instead mesopterygoid, anterior ceratohyal instead of ceratohyal, posterior ceratohyal instead epihyal, retroarticular instead articular, anguloarticular instead angular, accessory element of ceratobranchial four instead of epibranchial five. We use inner arm of the *os suspensorium* instead of *os suspensorium*, and outer arm of the *os suspensorium* instead of rib of fourth vertebra, following Conway & Britz (2007). Terminology of cartilaginous elements of caudal fin follows Fujita (1989) and terminology of the canals of cephalic lateral line system follow the unpublished master thesis of Pastana (2014). We used the classification of Characidae from the phylogenetic analysis of Mirande (2010) and Mariguela et al. (2013).

A total of 63 characids were analyzed herein. This number includes 36 species further analyzed by Benine (2004) and Mirande (2010) plus 27 species exclusively analyzed herein. Specimens examined for this study are deposited in the Museu de Zoologia da Universidade de São Paulo (MZUSP).

Results

Overview of the entire body and fin positions in *Moenkhausia lepidura* (Figure 1).

1. **Neurocranium**

1.1. **Olfactory region** (Figure 2): Anterior tip of mesethmoid triangular shaped, slightly sloped anteroventrally, in between bases of ascending processes of the premaxillae. Lateral wing of mesethmoid pointed distally and directed straight laterally. Vomer T- shaped, pointed posteriorly, limited anterolaterally by the mesethmoid and posteriorly by the anteriomost portion of the parasphenoid. Lateral ethmoid well developed, its anterior process slender, leaving a broad space between this portion and the lateral margin of posterior portion of the vomer. Nasal in form of an elongate bony tube and containing the anteriormost portion of the supraorbital canal lacking bony lamellae (Fig. 2B).

1.2. **Orbital region** (Figure 2): Frontal large and relatively long, bordering the upper portion of orbit, with the supraorbital lateral-line canal starting at the nasal and running along the lateral margin of frontal. Frontals connected by the epiphyseal bar, limiting the frontal and parietal fontanels. Frontal fontanel two-thirds length of parietal fontanel.

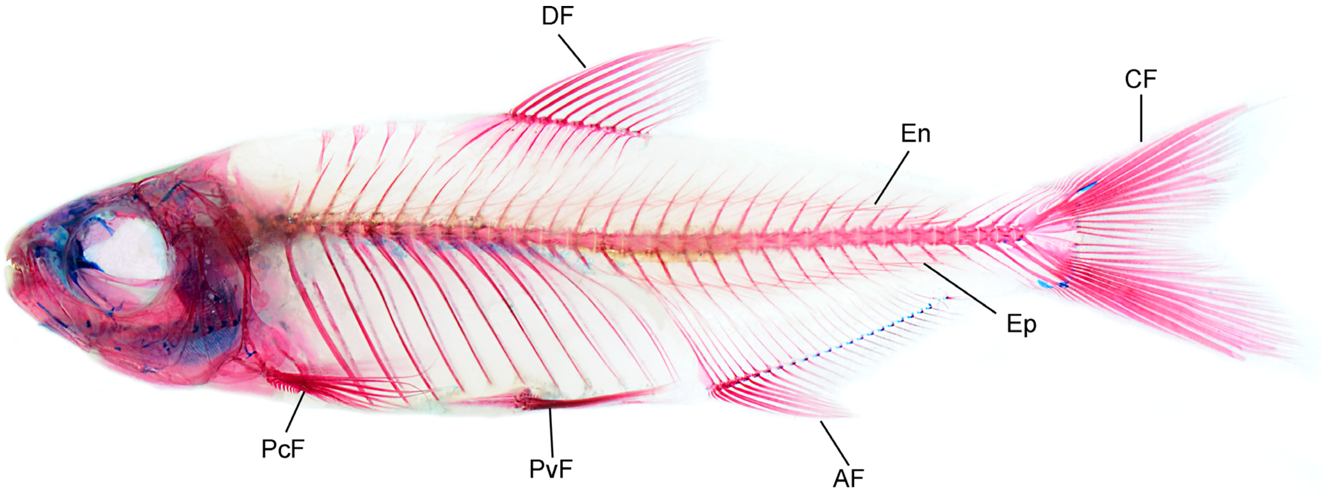

Figure 1. *Moenkhausia lepidura*, lateral view of entire skeleton, MZUSP 8181, 64.1 mm SL. **AF**, Anal fin, **CF**, caudal fin, **DF**, dorsal fin, **En**, epineural, **Ep**, epipleural, **PcF**, pectoral fin, **PvF**, pelvic fin.

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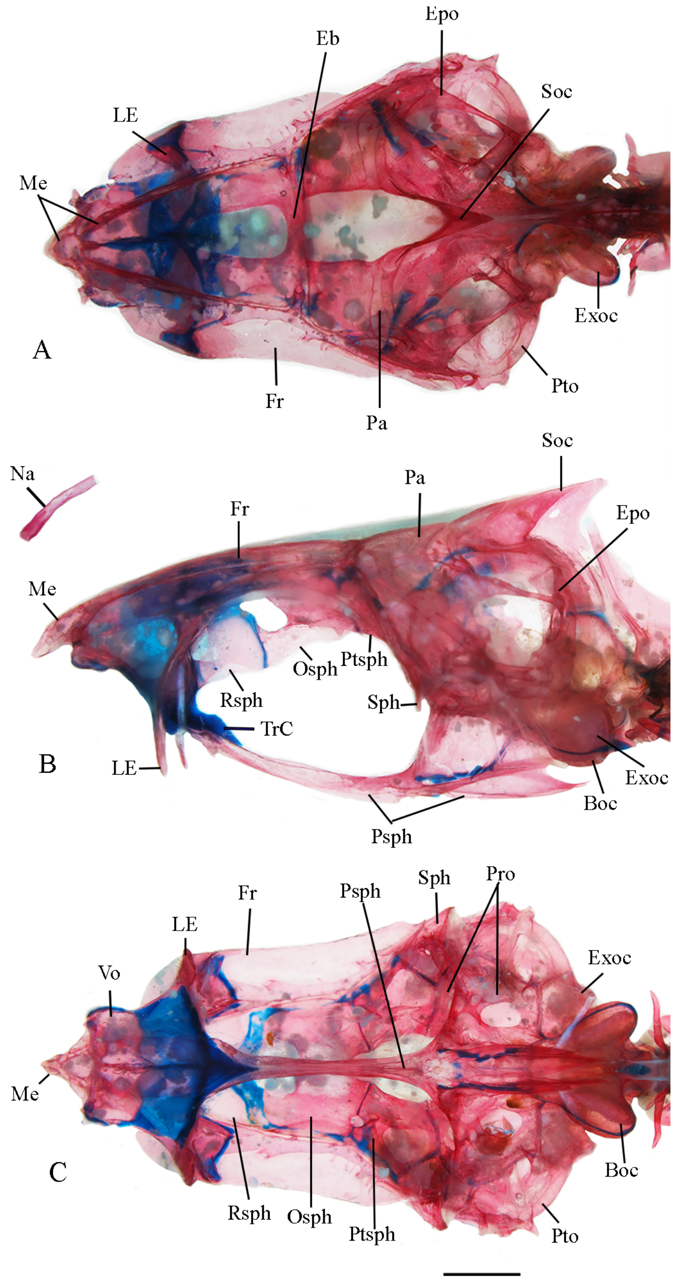

Figure 2. *Moenkhausia lepidura*, neurocranium, MZUSP 37458, 66 mm SL, **A:** dorsal view. **B:** lateral view. **C:** ventral view. **Boc**, basioccipital, **Eb**, epiphyseal bar, **Epo**, epioccipital, **Exoc**, exoccipital, **Fr**, frontal, **LE**, lateral ethmoid, **Me**, mesethmoid, **Na**, nasal, **Osph**, orbitosphenoid, **Pa**, parietal, **Pro**, prootic, **Pto**, pterotic, **Psph**, parasphenoid, **Ptsph**, pterosphenoid, **Rsph**, rhinosphenoid, **Soc**, supraoccipital, **Sph**, sphenotic, **Trc**, trabecula communis, **Vo**, vomer. Scale bar: 2 mm.

Orbitosphenoid in contact with frontal dorsally and pterosphenoid posteriorly, with median, slender horizontal process projecting anteriorly toward rhinosphenoid. Rhinosphenoid expanded posterodorsally, limited dorsally by posteroventral portion of mesethmoid cartilage anteriorly by trabecula communis cartilage and posteriorly by the orbithosphenoid. Pterosphenoid flat, anterior margin contacting the posterior margin of orbitosphenoid synchondrally, leaving a small foramen for the passage of trochlear nerve. Parasphenoid long, and narrow, wider on posterior portion, with a pair of ascending processes extending to approximately the vertical through the midlength of basioccipital.

1.3. **Otic region** (Figure 2): Prootic with two foramina, facial foramen and trigemino-facial foramen, displaced anteriorly to the large auditory foramen covering more than one-half of its posterior surface. Prootic in contact with pterosphenoid and sphenotic anteriorly and with pterotic, exoccipital and basioccipital posteriorly. Supraoccipital limiting the posterior margin of posterior cranial fontanel. Parietal branch of supraorbital canal over the posterior portion of frontal and spread along the anteroposterior extension of the parietal bone. Supratemporal canal bordering the posterior portion of parietal. Sphenotic small, with a conspicuous lateral sphenotic spine. Pterotic limited anteriorly by sphenotic, ventrally by prootic and posteriorly by exoccipital, articulating to the hyomandibula ventrally. Posterior spiniform projection present. The otic and postotic canals are associated to the pterotic anterior and posteriorly, respectively.

1.4. **Occipital region** (Figure 2): Main portion of epioccipital roughly rectangular, contacting supraoccipital dorsally and exoccipital ventrally. Epioccipital bridge cylindrical, located over the posttemporal fossa, expanded anteriorly and contacting parietal. Anterior margin of supraoccipital slightly concave forming the posterior margin of cranial fontanel. Supraoccipital spine short, extending posteriorly approximately one half extent of neural complex. Exoccipital large, its ventral region forming the dorsal surface of the legenar capsule. Basioccipital large, forming the ventral surface of the lagenar capsule. Intercalar absent.

2. **Infraorbital series** (Figure 3A): Antorbital roughly rectangular, pointed dorsoposteriorly, with expanded base. No laterosensory canal ossifications on antorbital. Six infraorbital bones, all bearing ossifications of the laterosensory canals. Infraorbitals one to five with laterosensory canal located near to inner margin of the infraorbital bones. Posterior portion of canal of infraorbital two and middle-anterior portion of canal of infraorbital three contacting the inner margin its respective bones. Infraorbital six with laterosensory canal located on its posterior margin, in contact with frontal dorsally. Ventral portion of infraorbital one overlapping the posterodorsal portion of maxilla. Infraorbital two elongated, with developed posteroventrally margin. Infraorbital three largest, with posteroventral margin bordering dorsally the angle of preopercle. Infraorbital four approximately square and bordered dorsally by infraorbital five. Infraorbital five rectangular. Infraorbital six with the anterodorsal portion slightly pointed. Supraorbital absent.

3. **Jaws**: Premaxillary teeth in two rows, both aligned in a straight line. Outer row with four tricuspid teeth (seven), inner row with five teeth (seven), in which the symphyseal and the posteriormost teeth are tetracuspid, remaining teeth pentacuspid. Maxilla elongated, with one (four) or two (three) conical or tricuspid teeth. Ascending portion of maxilla slender, with pointed tip, reaching the posterodorsal portion of premaxilla. Posterior portion of maxilla expanded, its tip almost reaching the vertical through the middle of infraorbital two. Posterior tip of maxilla reaching posterior end of Meckelian cartilage. Dentary slightly elongate, with four large pentacuspid teeth (seven) followed by a small tricuspid tooth and by a row of six or eight small conical teeth. Mandibular canal long, starting slightly below the base of first large pentacuspid teeth extending horizontally along the entire dentary and anguloarticular, ventral to Meckel's cartilage. Anterior portion

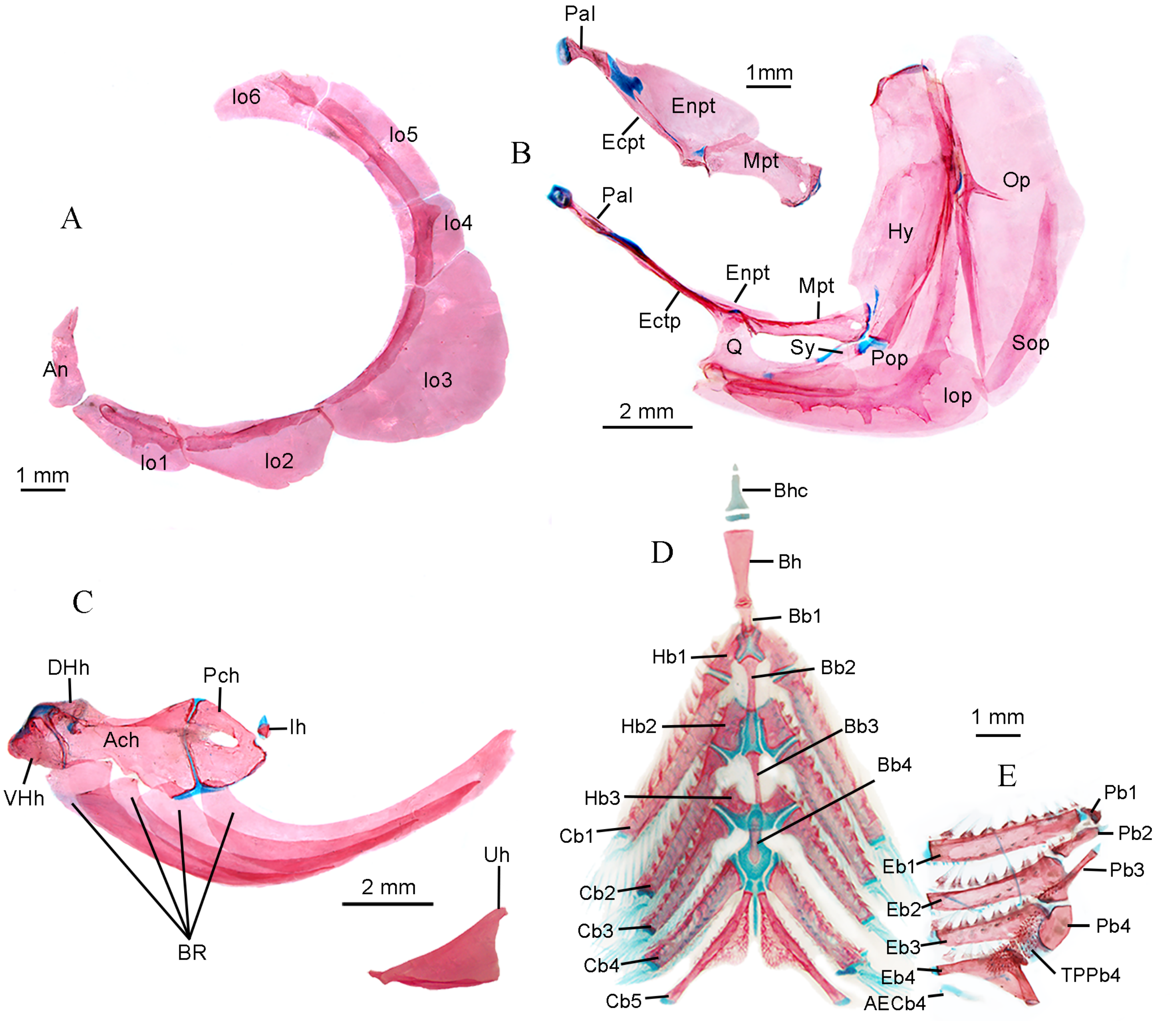

Figure 3. *Moenkhausia lepidura*, infraorbital and opercular series, branchial, hyopalatine and hyoid arches. **A:** infraorbital series, lateral view, left side, MZUSP 8181, 62.7 mm SL. **B:** hyopalatine arch and opercular series, lateral view, left side **C:** lateral view of hyoid arch, left side, MZUSP 6247, 61 mm SL. **D:** upper portion of branchial arch, right side, ventral view, MZUSP 109841, 60.2 mm SL. **E:** lower portion of branchial arch, dorsal view, MZUSP 6247, 61 mm SL. **ACh**, anterior ceratohyal, **AECb4**, accessory element of ceratobranchial 4, **An**, antorbital, **Bb1-4**, basibranchial 1-4, **Bh**, basihyal, **Bhc**, basihyal cartilage, **BR**, branchiostegal rays, **Cb1-5**, ceratobranchial 1-5, **DHh**, dorsal hypohyal, **Eb1-4**, epibranchial 1-4, **Ecpt**, ectopterygoid, **Enpt**, endopterygoid, **Hb1-3**, hypobranchial 1-3, **Hy**, hyomandibular, **Ih**, interhyal, **Io1-6**, infraorbital 1-6, **Iop**, interopercle, **Mpt**, metapterygoid, **Op**, opercle, **Pal**, palatine, **Pb1-4**, pharyngobranchial, **PCh**, posterior ceratohyal, **Pop**, preopercle, **Q**, quadrate, **Sop**, subopercle, **Sy**, symplectic, **TPPb4**, tooth plate of pharyngobranchial 4, **Uh**, urohyal, **VHh**, ventral hypohyal.

of dentary aligned vertically with the anterior portion of premaxilla. Posterior tip of dentary at vertical through the middle of infraorbital two. Bony interdigitations between dentaries, disposed horizontally and parallel to each other. Anguloarticular with vertical arm extending laterally on the posterodorsal portion of dentary and with horizontal arm extending on its medial surface. Meckelian cartilage along the medial portion of dentary, contacting the anguloarticular posteriorly in medial view. Coronomeckelian bone ovate, situated mainly lateral to the Meckelian cartilage. Retroarticular small and roughly triangular (see Marinho & Langeani, 2016: Figure 4).

4. **Hyopalatine arch** (Figure 3B): Hyomandibular large, elongate, with wide thin bony lamellae developed anteriorly. Hyomandibular fossa bordered by sphenotic and prootic anteriorly and pterotic dorsally. Hyomandibular with a condylar articulation posteriorly with opercle. Symplectic thin and elongated. Metapterygoid horizontally elongate, approximately rectangular. Posterior portion larger than anterior, slightly overlapping laterally a small portion of hyomandibular lamellae, with the foramen for afferent pseudobranchial artery completely encircled by metapterygoid. Quadrate contacting the anterior and posterior portions of metapterygoid by a cartilage. Anterodorsal portion of quadrate synchondrally articulated with metapterygoid. Posterior tip of quadrate reaching approximately the vertical through middle of sympletic, its tip separated from posteroventral portion of metapterygoid by remnants of palatoquadrate cartilage. Metapterygoid-quadrate fenestra

large and horizontally ovate. Endopterygoid lamellar, wide, tapering anteriorly. Posterior portion of endopterygoid slightly overlapping the anterodorsal margin of metapterygoid and quadrate, with a pointed lateroventral projection, directed to quadrate. Ectopterygoid elongate, narrow posteriorly, bordered laterally by the endopterygoid. Anterior portion of ectopterygoid wider, articulating synchondrally with palatine. Posterior portion of ectopterygoid in contact with the anterodorsal region of quadrate. Palatine roughly rectangular in dorsal view, shorter than a half-length of ectopterygoid. Palatine located lateroventrally to vomer.

5. **Opercular series** (Figure 3B): Opercle laminar, large, slightly concave posterodorsally, extending beyond the vertical through dorsal margin of hyomandibular dorsally, and reaching the horizontal through the ventral margin of quadrate ventrally. Preopercle large, inverted L-shaped, round on its anterior corner, bordering posteriorly and ventrally the hyomandibular arch. Well-developed preopercular laterosensory canal running along the central portion of preopercle. Dorsal portion of preopercle represented solely by the ossified preopercular canal tube, lacking bony lamellae, reaching the horizontal through the dorsal margin of hyomandibula. Interopercle elongated anteroposteriorly. Anterior portion narrow, extending slightly beyond the anterior end of preopercle. Subopercle elongated and slightly arched.

6. **Hyoid arch** (Figure 3C): Anterior ceratohyal slightly narrow at its medial portion, connected anteriorly with the hypohyals and posteriorly with the posterior ceratohyal. Ventral margin of anterior ceratohyal with two or three notches, for articulation of the anteriormost branchiostegal rays. A large canal containing the hyoid artery is present on the dorsal portion of the anterior and posterior ceratohyal. The hyoid canal opens on anterior portion of the anterior ceratohyal, continuing as a canal on posterior ceratohyal. Posterior ceratohyal triangular, with a central foramen through which the hyoid artery enters. Interhyal short, its tips cartilaginous connecting to the posterior margin of posterior ceratohyal to the suspensorium at the cartilaginous connection between hyomandibula and sympletic. Dorsal hypohyal with two arms, connecting with anterodorsal portion of anterior ceratohyal, forming a foramen dorsally. Ventral hypohyal triangular shaped, separated from dorsal hypohyal by a cartilage. Basihyal elongated (Figure 3D) with anterior portion wider. Urohyal triangular shaped, with small lateral bony lamellae on ventral portion (Figure 3E). Anterodorsal projection of urohyal short at insertion of ligaments connecting to ventral hypohyal. Four branchiostegal rays, anteriormost three articulated with anterior ceratohyal and posteriormonst ray articulating laterally on ventral portion of posterior ceratohyal.

7. **Branchial arches** (Figure 3D–E): Three (four) or four (three) ossified brasibranchials, separated by cartilages, each situated medial to hypobranchials. Anterior portion of basihyal with three blocks of cartilage. Anterior margin of first basibranchial articulating with the posterior margin of basihyal. Three hypobranchials with cartilaginous margins. Each hypobranchial bearing one to three gill rakers, basally expanded, completely covered with small denticles. Five well-developed ceratobranchials, decreasing in length from first to last ceratobranchial, all covered with gill rakers. Ceratobranchial one with nine or 10 elongated gill rakers in one series, situated anteriorly, with few small spines scattered on its surface. Ceratobranchial two to five with shorter gill rakers, each with a basal extension forming a lateral plate full of small denticles. Ceratobranchial two with nine gill rakers in one series located on its anterior margin. Ceratobranchial three and four with gill rakers in two distinct series. Anterior series with seven, eight or nine and posterior with six, seven or eight gill rakers. Ceratobranchial four with eight gill rakers on anterior series and six or nine gill rakers on posterior series. Acessory element of ceratobranchial four cartilaginous, small and slightly elongate. Four small pharyngobranchials with cartilaginous edges. Ceratobranchial five with seven gill rakers in a single anterior series, posterior margin of ceratobranchial five with a triangular tooth plate. Five epibranchials, first four ossified and the last one cartilaginous. Epibranchial one to three with two series of gill rakers and epibranchial four with only one series of gill rakers. Epibranchial one to four with small triangular gill rakers. Their basal and lateral expansions covered with small spines. Epibranchial one with eight gill rakers on anterior series and seven or eight gill rakers on posterior ones. Epibranchial two with six, eight or nine gill rakers on anterior series and seven on posterior ones. Epibranchial three forked dorsally, with seven gill rakers on anterior series and six on posterior ones. Epibranchial four wider and triangular shaped with five gill rakers on the anterior series. Pharyngobranchial one slightly round. Pharyngobranchial two elongate, some specimens with denticles on its base. Pharyngobranchial three mostly slender, wider on its base, bearing small denticles. Pharyngobranchial four cartilaginous (six) or ossified (one) (see Figure 3 E), with a tooth plate well developed connected with the tip of fourth epibranchial.

8. **Weberian ossicles** (Figure 4): Vertebral centrum one shorter than the remaining ones. Centrum two presenting well-developed lateral process, extending beyond the ventral anterior portion of tripus. Claustrum small, situated dorsally to scaphium. Scaphium rectangular shaped, located dorsally to vertebral centrum one. Intercalarium elongate. Tripus well developed, triangular shaped, displaced lateroventrally to neural arch three, with posterior pointed projection reaching the *os suspensorium* inner arm. Neural arch pedicle of centrum three elongate, well developed. *Os supensorium* outer arm arched ventrally, robust and flattened, and inner arm projecting ventrally, with expanded anterior tip almost meeting its counterpart in ventral midline. Neural complex well developed, roughly triangular and concave on its dorsal surface. Neural spine of fourth vertebrae well developed, reaching approximately one half-length of neural spine of centrum five.

9. **Post-Weberian axial skeleton** (Figure 1): Total of 31(1), 32(3) or 33(3) vertebrae consisting of 16(7) precaudal vertebrae and 15(1), 16(3) or 17(3) caudal vertebrae. Vertebral centra slightly longer than deep. Neural pre and postzygapophyses present in all vertebrae except the posteriormost three caudal vertebrae. Haemal pre and postzygapophyses present in all caudal vertebrae. Four (seven) supraneurals with expanded dorsal portion. Usually, the first supraneural displaced between neural spines of fourth and fifth vertebrae (Figure 5A). Parapophysis well developed on ventral portions of five–10 precaudal vertebrae. Ribs associated with fifth to 14th (three), 15th (three) or 16th (one) precaudal vertebrae. All ribs similar in size. Posterior ribs thinner. Second to eighth (one) or ninth (six) ribs with a dorsal triangular shaped lamellae expansion (Figure 1 and 8 C, see details in Discussion). Neural arch and spines present in all vertebrae posterior to the Weberian apparatus.

9.1 **Intermuscular bones** (Figure 1): 26(one), 27(four) or 28(two) epineurals and 16(one) or 17(six) epipleurals along body. Epineurals and epipleurals forked proximally from the first to eighth caudal vertebrae.

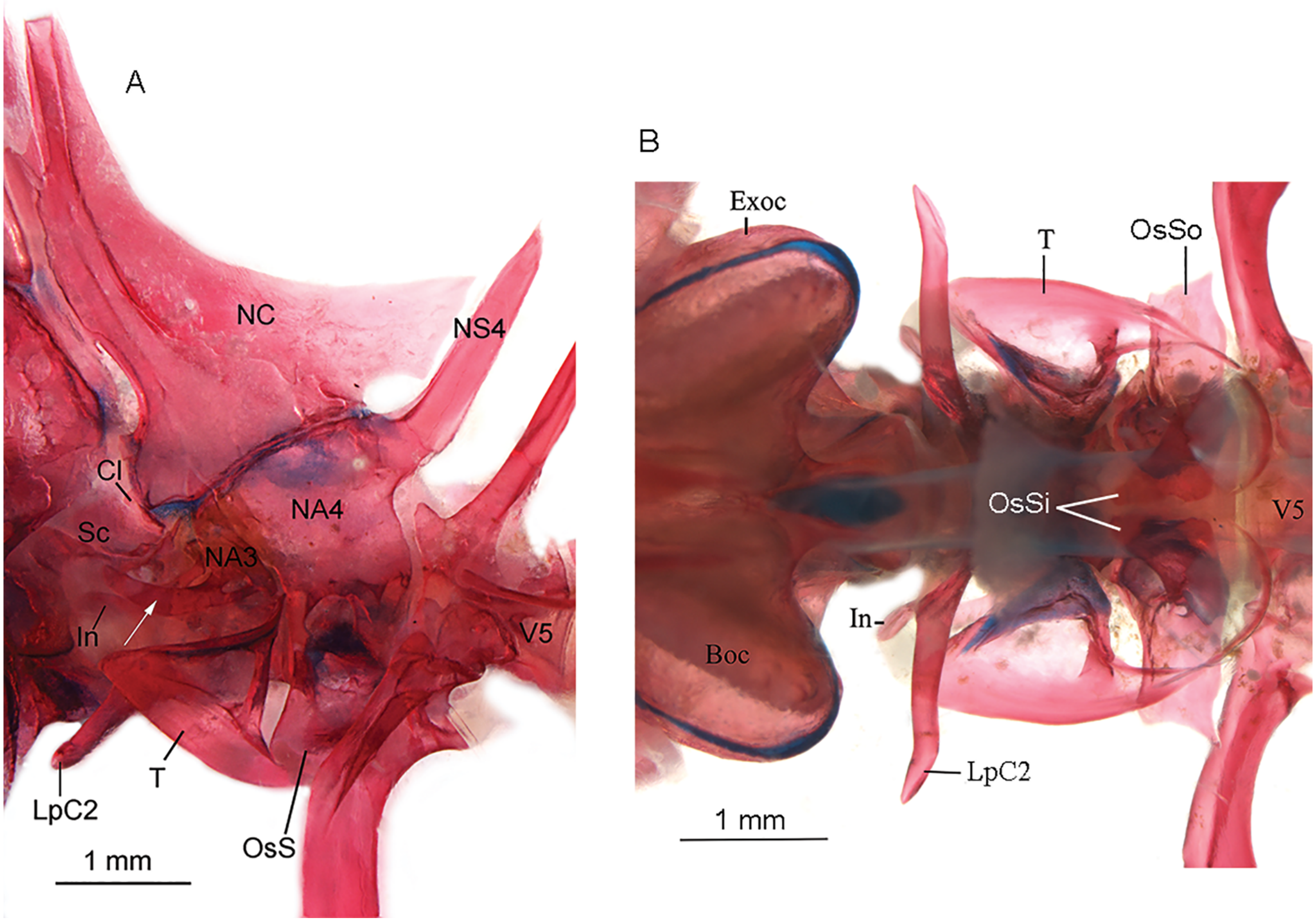

Figure 4. *Moenkhausia lepidura*, Weberian ossicles. **A:** Left lateral view, MZUSP 37458, 40 mm SL. White arrow indicates the transverse process of neural arch pedicle of third vertebra. **B:** Ventral view, MZUSP 37458, 66 mm SL; **Boc**, basioccipital, **Cl**, claustrum, **Exoc**, exoccipital, **In**, intercalarium, **LpC2**, lateral process of centrum 2, **NA3-4**, neural arch 3-4, **NC**, neural complex, **NS4**, neural spine 4, **OsSo**, *os suspensorium* outer arm, **OsSi**, *os suspensorium* inner arm, **Sc**, scaphium, **T**, tripus, **V5**, vertebrae 5.

10. **Dorsalfin** (Figure 5B): Ten pterygiophores supporting the dorsal-fin rays (seven). Anteriormost five proximal and middle radials are fused into one single structure and the remaining with proximal and middle radials separated by cartilage. First proximal-middle radial the longest, its tip extending frontward between the neural spine of ninth and 10th vertebrae (seven), with well-developed lateral flanges, supporting two unbranched dorsal-fin rays in supernumerary association (seven). All analyzed specimens with a small bony spine under skin anterior to first dorsal-fin ray, associated with the first proximal-middle radial. Laterally flattened bony lamellae associated to the anterior and posterior surface of all proximal radials, decreasing in size posteriorly. Last two(one), three(one) or four(one) dorsal-fin proximal radial presenting a small foramen distally. Bony stay L-shaped, vertically aligned with 17th neural spine (seven). Ventral tip of bony stay cartilaginous. Anteriormost unbranched dorsal-fin ray approximately half-length of second unbranched ray, which is the longest, followed by nine (seven) branched rays decreasing in length.

11. **Analfin** (Figure 5C): Anal-fin rays supported by 22(two), 23(two), 24(two) or 25(one) pterygiophores. First to fifth pterygiophores with proximal and middle radials fused into a single bone (proximal-middle radials). Remaining pterygiophores with proximal and middle radials separated by cartilage. Distal radial present as separate bone in all pterygiophores. Pterygiophores decreasing in size posteriorly. Anteriormost proximal-middle radial larger at base, longer, reaching the haemal spine of first caudal vertebrae, and supporting three(one) or four(six) supranumerary unbranched rays. Anteriormost supranumerary unbranched ray shortest. Analfin falcate. Last unbranched anal-fin ray the longest. Rays decreasing in size posteriorly from sisxth branched ray. Remaining rays smaller, and similar in size. Bony stay variable in shape: vertically elongate, its dorsal tip cartilaginous, reaching approximately half-length of posteriormost proximal-radial (five) or short and wide, its dorsal tip reaching approximately one-fourth length of posteriormost proximal-radial (two).

12. **Pectoral girdle** (Figure 6A-B): Extrascapular well developed and square shaped. Sensory canal contained in the extrascapula connecting to supratemporal canal dorsally, and to postotic canal anteriorly and posteroventrally. Posttemporal pointed dorsally, enlarged and rounded ventrally, with medial well-developed pointed projection. Sensory canal on its anteroventral portion. Supracleithrum elongate, aligned with posttemporal, thinner ventrally, overlapping the dorsal tip of cleithrum and dorsal portion of postcleithrum one. Postotic canal bypass the supracleithrum from its lateral to medial face and follows to the first pored lateral line. Cleithrum tapered dorsally, enlarged posteroventrally. Cleithrum contacting the coracoid anteriorly by

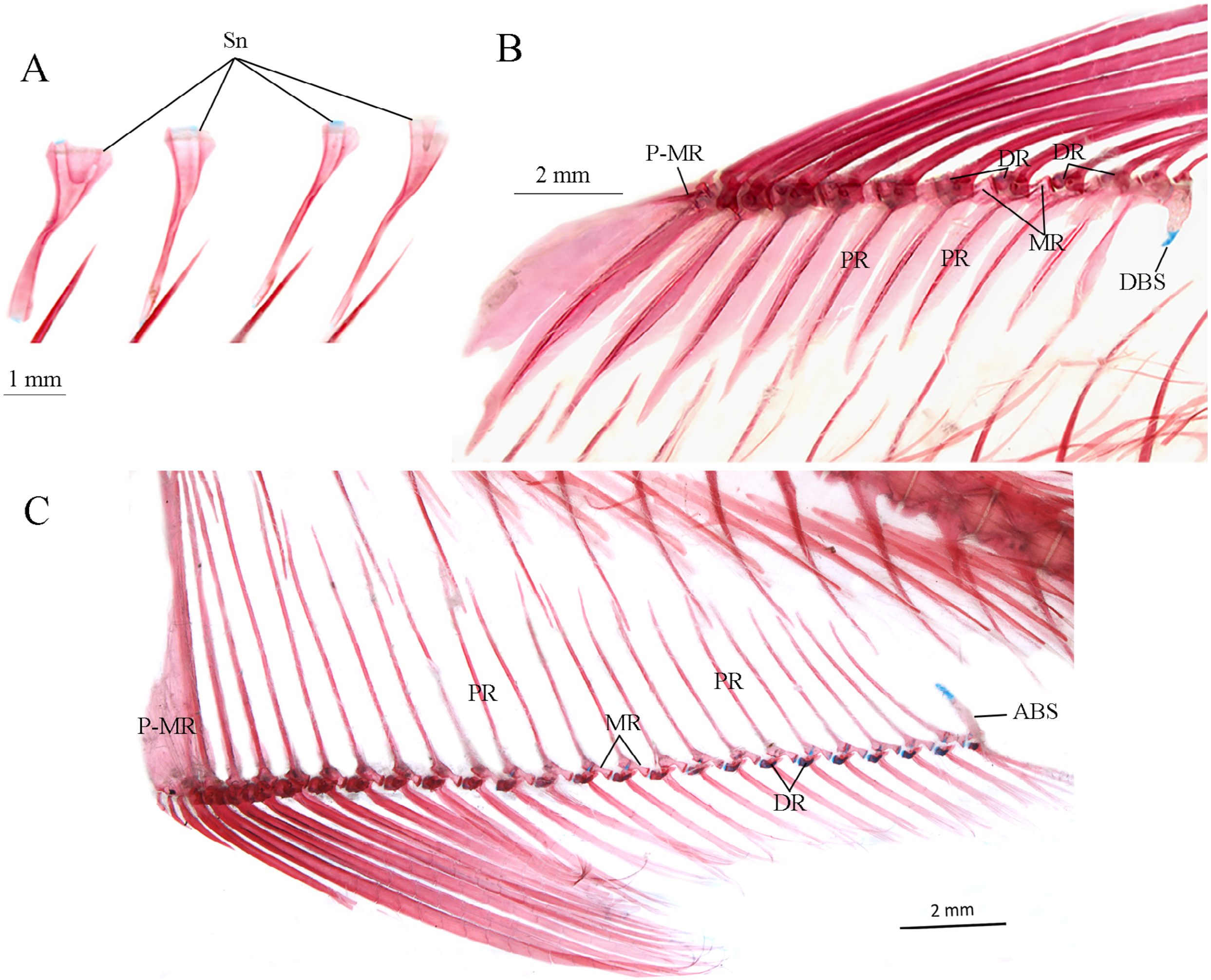

Figure 5. *Moenkhausia lepidura*, supraneurals, dorsal and anal fins. **A:** supraneurals, MZUSP 37458, 66 mm SL. **B:** dorsal fin and **C:** anal fin, MZUSP 8181, 62.7 mm SL. **ABS**, anal-fin bony stay, **DR**, distal radial of pterygiophore, **DBS**, dorsal-fin bony stay, **MR**, middle radial, **P-MR**, proximal middle radial, **PR**, proximal radial, **Sn**, supraneural.

interdigitating sutures, the scapula and mesocoracoid medially, pectoral-fin rays ventrally and poscleithrum two posteriorly. Postcleithrum one rounded, located ventral to the tip of supracleithrum. Postcleithrum two ovate, located medially to posterior tip of cleithrum, slightly overlapping anterodorsal tip of postcleithrum three. Postcleithrum three thin, elongated, with ovate, posterior bony lamella. Coracoid flat, located medially to cleithrum, connected to it anteriorly and laterally to the medial lamellae of cleithrum (cleithrum-coracoid bridge) to form the interosseous space. Coracoid connected with scapula and mesocoracoid posterodorsally. Round opening delimited by cleithrum-coracoid bridge anteriorly and scapula posteriorly. Mesocoracoid thin, elongate, enlarged basally, its dorsal tip contacting the anterior portion of cleithrum and its ventral tip the posterior portion of coracoid. Scapula located medially to the posteroventral portion of cleithrum. Dorsal portion of scapula bifurcated, with anterior and posterior projection. Rays on pectoral-fin i(seven), 12(four) or 13(three). Four proximal radials. Four distal radials partially ossified distally.

13. **Pelvic girdle** (Figure 6C): Basipterygium roughly triangular in shape, its tip situated posterior to vertical through ribs of sixth (three) or seventh (four) vertebrae. Ischiatic process with a posteriorly directed process, with cartilaginous tip.

14. **Caudalfin** (Figure 7): Dorsal procurrent caudal-fin rays 10 (two), 11(three) or 12(two) contacting the last three neural spines, two epurals and a pair of uroneurals. Ventral procurrent caudal-fin ray eight(two), nine(two), 10(one) or 11(two) contacting the last three haemal spines and parhypural. Principal caudal-fin rays i,9,8,i (seven). Compound centrum with dorsal specialized neural process well developed. First hypural not connected to the compound centrum. Second hypural thin, always connected with the compound centrum. First and second hypurals and parhypural supporting the ventral caudal-fin lobe. Third, fourth, fifth and sixth hypurals supporting the upper caudal-fin lobe. Relatively wide, distal gap between second and third hypurals. One specimen presenting the first and second hypurals fused, possibly representing an abnormal condition. Distal portions of hypurals, haemal spines of preural centra two and three and parhypural cartilaginous. Two ventral caudal radial cartilages; anterior one (inter-haemal spine cartilage of preural centrum four: CIHPU4) situated anterior to tip of haemal spine of preural centrum three, posterior one (inter-haemal spine cartilage of preural centrum three: CIHPU3) situated between tips of haemal spines of preural centra two and three. CIHPU3 smaller than anterior cartilage. Dorsal caudal radial cartilages absent. Opisthural cartilage present in all specimens at posterior tip of notochord.

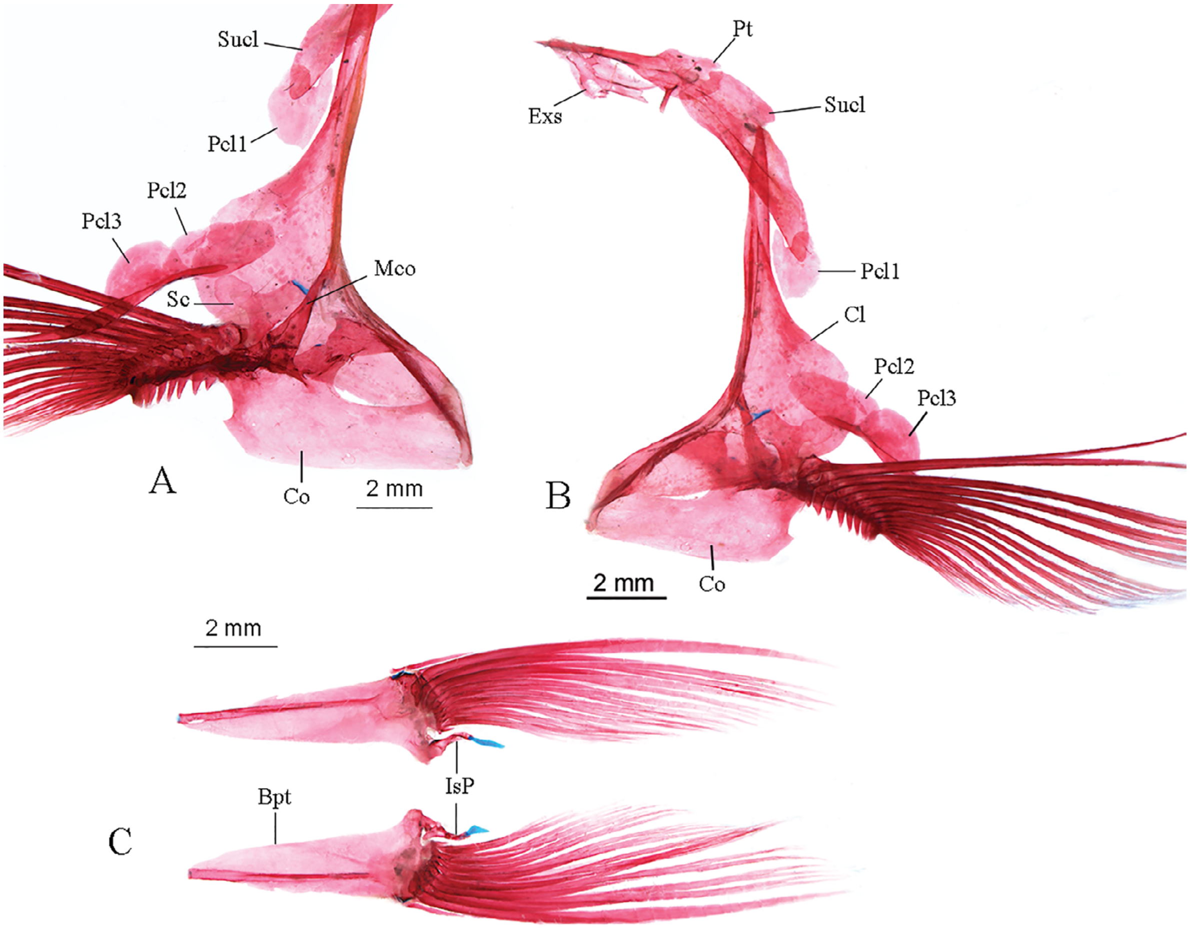

Figure 6. *Moenkhausia lepidura*, pectoral and pelvic fins. **A:** pectoral girdle, medial view and **B:** pectoral girdle, lateral view, MZUSP 37458, 66 mm SL. **C:** pelvic girdle, ventral view, MZUSP 8181, 62.7 mm SL. **Bpt**, basipterygium, **Cl**, cleithrum, **Co**, coracoid, **Exs**, extrascapular, **IsP**, ischiatic process, **Mco**, mesocoracoid, **Pcl1-3**, postcleithrum 1-3, **Pt**, posttemporal, **Sucl**, supracleithrum, **Sc**, scapula.

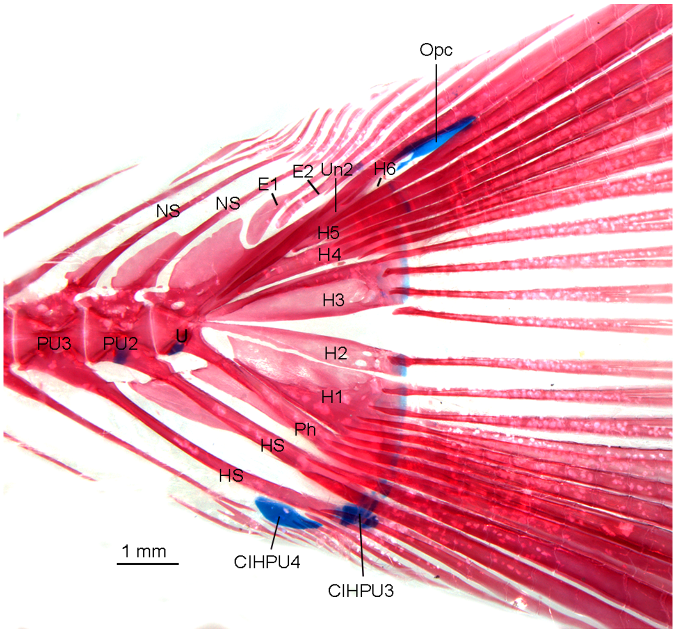

Figure 7. Caudal fin of *Moenkhausia lepidura*, MZUSP 8181, 64.1 mm SL. **CIHPU3-4**, inter-haemal spine cartilage of preural centrum 3 and 4, **E1-2**, epural 1 and 2, **H1-6**, hypural 1-6, **HS**, haemal spines, **NS**, neural spines, **Opc**, opisthural cartilage, **Ph**, parhypural, **PU2-3**, preural centrum 2 and 3, **U**, compound ural centrum, **Un2**, uroneural 2.

Discussion

A comparative survey with several other characids was performed in addition to the osteological description of *Moenkhausia lepidura* (see Material examined) in order to search for possibly informative phylogenetic characters. Extensive descriptions and illustrations of morphological conditions in the Characidae were investigated (e.g. Benine, 2004, Mirande, 2010, Mattox et al. 2014). The comparative analysis undertaken herein revealed two remarkable characters present in *Moenkhausia lepidura* that deserve further attention due its restricted distribution within the family.

In *Moenkhausia lepidura* there is a triangular shaped bony lamella on the dorsal margin of the ribs, directed slightly posteriorly along its distal portion (Figure 8C). These bony lamellae are located from the second to eighth (one) or ninth (six) ribs, and serves as the attachment site for fibers of the *obliquus superioris* muscle. In the present study, a total of 63 species of Characidae were analyzed, of which 36 were also analyzed by Benine (2004) and Mirande (2010), and the remaining 27 species were exclusively examined herein, which provided a deep and detailed view about the variation of this character in closely related species. In the material examined, the dorsal portion of the ribs exhibited the following morphological variations: (0) bony lamellae absent (Figure 8A), found in most characids, (1) bony lamellae present and small, with smooth surface along the dorsal margin of the ribs (Figure 8B), as observed in *Hemigrammus durbinae* Ota, Lima & Pavanelli, 2015, *H. ulreyi* (Boulenger, 1895), *H. unilineatus* (Gill, 1858) and *M. phaeonota*, (2) bony lamellae triangular in shape, slightly pointed posteriorly on the distal portion, present in *Astyanax multidens* Eigenman, 1908, *Hemigrammus marginatus, Hyphessobrycon diancistrus* Weitzman, 1977, *H. hebertaxelrodi* Gerý, 1961, *M. aurantia* Bertaco, Jerep & Carvalho, 2011, *M. bonita*, *M. collettii*, *M. costae*, *M. dichroura*, *M, intermedia*, *M. jamesi* Eigenmann, 1908, *M. lopesi* Britski & de Silimon, 2001, all species assigned to the *M. lepidura* group *sensu* Géry (1992), *Moenkhausia abyss* Oliveira & Marinho, 2016, *M. celibela* Marinho & Langeani, 2010, *M. gracilima*, *M. hasemani* Eigenmann, 1917, *M. hysterosticta* Lucinda, Malabarba & Benine, 2007, *M. icae* Eigenmann, 1908, *M. inrai* Géry, 1992, *M. megalops* (Eigenmann, 1907), *M. lata* Eigenmann, 1908, *M. lepidura*, *M. loweae* Géry, 1992, *M. mikia* Marinho & Langeani, 2010 and *Thayeria obliqua* Eigenmann, 1908, (3) very thin lamellae present, connected to a thin elongated bony projection on the proximal portion of the ribs, present only in *Parecbasis cyclolepis* (Figure 8D).

According to the distribution of the conditions of 'bony lamellae on the ribs' herein observed and the results of phylogenetic studies discussed below, it seems condition 1 is homologous to condition 2, but not condition 3, presented by *Parecbasis cyclolepis.* Lucena (1993) mentioned the presence of bony lamellae on the ribs as parallel autapomorphy of *M. lepidura* and *Parecbasis cyclolepis* Eigenmann, 1914. Benine (2004) also noticed similar condition in *Hemigrammus marginatus* Ellis, 1911, *Moenkhausia barbouri* Eigenmann, 1908, *M. bonita* Benine, Castro & Sabino, 2004, *M. browni* Eigenmann, 1909, *M. ceros* Eigenmann, 1908, *M. collettii* (Steindachner, 1882), *M. copei* (Steindachner, 1882), *M. costae* (Steindachner, 1907), *M. dichroura* (Kner, 1858), *M. gracilima* (Eigenmann, 1908), *M. intermedia* Eigenmann, 1908, *M. justae* Eigenmann, 1908, *M. lepidura, M. phaeonota* Fink, 1979, *M. shideleri* Eigenmann, 1909 and *Stichonodon insignis* (Steindachner, 1876) and proposed the presence of such bony lamellae as one of the three synapomorphies for a clade including all abovementioned species (Benine, 2004: fig. 62, Clade 42) with exception of *M. barbouri* and *S. insignis*. In a phylogenetic analysis of the Characidae based on morphological data, Mirande (2010) observed these lamellae in the ribs of *Moenkhausia intermedia, M. dichroura, Parecbasis cyclolepis* and *Stichonodon insignis* (character 224:1, synapomorphy for node 297 and paralleled in *P. cyclolepis* and *S. insignis*). In the molecular phylogeny of *Moenkhausia* species by Mariguela et al. (2013), a "*M. lepidura*" was recovered as closely related to *M. copei* (Clade 2), but it is a misidentification of *M. copei* (Petrolli et al. 2016). Although not including *M. lepidura*, most species (14 of 17) of clade 4 of Mariguela et al. (2013) present bony lamella in the ribs. Of which, 12 present the condition described for *M. lepidura* (condition 2)

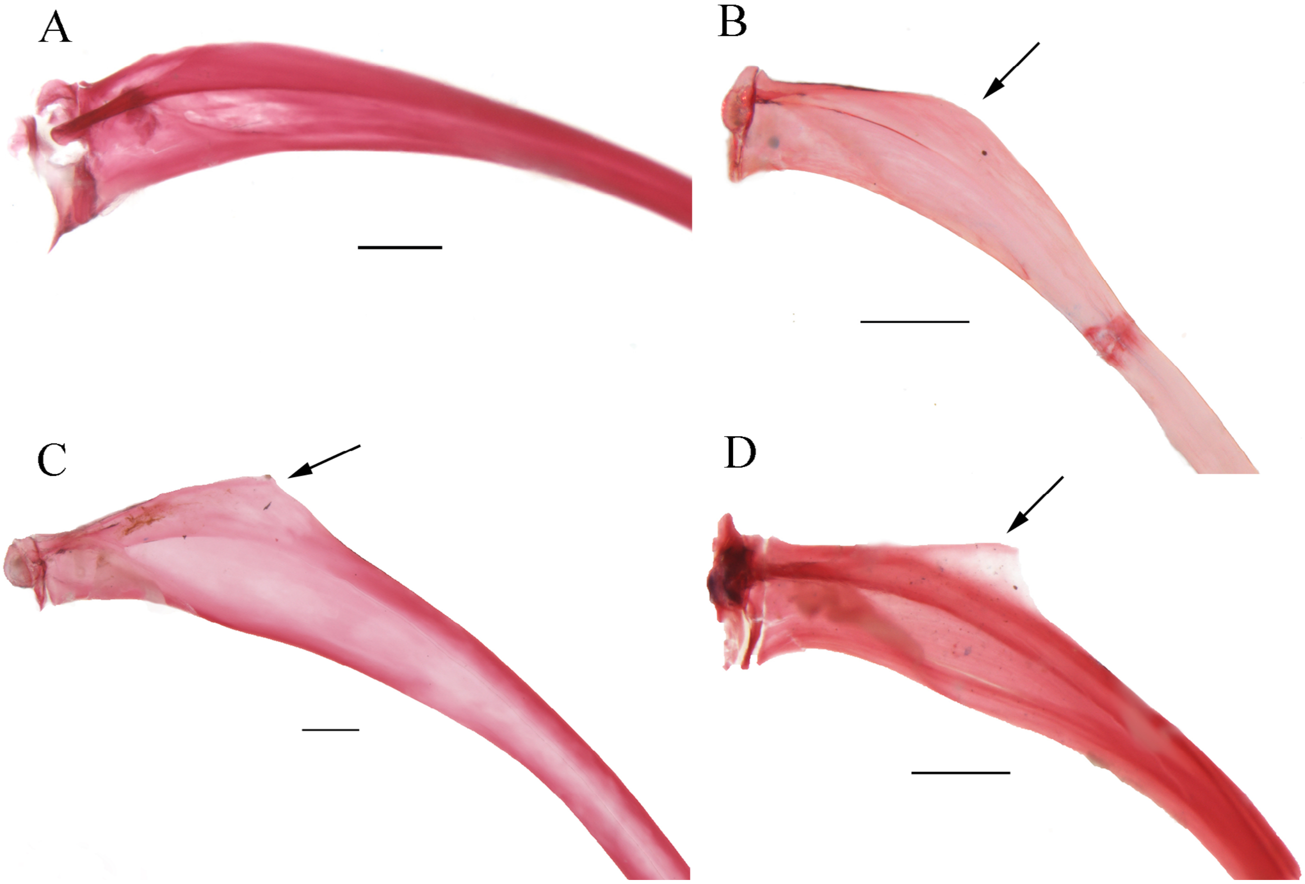

Figure 8. Lateral view, left side of proximal portion of third rib of **A:** *Moenkhausia pirauba*, MZUSP 73467, 40.8 mm SL. **B:** *Hemigrammus ulreyi*, MZUSP 59538, 29.3 mm SL. **C:** *Moenkhausia lepidura*, MZUSP 37458, 66 mm SL. **D:** *Parecbasis cyclolepis*, MZUSP 25942, 41.7 mm SL. Arrow indicates the distinct conditions found in bony rib lamella of characids. Scale bar: 0.5 mm.

and two present the condition describe for the type species of the genus, *M. xinguensis* (condition 1). Therefore, all aforementioned phylogenetic analysis indicate that there is a monophyletic assemblage within the Characidae sharing the presence of bony lamellae dorsally to the ribs.

Interestingly, most species of the assemblage of Clade 4 (Mariguela et al. 2013) bearing bony lamellae described as condition 2 (bony lamellae triangular, slightly pointed posteriorly on the distal portion), similar to *M. lepidura*, have pigmented caudal-fin lobes. They are *M. celibela*, *M. gracilima*, *M. lata*, *M. costae*, in which the upper caudal-fin lobe is dark as in *M. lepidura*, and *Hemigrammus marginatus*, *M. bonita*, *M. dichroura*, and *M. intermedia* in which both lobes are black marked. Other species sharing both characters herein examined are *Moenkhausia abyss*, *M. hasemani*, *M. hysterosticta*, *M. icae*, *M. inrai M. megalops*, *M. loweae*, *M. mikia*, all pertaining to the *Moenkhausia lepidura* group (*sensu* Géry, 1992), which may indicate that they are closely related. However, all these assumptions must be tested in a broad phylogeny encompassing all species.

Another remarkable feature of *M. lepidura* is the presence of well-developed gill rakers with a basal expansion covered with small denticles (Figure 9), which were used by Marinho & Langeani (2016) as one of the diagnostic features of the species (*vs.* all the other species herein analyzed have gill rakers slender, with no basal expansions and with few spines scattered along its surface). Herein, we observed that these well-developed gill rakers are present in all branchial arches, except in ceratobranchial of the first arch. Such unique morphological condition is most likely an autapomorphy of *M. lepidura*. Toledo-Piza (2007) reported similar condition, but in the first branchial arch, in *Acestrohynchus* (Agassiz, 1829) and Cynodontinae as a synapomorphy uniting both taxa (character 65:1). Mirande (2010, character 197:2) also reported "short, broad and strongly denticulated gill rakers", but in the first branchial arch (specifically in first ceratobranchial) of *Acestrorhynchus pantaneiro* Menezes, 1992 and *Rhaphiodon vulpinus* Spix & Agassiz, 1829. Furthermore, Mirande (2010, character 199:1) coded "broad and laminar lateral base of gill rakers on first ceratobranchial" for *Acestrorhynchus pantaneiro*, *Brycon* spp., *Rhaphiodon vulpinus, Salminus brasiliensis* (Cuvier, 1816) and *Triportheus* spp. These are all piscivorous species considered basal lineages in Characidae (Malabarba & Weitzman, 2003; Calcagnotto et al., 2005; Mirande, 2010). Although they also present basal expansion on gill rakers similar to *M. lepidura*, such structures are not located in the same branchial elements and seems not to be homologous.

As observed, the comparative morphological analysis presented herein, along with the molecular and morphological based phylogeny of the Characidae available (e.g. Benine, 2004, Mirande, 2010, Mariguela et al. 2013) suggest the relationships of *M. lepidura* are among the species of *Moenkhausia* (and related small characids such as *Hemigrammus marginatus*) with the caudal fin black marked and bony lamella on the ribs, such as those belonging to the *M. lepidura*

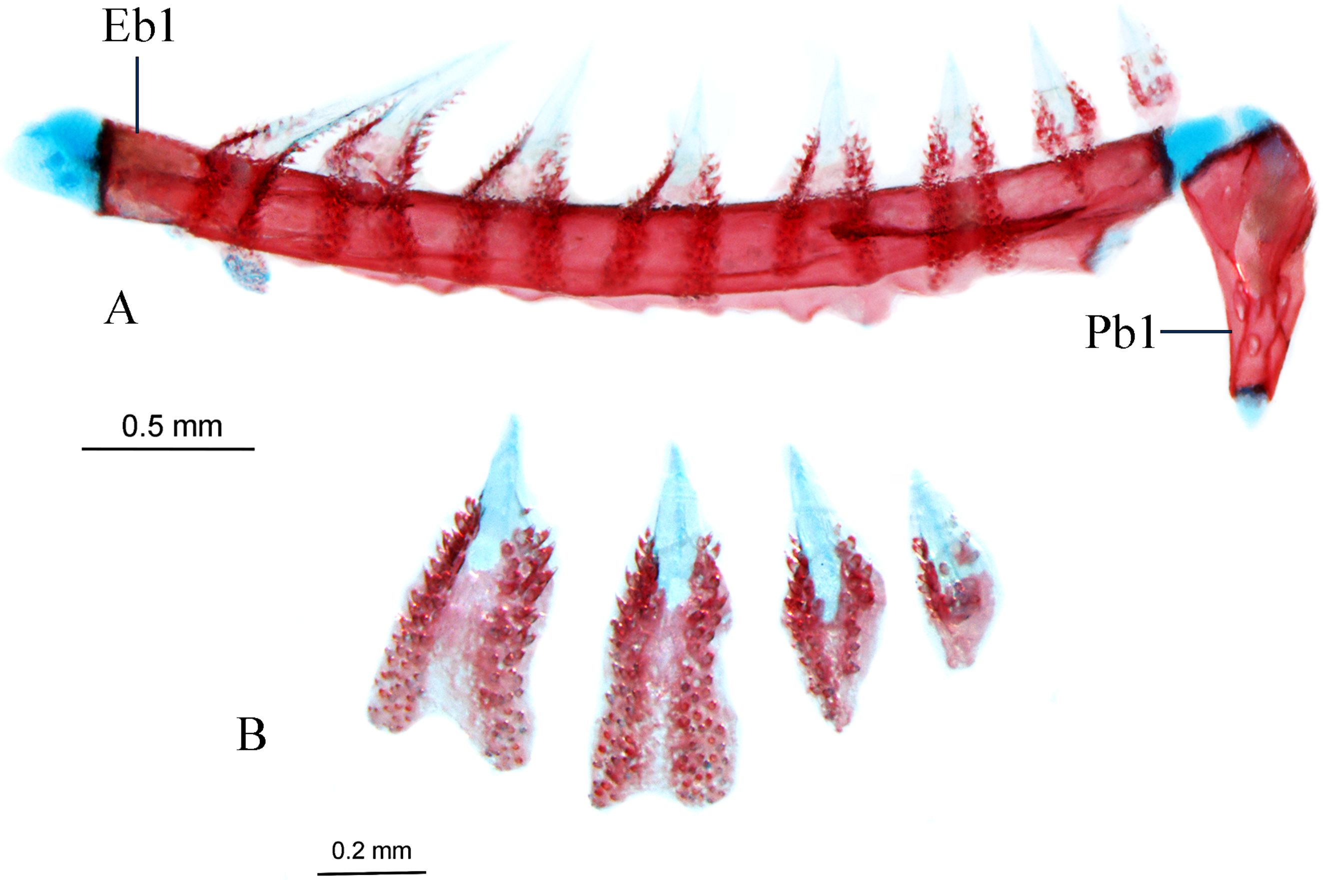

Figure 9. Gill rakers of *Moenkhausia lepidura* showing distinct basal expansions full of spines, MZUSP 109841, 60.2 mm SL. **A:** epibranchial and pharingobranchial of first branchial arch, right side, ventral view. **B:** close up of the gill rakers of epibranchial 1 showing the presence of a basal expansion full of small spines. **Eb1**, epibranchial 1, **Pb1**, pharingobranchial 1.

group. Considering the vast diversity of the Characidae, such hypothesis still needs to be tested through a cladistics analysis, but the features highlighted will certainly be useful in future phylogenetic studies of the Characidae.

Material examined. *Acestrocephalus sardina*: MZUSP 29241 (1 c&s, 64.1 mm SL), rio Negro basin, rio Marauiá, near to mouth, Amazonas State, 0°24'S 65°12'W, Brazil. *Agoniates halecinus*: MZUSP 34332 (1 c&s, 119.2 mm SL), rio Xingu basin, Belo Monte, Pará State, 3°7'S 51°42'W, Brazil. MZUSP 103245(1 dry skeleton, 134.8 mm SL), rio Jari basin, rio Iratapuru, tributary of left margin of rio Jari on community of Iratapuru, Amapá State, 0°33'59'S 52°34'43"W, Brazil. MZUSP 94366 (1 dry skeleton, 164.65 mm SL), rio Xingu basin, Miriam Lake, right margin of rio Culuene, Mato Grosso State, 13°25'48"S 53°2'24"W, Brazil. *Astyanax guianensis*: MZUSP 109674 (4 c&s, 31.5–33.7 mm SL), rio Negro basin, right margin of downstream of rio Jurubaxi, Amazonas State, 0°30'6"S 64°49'11"W, Brazil. MZUSP 104823, (2 c&s, 36.8–38.4 mm SL), left margin of rio Jari, at pier of Santo Antônio community, Amapá State, 0º38'22"S 52º30'33"W, Brazil. *Astyanax multidens*: MZUSP 96726 (2 c&s, 34.4–34.8 mm SL), rio Tapajós basin, rio Teles Pires near to float of MT-416 road, Mato Grosso State, 9°27'7"S 56°30'46"W, Brazil. *Brycon nattereri*: MZUSP 59623 (1 c&s, 130.0 mm SL), rio Tocantins basin, tributary stream of rio Tocantizinho, Goiás State, 14°2'27"S 48°12'22"W, Brazil. *Bryconops caudomaculatus*: MZUSP 84978 (1 c&s, 74.8 mm SL), rio Tiqué, between waterfall of Pedra Curta and village of São Pedro, Amazonas State, 0°16'S 69°58'W, Brazil. *Coptobrycon bilineatus*: MZUSP 87723 (2 c&s, 31.9–33.5 mm SL), Ribeirão Venerando, rio Tietê drainage, São Paulo State, 23°39'8"S 45°53'21"W, Brazil. *Charax stenopterus*: MZUSP 9616 (1c&s, 69.4 mm SL), rio Patos, Pelotas, Rio Grande do Sul State, 31°46'S 52°20'W, Brazil. *Cynopotamus gouldingi*: MZUSP 77268 (1 c&s, 113.2 mm SL), rio Madeira basin, rio Roosevelt, above the waterfall, Mato Grosso State, 9°11'29"S 60°44'9"W, Brazil. *Galeocharax goeldii*: MZUSP 92436 (1 c&s, 35.8 mm SL), rio Madeira basin, Igarapé Karipuna, upper rio Madeira, Rondônia State, 9°15'10"S 64°38'31"W, Brazil. *Hemigrammus bleheri*: MZUSP 29435 (3, 24.4 – 26.7 mm SL), rio Negro basin, confluence with rio Urubaxi, Amazonas State, 0°31'S 64°50'W, Brazil. *Hemigrammus durbinae*: MZUSP 50074 (1 c&s, 20.5 mm SL), rio Solimões basin, Januacá lake, Amazonas State, 3°25'S 60°17'W, Brazil. *Hemigrammus marginatus*: MZUSP 113913 (1 c&s, 26.3 mm SL), rio São Francisco basin, Rio Porto Alegre, tributary of rio Grande, Bahia State, 12°47'49"S 44°67'54"W, Brazil. *Hemigrammus ulreyi*: MZUSP 59538 (1 c&s, 29.3 mm SL), rio Verde of Mato Grosso, Mato Grosso do Sul State, 19°9'67"S 55°17'86"W, Brazil. *Hemigrammus unilineatus*: MZUSP 38713 (1, 34.8 mm SL), first north tributary of rio Tarapuy, rio Negro basin, Napo, Equador. *Hyphessobrycon bifasciatus*: MZUSP 55159 (6 c&s, 21.6–42.2 mm SL), Ribeira de Iguape, São Paulo State, 24°18'42"S 42°35'50"W, Brazil. *Hyphessobrycon compressus*: MZUSP 28535 (1 c&s, 22.1 mm SL), Orangewalk District, Baba Creek near New River Lagoon, Belize State, 17°46'44"S 88°38'29"W, Mexico. *Hyphessobrycon diancistrus:* MZUSP 29846 (2 c&s, 27.0–29.7 mm SL), MZUSP 29847 (1 c&s, 29.6 mm SL), rio Negro basin, island of Tamanquaré, 0°30'S 64°55'W, Brazil. *Hyphessobrycon elachys*: MZUSP 59415 (9 c&s, 15.5–19.5 mm SL), lower rio Negro, rio Paraguai basin, Mato Grosso do Sul State, 19°20'29"S 56°58'10"W, Brazil. *Hyphessobrycon eques*: MZUSP 102973 (3 c&s, 15.8–22.8 mm SL), rio Solimões basin, Lago do Castanho, Amazonas State, Brazil. *Hyphessobrycon hebertaxelrodi*: MZUSP 103764 (3 c&s, 20.6–28.8 mm SL), rio Paraguai basin, Mato Grosso do Sul State, 18°28'50"S 54°40'37"W, Brazil. *Hyphessobrycon luetkenii*: MZUSP 19021 (4 c&s, 24.5–44.9 mm SL), road to Grande/Cassino, rio Patos drainage, Rio Grande do Sul State, 32°5'S 52°12'W, Brazil. *Hyphessobrycon megalopterus*: MZUSP 96690 (6 c&s, 12.8–17.1 mm SL), rio Paraguai basin, rio Mutum, between Mimoso Village and Joselândia, Mato Grosso State, 16°19'30"S 55°49'59"W, Brazil. *Hyphessobrycon pulchripinnis*: MZUSP 92682 (6 c&s, 25.1–31.9 mm SL), igarapé afluente do rio Tapajós, rio Amazonas basin, Pará State, 4°33'48"S 56°15'40"W, Brazil. *Hyphessobrycon socolofi*: INPA 32626 (1 c&s, 33 mm SL), rio Negro basin, Roraima State, Brazil. *Moenkhausia abyss*: MZUSP 29422 (2 c&s, 34.0–36.7 mm SL), rio Negro basin, Brazil. *Moenkhausia aurantia*: MZUSP 113875 (1 c&s, 30.1 mm SL), rio Tocantins basin, rio Arraias above bridge of road TO-050, Tocantins State, 12°81'94"S 47°6'36"W, Brazil. *Moenkhausia bonita*: MZUSP 38086 (1 c&s, 34.9 mm SL), rio Paraguai basin, Ponte e Lacerda Farm, rio Jauru, Mato Grosso State, 16°8'S 58°1'W, Brazil. *Moenkhausia celibela*: MZUSP 30309 (1 c&s, 35.0 mm SL), rio Tapajós basin, Jacareacanga-Itaituba road, Pará State, Brazil. MZUSP 97629 (3 c&s, 27.7–31.3 mm SL), rio Xingu basin, flood at BR163 bridge, near Castelo dos Sonhos, Pará State, 8°15'17"S 55°6'40"W, Brazil. *Moenkhausia collettii*: MZUSP 109450 (1 c&s, 30.9 mm SL), rio Negro basin, Igapó on tributary of rio Jaradi, near to joint of rio Jaradi and rio Marauiá, Amazonas State, 0°22'44"S 65°12'39"W, Brazil. *Moenkhausia cosmops*: MZUSP 93556 (3 c&s, 26.1–28.1 mm SL), rio Juruena, rio Tapajós basin, Mato Grosso State, 13°14'47"S 59°0'52"W, Brazil. *Moenkhausia costae*: MZUSP 90893 (1 c&s, 29.5 mm SL), Itapicurú basin, rio Itapicurú on city of Queimadas, below to Barragem Grande, Bahia State, 10°59'2"S 39°40'9"W, Brazil. *Moenkhausia cotinho:* MZUSP 55125 (3 c&s, 29.5–39 mm SL), rio Negro basin, Igarapé in São João, near Tapurucuara, Amazonas State, 0°24'S 65°2'W, Brazil. *Moenkhausia dichroura*: MZUSP 90191 (1 c&s, 28.1 mm SL), rio Paraguai basin, rio Sepotuba, Mato Grosso State, 15°47'33"S 57°39'20"W, Brazil. *Moenkhausia gracilima*: MZUSP 5447 (2 c&s, 44.9–46.4 mm SL) rio Trombetas basin, Oriximiná, Pará State, 1°46'S 55°52'W, Brazil. *Moenkhausia hasemani*: MZUSP 99015 (2 c&s, 64.3–70.2 mm SL), rio Tefé, tributary of rio Amazonas, Juruapari, Amazonas State, 3°22'S 64°43'W, Brazil. *Moenkhausia hysterosticta*: MCP 32561 (4 c&s, 40.5–47.5 mm SL), rio Tocantins basin, Legeado, Funil, Tocantins State, 9°45'2"S 48°21'56"W, Brazil. *Moenkhausia icae*: MZUSP 104338 (1 c&s, 33.9 mm SL) rio Japurá basin, Paraná da Jacintara, Amazonas State, 1°57'S 65°10'W, Brazil. *Moenkhausia* cf. *intermedia*: MZUSP 40918 (1 c&s, 35.7 mm CP), rio Tocantins basin, Poço do Gandaia, marginal lake of rio Paranã, Olho d'água farm, Goiás State, 14°26'S 47°3'W, Brazil. *Moenkhausia jamesi*: MZUSP 17352 (1 c&s, 53.8 mm SL), Sorubim Island, above Coari, rio Solimões basin, Amazonas State, 3°55'S 63°20'W, Brazil. *Moenkhausia lata*: MZUSP 7921 (2 c&s, 53.2–55.3 mm SL), rio Amazonas basin, Igarapé of rio Jamari, above Terra Santa, Pará State, 2°7'S 56°29'W, Brazil. MZUSP 18047 (1 c&s, 49.4mm SL), Igarapé Inó, Furo de Panaquera, Pará State, Brazil. *Moenkhausia lepidura*: MZUSP 6247 (1 c&s, 61 mm SL), rio Negro basin, above Manaus, Amazonas State, 3°10'S 60°W, Brazil. MZUSP 8181 (2 c&s, 62.7–64.1 mm SL), rio Trombetas, Jacuapá Lake, Oriximiná, Pará State, 1°46'S 55°54'W, Brazil. MZUSP

37458 (2 c&s, 66–70 mm SL), rio Madeira basin, rio Alegre, tributary of rio Guaporé, approximately 30 km from Vila Bela da Santíssima Trindade, Mato Grosso State, 15°30'S 59°20'W, Brazil. MZUSP 109841 (2 c&s, 60.2–64.2 mm SL), rio Amazonas basin, rio Tefé, tributary of rio Amazonas, Juruapari, Amazonas State, 3°22'S 64°43'W, Brazil. *Moenkhausia lopesi:* MZUSP 82057 (1 c&s, 33.7 mm SL), tributary of rio Culuene, upper rio Taquari, Sonora, Mato Grosso do Sul State, Brazil. *Moenkhausia loweae*: MZUSP 91869 (2 c&s, 47.4–45.5 mm SL), stream of farm of Lício, tributary of rio Culuene, city of Paranatinga, Mato Grosso State, 13°49'S 53°15'W, Brazil. *Moenkhausia megalops*: MZUSP 97314 (1 c&s, 46.2 mm SL), rio Tapajós basin, rio Jamanxim, near Vila Mil, Pará State, 7°43'51''S 55°16'36''W, Brazil. *Moenkhausia mikia:* MZUSP 81198 (1 c&s, 46.7 mm SL), rio Tiquié, sand beaches downstream waterfall, Caruru village, Amazonas State, 0°16'29''S 69°54'54''W, Brazil. MZUSP 81219 (2 c&s, 33.7–37 mm SL), rio Tiquié, port between São Domingos Sávio and Jabuti village, Amazonas State, 0°4'59''S 68°25'W, Brazil. *Moenkhausia nigromarginata*: MZUSP 118180 (1c&s, 39.7 mm SL), rio Papagaio, after rio Sacre, Mato Grosso State, 13°37'32.6''S 58°17'38.7''W, Brazil. *Moenkhausia oligolepis*: MZUSP 092942 (2 c&s, 41.9–56 mm SL), rio Negro basin, Igarapé Castanha tributary of rio Tiquié, Sítio Belém, slightly below of comunity of Santa Rosa, Amazonas State, 0°5'23''S 69°39'57''W, Brazil. *Moenkhausia phaeonota*: MZUSP 45301 (2 c&s, 30.1–35.9 mm SL), headwater of rio Preto, Cuiabá-Santarém road, rio Tapajós basin, Mato Grosso State, 14°20'S 56°13'W, Brazil. *Moenkhausia pirauba*: MZUSP 73467 (3 c&s, 40.8–50.9 mm SL), rio Tapajós basin, Arinos river, Igarapé about 30 Km above Ponte dos Gaúchos, Mato Grosso State, Brazil. *Moenkhausia sanctaefilomenae*: MZUSP 96038 (3 c&s, 29.6–38.3 mm SL), rio São Francisco basin, Bahia State, Brazil. *Moenkhausia tergimacula*: MZUSP 97940 (1 c&s, 57.1 mm SL), rio do Sono, Tocantins State, 10°15'40''S 46° 53'3''W, Brazil. *Moenkhausia xinguensis*: MZUSP 111531 (1 c&s, 44.8 mm SL), rio Xingu, in Cachoeira do Espelho, Pará state, 3°39'5''S 52°22'42''W, Brazil. *Nematocharax venustus*: MZUSP 102635 (2 c&s, 36.8–46.8 mm SL), rio Água Preta do Mocambo, rio Almada drainage, Bahia State, 14°34'53''S 39°17'56''W, Brazil. *Parecbasis cyclolepis*: MZUSP 25942 (1 c&s, 41.7 mm SL), Rio Ucayali, Coronel Portillo, Perú. *Phenacogaster tegatus*: MZUSP 35889 (1 c&s, 35.5 mm SL), rio Paraguai basin, Piquiri river mouth, Santo Antônio do Paraíso, Mato Grosso State, 17°12'S 54°9'W, Brazil. *Poptella paraguensis*: MZUSP 59914 (1 c&s, 49.64 mm SL), rio Negro, road between Nhecolândia and road BR-262, Mato Grosso do Sul State, 19°17'16''S 57°3'39'' W, Brazil. *Rhaphiodon vulpinus*: MZUSP 92008 (1 dry skeleton, 306–385.6 mm SL), rio Tocantins basin, hidroeletric, Serra da Mesa, Brazil. *Roeboides paranensis*: MZUSP 19830 (1 c&s, 35 mm SL), rio Paraguai basin, rio Cuiabá, mouth of rio Croará, 20km from Barão de Melgaço, Mato Grosso State, 16°11'S 55°57'W, Brazil. *Roeboides xenodon*: MZUSP 54684 (1 c&s, 56.7 mm SL), rio São Francisco basin, Santana stream, 31 km Southern from Jesus da Lapa, at road to Malhada, Bahia State, 13°31'13''S 43°21'28''W, Brazil. *Salminus brasiliensis*: MZUSP 19439 (1 c&s, 132.2 mm SL), rio Grande basin, rio Mogi Guaçu, Emas, São Paulo State, 21°55'S 47°23'W, Brazil. *Thayeria obliqua*: MZUSP 29391 (1 c&s, 32.2 mm SL), rio Negro basin, joint with rio Urubaxi, Amazonas State, 0°31'S 64°50'W, Brazil. *Triportheus nematurus*: MZUSP 85808 (1 c&s, 116.6 mm SL), rio Paraguai basin, Taimã island, rio Paraguai, Mato Grosso do Sul State, Brazil.

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Authors' Contributions

Gustavo Darlim: Description of the bony complexes of the species; contributions to the photographs and laboratory preparations of cleared and stained specimens; contributions to data collection; contributions to data analysis and interpretation; contribution to manuscript preparation, concept and design of the study; contribution to critical revision, adding intellectual content.

Manoela Maria Ferreira Marinho: Support for orientation methodology and scientific theoretical contributions; contributions in the manuscript preparation, concept and design of the study; contributions to the photographs of the bones and laboratory preparation of cleared and stained specimens; contribution to data analysis and interpretation; contributions adding intellectual content; contribution to critical revision and intellectual content.

Conflicts of interest

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

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Article

Matter turnover in the oligotrophic restinga ecosystem and the importance of the key species *Clusia hilariana*

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Abstract: Restingas are sandy areas spread along the Brazilian coast made up of a mosaic of forest and open woodland vegetation adapted to varying conditions of aridity, oligotrophy and salinity. Two vegetation types are very common in southeast Brazilian restingas, open *Clusia* formations and seasonally dry forest formation. Litter production and nutrient (C and N) turnover were studied comparatively in forest formations and *Clusia* formations, in vegetation patches with and without *Clusia hilariana*. The results showed that the breakdown process is extremely retarded in *Clusia* formations, with or without *C. hilariana*, leading to C accumulation in the soil. Microbial and soil fauna activity is lower in *Clusia* formations in comparison to forest formations; patches without *Clusia hilariana* showed intermediate conditions regarding total matter and carbon loss. Nitrogen loss was lowest in patches without *C. hilariana*, where soil micro-organisms accumulate N during the decomposition process, such as in the forest. The ratio of lignin in litter and the slow release of N reinforce the importance of the dominant tree *Clusia hilariana* as a potential key species for organic matter turnover. The accumulation of organic matter under the *Clusia* formation vegetation may be determinant for the humus richness of some bodies of water in the restinga, demonstrating the importance of this species to the ecosystem.

Keywords: *Coastal ecosystem, decomposition; litterfall, nutrient cycling, nurse plant.*

Ciclagem de matéria nos ecossistemas oligotróficos de restinga e a importância da espécie chave *Clusia hilariana*

Resumo: Restingas são terraços arenosos dispostos ao longo da costa brasileira. Dois tipos de vegetação são muito comuns nas restingas do sudeste brasileiro, formações *Clusia* e formação florestal sazonalmente seca. A produção de serapilheira e o turnover de nutrientes (C e N) foram estudados comparativamente na formação florestal e na formação aberta de *Clusia*, em moitas de vegetação com e sem *Clusia hilariana*. Os resultados mostraram que a decomposição é muito lenta na formação de *Clusia* levando a acumulação de C no solo. A ação microbiana e da fauna do solo também é mais lenta nessa formação em comparação com a formação florestal; moitas sem *Clusia hilariana* apresentaram condições intermediárias com relação a perda de carbono total. A liberação de nitrogênio também é menor em moitas sem *C. hilariana*, onde os microrganismos do solo acumulam o N durante o processo de decomposição, como na floresta. A proporção de lignina na serapilheira e a liberação lenta de N reforçaram a importância da espécie dominante *Clusia hilariana* como uma espécie chave para o turnover da matéria orgânica do ecossistema. Por fim o acúmulo de altos teores orgânicos sob a vegetação da formação de *Clusia* pode ser o fator responsável pela riqueza de húmus de alguns corpos de água na restinga, demostrando a importância desta espécie para o ecossistema.

Palavras-chave: *Ecossistemas costeiros, decomposição; serapilheira; ciclagem de nutrientes, planta facilitadora.*

Introduction

Approximately 70% of the Brazilian coastline is covered by restinga. Restinga is defined geomorphologically by sandy marine deposits during Quaternary changes of the sea-level (Martin et al. 1993), which form sandy coastal plains of approximately 8,000 km in length (Tessler & Goya 2005). These coastal plains consist of a mosaic of bare sandy areas, sections loosely covered by low vegetation, patches of low woody vegetation, spots covered by dry or wet woods, swales, artificial channels and lagoons which originate in ancient river delta depressions (Martin et al. 1993) with or without outflow to the ocean.

Three decades of studies of restinga ecology have shown that these ecosystems are exposed to extreme micro-climatic conditions. Studies on restinga vegetation describe different plant communities determined by distinct combinations of stressful conditions such as oligotrophy, drought, temperature, salinity and flooding (Scarano et al. 2005). Plant species, which affect the nutrient content of the soils and coastal lagoons, have physiologically adapted to these extreme conditions (Dias & Scarano 2007; Hay & Lacerda 1984). A few sets of species are extremely dominant and, consequently, predominate the functioning of these ecosystems (Scarano et al. 2004).

Open woodlands sparsely covered by shrubs are very common in the southeast Brazilian restinga. When *Clusia hilariana* Schltdl. (Clusiaceae) is the dominant tree species over an area, this area is defined as a *Clusia* formation. This tree has attracted scientific attention in recent years because of its local role as a nurse plant in this formation due to its: (1) attraction of seed dispersers, consequently affecting seedling density and richness of the understory (Dias & Scarano 2007); (2) high contribution to biomass production (Dias et al. 2006); and (3) for its crassulacean acid metabolism (CAM) (Scarano et al. 2005). Scarano et al. (2004) proposed a succession model for *Clusia* formations driven by the senescence and death of *Clusia* in a patch, promoting the growth of understory juveniles and a change in architecture to a short and dense vegetation type. Dias et al. (2006) and Dias (2008) described a slow development of seedlings in *C. hilariana* understory until its senescence, after that the dynamics of nutrients changes. Dias et al. (2006) indicated that *C. hilariana* may promote the accumulation of organic matter due to the relatively high production of litter with slow decomposition rates.

The present study highlights questions related to the nutrient cycling in restinga environments considering the differences between vegetation types and the influence of *Clusia hilariana* biomass, considering this as a key species for organic matter turnover. We aimed to understand differences in biomass production and decomposition, considering carbon and nitrogen content in litterfall and soil. The ratio of lignin in litter and the presence of macroinvertebrates on the soil were also considered as vector that interferes in matter turnover. We hypothesized that the vegetation of open areas presents lower productivity when compared to the forest formations, what is cause and consequence of nutrients availability in the soil and the litter, being the *Clusia hilariana* one of the most recalcitrant species, promoting differences between vegetation patches with and without *C. hilariana*.

Material and Methods

1. *Study site*

The investigation was performed in an area located in the restinga of Jurubatiba National Park, north of the city of Macaé, Rio de Janeiro State, Brazil (22º15'47-58" S, 41º36'16-27"W).

The restinga vegetation in the National Park was characterized by different vegetation types with plants primarily originating from the Atlantic rain forest (Scarano 2009). *Clusia* formations make up 32% of the total area in the National Park, followed by Ericaceae formations (ca. 29%) represented by sparse herbaceous vegetation, and forest formations (ca. 16%) that occur at the periphery of the swales, lagoons or channels (ca. 10%) (Caris et al. 2013). Araujo et al. (1998) differentiated two forest formations: seasonally dry and long-lasting flooded forests.

Clusia formations are patchy clumped patterns of vegetation that cover approximately 40% of the area interlaced with white sandy areas (Oliveira-Galvão et al*.* 1990). *Clusia hilariana* was the dominant plant species, and was the central tree in most woody patches. Patches that had no *Clusia* as the central tree were covered by C_3 shrubs.

The soil and subsoil consisted of light brownish marine quartz sands, excessively drained and poor in nutrients, clay, and organic matter (Hay & Lacerda 1984). The soil composition differed in the stream and channel valleys, probably due to the deposition of silt and clay carried by running waters from outside of the restinga (Magnago et al. 2010).

Three vegetation types were studied in this investigation: two types of *Clusia* formations and the seasonally dry forest formation. The two types of the heterogeneous *Clusia* formation were woody vegetation patches: 1) dominated by *Clusia* and 2) without *Clusia*. The studied formations were disposed in an equidistant point between two lagoons (towards 2.5 km from Carapebus and Comprida lagoons) and approximately one kilometer from the beach. Sampling and experiments were performed around coordinates 22º15'56.57"S - 41º38'14.39"W in *Clusia* formation and 22º15'44.98"S - 41º38'20.09"W in seasonally dry forest formation. Patches dominated by *Clusia* and without *Clusia* were randomly distributed in an area of approximately 30.000 m^2 at *Clusia* formation site. These two different types of patches included in the study were also in random distribution, with approximately 65 m^2 (standard deviation ± 25 m^2) and with a hemispherical shape.

2. *Experimental design and chemical analyses*

The study on litter production was performed from May 2012 to May 2014 (Figure 1). Fifteen replicate sites of each of the three vegetation types were selected (Figure 2). At each site, one litter sampler was installed 50 cm above the ground consisting of a wooden frame of 50 cm x 50 cm x 15 cm. Litter was sampled at 15 day intervals from May 2012 to April 2014, totalizing 53 samples per site. The collected litter was oven dried at 80°C to a constant weight and subsequently weighed (precision reading of 0.1 g). The following litter fractions were weighed separately: (1) leaves, (2) wood (a set of recognized branches small than 50 cm), (3) reproductive parts (flower, fruits and seeds), and (4) unidentified solid objects. Subsequently, the dried samples were stored in a freezer for later analyses. Only leaves with least than 15 days sampled by the litter sampler were used in this study to avoid effects of decomposition time. Leaves sampled during May 2012 to November 2012 were used for decompositions experiments, whereas samples from December 2012 to November 2013, were used for chemical analyses of carbon and nitrogen decay (Figure 1).

Temperature and precipitation data obtained from the National Institute of Meteorology (INMET) at the Macaé-Rio de Janeiro station. The climate in Macaé is characterized by hot summers (January to March) and warm winters (July to September), markedly seasonal mainly due to differences in rainfall (Folharini 2015). During our study (May 2012 to April 2014) the mean annual daytime temperature was 23.5 °C, 25.7 °C in summer and 21.1 °C in winter. Total precipitation was 640 mm and 140 mm, during the summer and winter, respectively, with a mean annual sum of approximately 1060 mm.

Original dried litter collected from May 2012 to November 2012 for the litter production study was thawed, dried and mixed. Because of the requirement for 7 g of dried litter for decomposition analysis, sufficient litter bags were available for distribution and decomposition analysis in only 5 randomly chosen sites within each of the three vegetation types (Figure 2). The mesh bags were closed and exposed to the soil surface in the field. To roughly differentiate between soil fauna and micro-organism effects on the litter breakdown, two types of mesh bags were used. Five (5) mm mesh size bags allowed access to all soil organisms to the breakdown process and 0.02 mm mesh sizes allowed only access to micro-organisms (bacteria, fungi and soil micro-fauna) (Kampichler & Bruckner 2009). Due to differences in decomposition rate requiring more frequent sampling from the 5 mm bags, 12 mesh bags of the 5 mm size and 4 mesh bags of the 0.02 mm size were exposed on

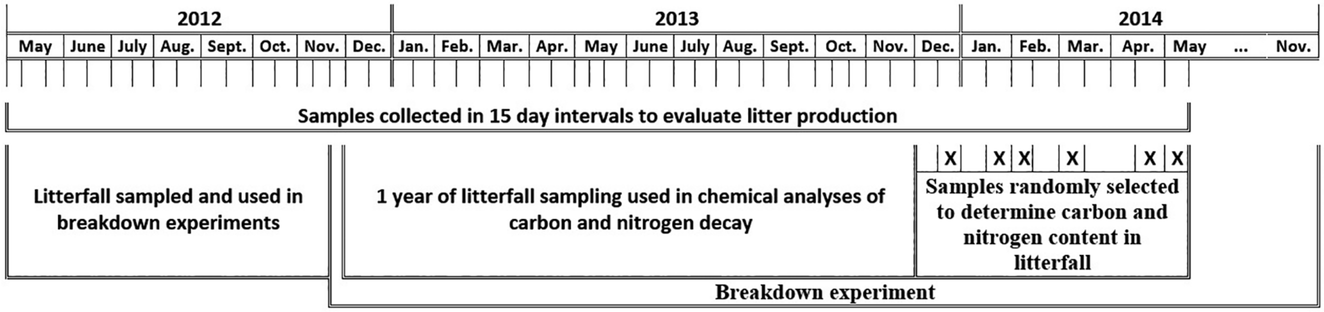

Figure 1. Sampling calendar for litterfall production, breakdown experiments and analysis of carbon and nitrogen content and decay.

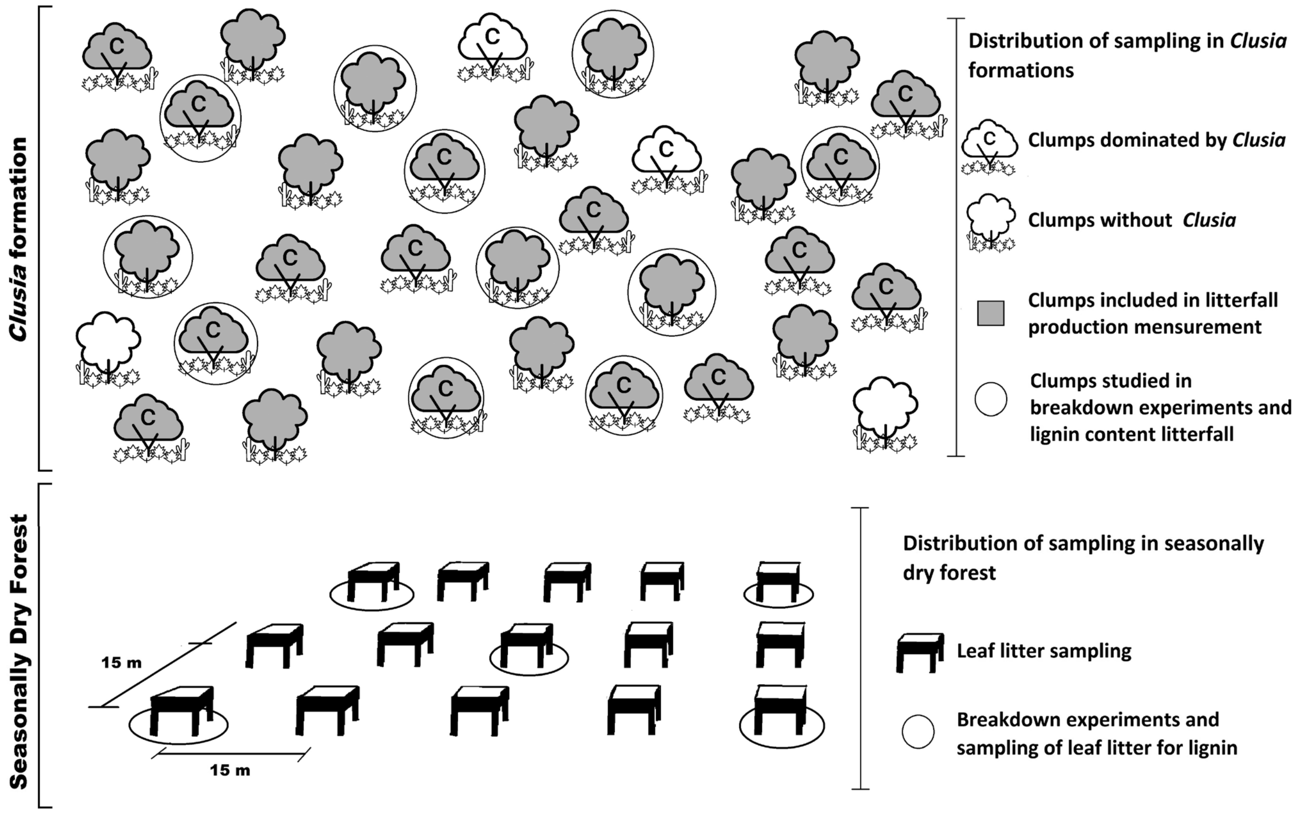

Figure 2. Distribution of sampling for litterfall production, breakdown experiments and lignin content among heterogeneous *Clusia* formations, with and without *Clusia*, and seasonally dry forest.

November 28th, 2012, at each of the 15 selected sites. The bags with 5 mm mesh size were successively sampled at two months intervals. The bags with 0.02 mm mesh sizes were sampled at 4 to 5 months intervals. The last sampling date was November 8th, 2014, approximately two years after initial exposure (Figure 1). The litter within the mesh bags was oven dried at 60°C for one day and weighed (precision reading of 0.1 g). After weighing, the material in the mesh bags was ground to powder for the chemical analyses.

For measurement of carbon and nitrogen content in litterfall, material of one sample day was combined for five random sites of each vegetation type. This procedure was repeated six times from December 2013 to May 2014, providing six replicate samples from each of the three vegetation types (as shown in Figure 1). Total carbon was measured using a Carbon Analyser (unit TNM-1, TOC-5000, Shimadzu). Nitrogen concentration was determined spectrophotometrically using a modification of the standard Kjeldahl-N method for plant samples as described by Graça *et al*. (2005).

Analyses for lignin content were conducted at each of the five sites chosen for the litter breakdown experiment, considering five samples of each site as one replicate, with a total of 5 replicates per vegetation type (Figure 2). Lignin content was determined by Agrolab Group (Bruckberg, Germany) using the Acid Detergent Lignin Procedure (ADL) (according to VDLUFA 2006). Nitrogen values of the freshly fallen leaves were used to calculate the lignin/nitrogen ratio, which is considered a measure of decomposition resistance (Melillo et al. 1982).

Soil (0-10 cm depth) was physically and chemically analysed at each site of the litter breakdown experiment (5 replicates per vegetation type) (Figure 2) and in 5 replicate sites of the bare sandy areas between vegetation patches. Five samples of each site were mixed to one replicate sample for further analysis. The soil analyses were performed by the Soil Analyses Center of the Federal Rural University of Rio de Janeiro (UFRRJ).

3. *Statistical Analyses*

Differences in litter production, carbon and nitrogen content and lignin/nitrogen ratio between the vegetation types were statistically tested using the non-parametric Kruskal-Wallis rank test for multiple pairwise comparisons and Monte Carlo for pairwise comparison.

Friedman ANOVA was used to test the differences in the proportion of litter fractions of each vegetation type (leaves, wood, reproductive parts and unidentified). Dry litter weight and carbon content in each sample were correlated with the following sums of rainfall before litter sampling: 15, 30, and 45 days. The 15 day intervals were selected because litter sampling was also conducted at 15 day intervals. The effect of mean temperature of 15 days before litter sampling was correlated with dry litter weight considering two years of sampling. Mean of temperature were also correlated with carbon content in each sample from the second year (chemically analyzed) and linear regressions were used to test relationships between these carbon contents and litter production.

The breakdown rate was calculated using the exponential function of Olson (1963): $x_t = x_0\ e^{-kt}$, where x_t = mass at time t, x_0 = mass at time t_0, and k the breakdown rate. To compare rates, k was calculated for one year using the formula: $k = -\ln(x_t/x_0)/(t_t-t_0)*365$. Half time of litter breakdown was calculated using the formula 0.693/k, and 95% breakdown using the formula 3/k (Olson 1963). The soil fauna impact was determined using the formula $k_f = k_{tot}-k_m$, where k_f is the role of the soil fauna on the breakdown process, k_m the role of the soil micro-organisms derived from the 0.02 mm mesh bags, and k_{tot} the role of all soil organisms derived from the 5 mm mesh bags. For the calculation of the breakdown rate at each site, the first two rates were omitted due to adaptation processes in the early period of the experiment during which bacteria and fungi are allowed time to grow and find a natural balance with micro-organism grazers. The rates were compared using a Kruskal-Wallis ANOVA with subsequent Tukey's post hoc tests, however all statistic comparisons considered data obtained until 74 weeks, when the experiments with 0.02 mm mesh bags was over.

Soil analyses were compared using a nonparametric Kruskal-Wallis rank test for multiple comparisons of mean ranks for all groups, followed by Monte Carlo for pairwise comparison. Statistical analyses were performed using the program PAST (Hammer et al. 2014).

Results

1. *Litter Production*

The litter production showed significant differences among seasonally dry forests and both types of *Clusia* formation (N = 159, df = 2, H = 38.23, $p < 0.001$); however, no differences were observed between the two vegetation types of the *Clusia* formation (N = 4, df = 2, Friedman ANOVA = 1.64, $p < 0.05$) (as shown in Table 1). Litter production was higher in seasonally dry forests. No significant differences between litter fractions were found in the vegetation types considered.

In *Clusia* patches, litter production significantly increased as accumulated rainfall decreased over periods of 30 and 45 days (sum of rainfall for 30 days before litter collection: $t = -2.33$, $r^2 = 0.096$, df = 51, $p = 0.02$; and 45 days before litter collection: $t = -2.34$, $r^2 = 0.097$, df = 51, $p = 0.02$), though this relationship was not observed over a 15 day period of decreasing rainfall. No relationship between litterfall and temperature was observed within *Clusia* patches. No relationship was observed between litterfall and temperature or accumulated precipitation in forest or patches without *Clusia*.

2. *Breakdown*

Breakdown rates were equal between the patchy sites dominated by *Clusia* or without *Clusia*, but differed between these patchy vegetation types and the forest (df: 5, Mean Squares between groups: 0.22, Mean Squares within groups: 0.01, $p < 0.001$) (as shown in Table 2). No significant differences between mesh bags giving access to all soil organisms and those giving access only to the soil micro-organisms were found in the two patchy vegetation types. In contrast, the rates differed significantly between the two mesh bag types in the forest. With one exception, breakdown in the forest was even faster in the 0.02 mm mesh bags than in the 5 mm mesh bags for the two vegetation types of the *Clusia* formation. The importance of the soil fauna on the breakdown process in the forest may also be seen in the significant difference of the k*f*-values between these sites and the patchy vegetation types (df: 2, MS between groups 0.11, MS within groups: 11, $p = 0.04$) (Table 2). Regarding the time required for 95% mass loss, breakdown in the forest takes only one third as long as in the *Clusia* dominated sites. Even the breakdown with only microbial access to the process is two times faster in the forest than in the *Clusia* dominated patches with access to all soil organisms.

Table 1. Litter characterization and Chemical and physical variables of soil (0-10 cm) of two formations of the Restinga de Jurubatiba National Park, Brazil. Equal superscript letters indicate insignificance difference at $p > 0.05$. ^{1}Clusia formation, ^{2}Seasonally dry forest.

	Unit	^{2}Forest	^{1}*Clusia* dominated	^{1}Without *Clusia*	^{1}Areas between tussocks
			LITTER		
Content of leaves	%	57.7	81.4	64.8	-
Content of wood		17.2	12.6	21.3	-
Reproductive parts		2.4	1.2	6.8	-
Unidentified solid object		22.7	4.7	7.1	-
Production	$t.ha^{-1}.yr^{-1}$	10.119^{b}	4.688^{a}	3.986^{a}	-
Carbon		4.494	2.114	1.694	-
Nitrogen		0.106	0.029	0.032	-
Carbon	% ± sd	^{a}44.41 ± 0.78	^{a}45.11 ± 1.46	^{a}45.25 ± 2.30	-
Nitrogen		^{b}1.05 ± 0.14	^{a}0.63 ± 0.13	^{a,b}0.82 ± 0.05	-
Lignin		^{a}47.12 ± 3.51	^{a}45.52 ± 3.69	^{a}40.56± 6.76	-
Lignin/nitrogen	ratio ± sd	^{b}45.30 ± 7.65	^{a}73.42 ± 12.32	^{a,b}49.68 ± 10.35	-
			SOIL		
	pH ± sd	^{a}3.92 ± 0.23	^{a}3.84 ± 0.17	^{a,b}4.18 ± 0.33	^{b}4.76 ± 0.18
Sand	g/kg	97.2	93.4	97.8	100
Silt		0.4	1.5	0.5	0
Clay		2.4	5.1	1.7	0
N total	% ± sd	^{a}0.20 ± 0.06	^{a}0.39 ± 0.44	^{a,b}0.18 ± 0.07	^{b}0.10 ± 0.02
C		^{a,b}2.75 ± 1.16	^{a}3.73 ± 1.52	^{a,b}3.29 ± 2.55	^{b}0.19 ± 0.05
OM	g/dm^{3} ± sd	^{a,b}47.48 ± 20.05	^{a}64.24 ± 26.20	^{a,b}56.72 ± 43.88	^{b}3.28 ± 0.84
S.B.*	$cmol_{c}/dm^{3}$ ± sd	^{a,b}2.59 ± 1.67	^{a}2.47 ± 0.55	^{a,b}2.34 ± 0.45	^{b}0.38 ± 0.14
T**		^{a,b}15.11 ± 8.33	^{a}23.27 ± 12.64	^{a,b}15.18 ± 16.71	^{b}0.38 ± 0.14

* sum of the bases (Ca+Mg+K+Na); ** cation-exchange capacity at pH 0.7.

Table 2. Breakdown rates (k) with half time and time for 95 % breakdown (years) in three vegetation types in the Restinga of Jurubatiba National Park, Brazil; equal superscript letters indicate insignificance difference at $p > 0.05$. ^{1}Clusia formation, ^{2}Seasonally dry forest.

	^{1}*Clusia* dominated		^{1}without *Clusia*		^{2}Forest	
	Mean	**s.d.**	**Mean**	**s.d.**	**Mean**	**s.d.**
k_{tot}	^{a}0.24	0.03	^{ab}0.33	0.02	^{c}0.76	0.25
k_{m}	^{a}0.22	0.05	^{a}0.23	0.04	^{ab}0.49	0.03
k_{f}	^{a}0.02	0.05	^{a}0.11	0.05	^{b}0.33	0.29
Half $time_{tot}$	2.91	0.35	2.09	0.11	0.91	0.23
Half $time_{m}$	3.12	0.55	3.07	0.50	1.42	0.08
$95\%_{tot}$	12.6	1.5	9.0	0.5	3.9	1.0
$95\%_{m}$	13.5	2.4	13.3	2.2	6.2	0.3

tot rates for 5 mm mesh bags; *m* rates for 0.02 mm mesh bags; *f* part of soil fauna on breakdown.

3. *Carbon and Nitrogen Turnover*

Carbon in litter was not correlated with the variations in litter production or with the climatic variables considered. Carbon concentrations were statistically similar in all vegetation types (as shown in Table 1). Nitrogen and lignin/nitrogen ratios were significantly different between *Clusia* dominated shrub patches and forest, with these levels being intermediate in shrub patches without *Clusia* (Table 1). Though nitrogen level was significantly lower in *Clusia* dominated patches than in forest vegetation, relatively high levels of lignin resulted in a significantly higher ratio of lignin/nitrogen in *Clusia* dominated patches than in forest vegetation.

Carbon loss in the litter bags may be separated into three processes: loss of C by microbial respiration and loss of stable C matter by food uptake of soil fauna or transportation to deeper soil layers. The succession of carbon turnover showed that carbon concentration decreased during the breakdown process (see Figure 3). At the end of the experiment, carbon concentration was approximately 93% of the initial concentration in the forest but approximately 99% of the initial concentration in the two patches of the *Clusia* formation (as shown in

Table 3). Total carbon loss was approximately 25% for both 0.02 and 5 mm mesh bags in the patches dominated by *Clusia* and approximately 45% in 0.02 mm mesh bags versus 60% in 5 mm mesh bags placed within the forest (Table 3). In the forest, approximately 10% of the total 70% C loss could be attributed to loss by concentration and another 10% to the soil fauna activity. In the *Clusia* dominated patches, approximately 1% of the total 35% could be attributed to loss by concentration without any additional effect of the soil fauna. Intermediate processes occurred in the patches without *Clusia* but were not significantly different from the patches dominated by *Clusia.* Nevertheless, regarding the total C loss, the faunal effect seemed to be higher than in the patches dominated by *Clusia* (as shown in Table 3).

The nitrogen process was very different compared to the carbon process. A loss of approximately 50% of total nitrogen content was detected in the *Clusia* dominated patches, whereas an increase of nitrogen was exhibited in the patches without *Clusia* and in the forest (as shown in Table 3). No differences were observed between large mesh bags and fine mesh bags. The decrease in N concentration in the *Clusia* dominated patches indicated that micro-organisms were inhibited and could not accumulate nitrogen in their biomass. In contrast, micro-organisms accumulated nitrogen higher than the initial values in the two other systems and, thus, reduced losses in comparison with *Clusia* dominated patches (see Table 3 and Figure 4a). Soil fauna was at least partly responsible for a slight nitrogen release in these two systems as could be seen by the differences between the two mesh bags.

The breakdown process showed that the nitrogen losses occurred mainly during the first weeks (see Figure 4). However, losses in the *Clusia* dominated patches lasted longer than in the forest, which indicated that the growing process of micro-organisms in the *Clusia* patches was slower than in the forest. After this initial process of nitrogen concentration loss, the accumulation process started in the forest, whereas under *Clusia* only a steady state level was exhibited.

4. *Soil*

Patches with *Clusia* showed higher nutrient content and higher proportions of silt and clay; leaching was probably less than in other areas with higher sand content. However, differences were only significant for the bare sandy areas in the *Clusia* formation (as shown in Table 1). Nitrogen and pH in the forest also exhibited significantly higher values when compared with the bare sandy areas.

Table 3. Carbon and nitrogen concentrations (% of original C, N) and losses at the end of the 74 weeks with results of the ANOVA and Tukey's post-hoc test; equal superscript letters indicate insignificance difference at $p > 0.05$. ^{1}Clusia formation, ^{2}Seasonally dry forest.

	^{1}***Clusia* dominated**				^{1}**Without *Clusia***				^{2}**Forest**			
	5mm		**0.02 mm**		**5mm**		**0.02 mm**		**5mm**		**0.02 mm**	
	Mean	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**	**Mean**	**S.d.**
C (% of initial)	^{a}98.9	1.7	^{a}98.5	2.7	^{a}98.7	1.8	^{a}99.8	1.1	^{b}91.1	6.5	^{ab}95.8	4.9
ANOVA	F: 3.96; df: 5, 23, 28; $p = 0.01$											
Total C loss (%)	^{a}23.5	10.1	^{a}28.5	10.6	^{ab}37.5	5.1	^{a}24.5	4.4	^{c}57.1	18.0	^{bc}45.4	4.2
ANOVA	F: 8.28; df: 5, 23, 28; $p < 0.001$											
N (% of initial)	^{a}68.6	9.2	^{a}62.0	20.1	^{b}139.6	54.2	^{b1}54.7	29.2	^{b}108.3	14.1	^{b}132.3	22.3
ANOVA	F = 10.49; df = 5, 18, 23; $p = 0.003$											
Total N loss (%)	^{a}48.8	2.8	^{a}54.5	16.1	^{ab}10.6	29.0	^{b}-17.3	21.3	^{a}48.3	34.8	^{a}26.3	14.8
ANOVA	F=8.94; df= 5, 23,28; $p = 0.005$											

Discussion

Results showed that litter production differed between forest and *Clusia* formations and that this difference would be even greater, if the area which was actually covered by vegetation was taken into account. In the *Clusia* formation, only approximately 40% is covered by vegetation, whereas the whole area is covered by vegetation in the forest (Oliveira-Galvão et al. 1990). These differences in production between restinga vegetation types have also been found in previous studies, e.g. maximum values of production (t.ha^{-1}.yr^{-1}) and their respective percentage of leaves fractions were: 4.5 – 66% in patches with *Clusia* (Silva et al. 2005) and 7.7 - 73% on periodically dry forests (Brietz et al. 2005). Litter production in the investigated forest was higher than in those reported by Brietz et al. (2005) and Silva et al. (2005). They were, however, in agreement with productions for equatorial areas, e.g. 10.9 t. ha^{-1}.yr^{-1} and 62% of leaves (Bray & Gorham 1964) and forests established on soils of moderate fertility of the Atlantic Forest, with a mean of 9.1 ± 1.24 t. ha^{-1}.yr^{-1} (n = 5) (Morellato 1992). The values obtained for the *Clusia* formation were similar to values reported for other Brazilian ecosystems with oligotrophic soils, scleromorphisms and seasonality, such as in both the Cerrado and Caatinga, with 3.2 and 5.1 t. ha^{-1}.yr^{-1}, respectively (Pires et al. 2006).

Differences in productivity of vegetation types contributed to dissimilarities in the vegetation composition driven by stressful abiotic conditions (Hay & Lacerda 1984; Scarano et al. 2004). The differences in plant productivity and the response to rainfall events in patches with and without *Clusia* endorse the relative importance of vegetation composition for litter production. Variation of litter production in patches dominated by *Clusia* was described as seasonally variable by Silva et al. (2005), who reported highest litter production at the end of the dry season in September. Results obtained in the current study do not suggest a well-defined periodicity on litter production, but a cumulative effect of drought. In the restinga, rainfall scarcity strongly affects water table levels, which are directly determined by the levels of coastal lagoons and the tide in coastal areas (Magnago et al. 2013; Umbelino 2008).

Nitrogen in the litter leaves was very low; values for *Clusia* formation were lower than the mean found for tropical forests on Spodosols and sandy soils (0.048 t.ha^{-1}.yr^{-1}), while the seasonal dry forest reaches values closer than found for tropical forest on infertile soils (0.108 t.ha^{-1}.yr^{-1}) (Vitousek & Sanford 1986). Mean values of

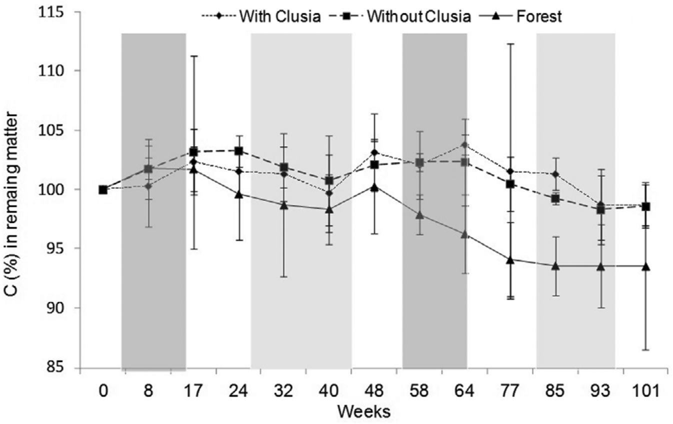

Figure 3. Carbon concentration during the breakdown process in 5mm mash bags showing different patterns for vegetation patches dominated by *Clusia* and without *Clusia*, and for Seasonally dry forest. Bars indicate standard deviation; dark grey: main rainy season, light grey: main dry season.

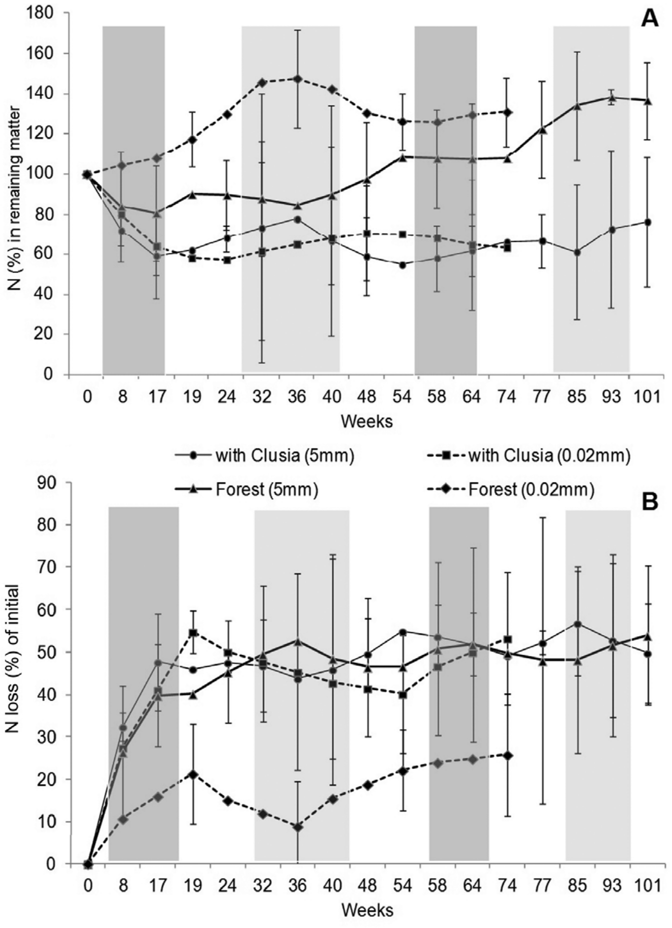

Figure 4. Nitrogen concentration during the breakdown process (A) and loss of nitrogen during the breakdown process (B).

nitrogen concentration in leaf litter of tropical plants were 1.46% ± 0.88 (Aerts 1997). However, Dias (2008) studied five species from the *Clusia* formation and found lower values, with means of 0.79% ± 0.19. According to Vitousek & Sanford (1986) the amount of nutrient cycling in regions of high productivity was high, whereas forests on the poor sandy soils were more efficient in cycling lower quantities of nitrogen.

Litter breakdown of restinga forests as well as nearby Atlantic and semi-deciduous forests in the São Paulo region was investigated by Castanho et al. (2012). These authors used different models to calculate the k-value. Their single exponential model was equal to the model used here. According to this model, the yearly k-values ranged between 2.0 and 2.9 which were much faster than values found in the forests of the restinga ecosystems investigated here, with k values of 0.24 to 0.76. The 95% breakdown in the São Paulo region lasted only 1.0 to 1.4 years in comparison to 13 years in the restinga of Jurubatiba National Park. This difference might be attributed to higher precipitation in the São Paulo region without dry seasons or with shorter dry seasons than in the currently investigated area. Litter breakdown in the Atlantic forest was also investigated by Gießelmann et al. (2010), who found strong effects for the composition of plant species. According to the k-values given by Gießelmann et al. (2010), 95% breakdown in the Atlantic forest ranged from 0.7 to 4 years depending on the leaves of the plant species. Only the investigated seasonally dry forest was in the range of the values found in the Atlantic forest. Castanho et al. (2012) and Gießelmann et al. (2010) also emphasized that, in general, the exclusion of invertebrates decreased litter decomposition but the effect depended mainly on litter composition. These results correspond with our findings in the restinga ecosystem where invertebrates only affect the breakdown in the forest formation but not in the *Clusia* formation. Peña-Peña & Irmler (2016) investigated hard-leafed Cerrado forests and found significant differences between dry and rainy seasons. They found 95% decay after 1 year for the rainy season and 7.6 yr for the dry season. Much lower effects of 6% to 13% were found by Vasconcelos et al. (2007) in their dry season irrigation experiment under the wetter conditions of eastern Amazonia. However, a periodical change in the course of the litter breakdown in the investigated restinga was only found in the forest formation.

The relation between nitrogen and carbon during the breakdown process showed that the *Clusia* dominated patches distinctly differ from the processes occurring in the other two sites. Under *Clusia*, the microbial soil system grew slowly and needed at least half a year to keep the nitrogen at a steady state level. The microbial soil system was not able to accumulate nitrogen during the decomposition of carbon at the beginning of the process. The micro-organisms in the two other systems grew faster and were able to accumulate nitrogen quickly, which contributed to the incorporation of nitrogen in the micro-organisms (Aber & Melillo 1982). According to Aber & Melillo (1982), this process was also related to the lignin content in the litter. Thus, *Clusia* seems to affect the whole decomposition process including the microbial potential and its effect on the immobilization of nitrogen (Bosatta & Staaf 1982). The ability to accumulate nitrogen in the microbial systems of the non-*Clusia* patches and the forest were responsible for the higher decomposition and losses of carbon in these systems. Soil fauna was also responsible for 20 to 30% nitrogen release during the late breakdown process in the forests. Anderson et al. (1983) found several soil fauna groups that accelerated the nitrogen release in litter, but Collembola were the most effective group. According to Irmler (2000), soil fauna only mediated the release of nitrogen between micro-organisms and roots.

Meentemeyer (1978) stated that lignin concentration and actual evapotranspiration are the main drivers controlling the litter decomposition. Thus, a main factor for the slow breakdown in the *Clusia* formation may be the high lignin concentration and the high lignin/N ratio of leaves. Castanho & de Oliveira (2008) reported similar values of lignin for another Clusiaceae, *Calophyllum brasiliense*, with approximately 48% lignin and a lignin/N ratio of 62.7, which was by far the highest lignin concentration of their investigated plants in southern Brazil. These were distinctly lower than in the *Clusia* dominated patches, with a lignin/N ratio of 73.4. The breakdown in the restinga investigated by Castano & de Oliveira (2008) was still faster than in the Cerrado. Thus, the slow breakdown rates in the *Clusia* formation without seasonal changes may be attributed to high lignin concentrations of *Clusia* leaves as well as to the poor sandy soils, which cause extreme physiological constraints throughout the year.

According to Dias et al. (2006), *C. hilariana* is also of great importance due to its higher production of biomass in the nutrient-poor coastal vegetation. Approximately 65% of the litter in patches dominated by *C. hilariana* were composed of leaves of this species (Silva et al. 2005), which was twice as much leaf biomass of *C. hilariana* in litter composition than on the live biomass stock (Dias et al. 2006). The oligarchic structure of the *Clusia* formation suggests that this community is driven to a high extent by *Clusia hilariana*, although obtained data have found no significant differences in the nutrient production between patches with and without *Clusia*. The present study suggests that this species also affects nutrient cycling, promoting a slow release of nutrients important for the development of understory plants in the oligotrophic soil of the restinga. However, the inhibition of microbial and soil fauna activity that minimize matter loss in patches with *Clusia hilariana* is combined with losses of N concentration, and is likely due to leaching processes in the rainy season after leaf fall. Overall, the N losses were higher in patches without *Clusia hilariana* but less than in those in the forest. Thus, for inhibition of nitrogen losses, the patches without *Clusia hilariana* were more efficient than the patches with *Clusia hilariana*. After two years of decomposition, N losses in the patches with *Clusia hilariana* amounted to approximately 50%, which is similar to the forest. Nearly no N loss was observed in the patches without *Clusia hilariana*. It is still not clear which role these plants play in N uptake. In contrast to the patches without *Clusia*, the lower immobilization of N by soil micro-organisms facilitated the N uptake by plants in the patches with *Clusia* from the released N at the beginning of the decomposition process. Thus, plants in the patches without *Clusia* might suffer N deficits.

The long-lasting decomposition process in the *Clusia* formation leads to carbon accumulation in the soil, which may be seen in the high carbon contents, particularly under patches with *Clusia* plants, which is approximately twice as high as in the forest formation. The C accumulation might also be responsible for the input of humic substances into adjacent lagoons. During the rainy season, water tables of lagoons and ground water tables rise and promote a kind of bottom-up fertilization (Brietz 1994). Suhett et al. (2004) found high concentrations of dissolved organic carbon in the restinga's water tables, with up to 168 mg C l^{-1} and 72% of the dissolved organic carbon coming from humic substances originating from the surrounding area. The accumulation of humic compounds in the water table results from continual leaching from the litter layer, which may increase the residential time of carbon since humic compounds are very refractory to decomposition (Tranvik 1998). Similar processes were already found as reason for the humus-rich water body of the Rio Negro in the Amazon basin (Klinge 1967). The development of large Podzol soils with high contents of organic matter in the upper Rio Negro seemed to be responsible for the black colour of the river. Although no Podzol soils have developed in the investigated restinga, the accumulation of high organic contents under the vegetation of the *Clusia* formation caused by the retarded litter decomposition may be the reason for the humus richness of some bodies of water in the restinga ecosystem (Farjalla et al. 2009).

The results describing *Clusia* formation as a vegetation associated to sandy soils with low amount of organic matter, commonly associated to poor vegetation with low amount of biomass. This formation is different from seasonally dry forest in which soil is formed also by clay and organic matter. Poor soil is also associated with strong competition effect, but, surprisingly, the recalcitrant character of the dominant *Clusia* species seems to control the turnover of matter in the system, probably due to the lignin content and its effect on nitrogen release affecting the understory growth (Dias & Scarano 2007, Dias 2008). The biomass of this formation is structured in patches due the effect of the arboreal habit of *C. hilariana* producing layers, with a high density and species richness of seedlings underneath, probably due to the greater activity of dispersers (Dias & Scarano 2007). Finally, the results highlight the importance of *Clusia* formation for restinga ecosystem and suggest that changes in water table level and the leaching should relief the nutrient control promoted by *Clusia hilariana*, resulting in differences between vegetation patches dynamics.

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Authors' Contributions

Letícia da Silva Brito - Contribution in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Ulrich Irmler and Rodrigo Lemes Martins - Orientation in the concept and design of the study; Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation.

Tatiane Pereira Xavier and Bruno Vasconcellos Guimarães Forte - Contribution to data collection.

Conflicts of interest

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

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Floristic and structure of the arboreal community of an Ombrophilous Dense Forest at 800 m above sea level, in Ubatuba/SP, Brazil

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Abstract: Undoubtedly, the publication of floristic lists and phytosociological studies are important tools for metadata generation, quantification and characterization of the megadiversity of Brazilian forests. In this sense, this work had the objective of describing the composition and the structure of the tree community of one hectare of Dense Atlantic Rainforest, at an altitude of 800 m. All individuals, including trees, palm trees, arborescent ferns and dead and standing stems, with a diameter at breast height (DBH) of ≥ 4.8 cm were sampled. After the identification of the botanical material, we proceeded to calculate the usual phytosociological parameters, besides the Shannon diversity index (H') and Pielou equability index (J). A total of 1.791 individuals were sampled, of which 1.729 were alive, belonging to 185 species, 100 genera and 46 families. The results obtained showed a strong similarity of structure and floristic composition with plots of both Montana and Sub Montana Ombrophilous Dense Forest studied in the same region. This reinforces the hypothesis that the transition between the phytophysiognomies of the Atlantic Ombrophylous Dense Forest is gradual, and that the boundaries between them cannot be clearly established.

Keywords: *Biodiversity conservation, Megadiversity, Atlantic forest, Serra do Mar State Park, PELD/ILTER FGAF Site.*

Florística e estrutura da comunidade arbórea de uma Floresta Ombrófila Densa a 800 m acima do nível do mar, em Ubatuba/SP, Brasil

Resumo: Indiscutivelmente a publicação de listas florísticas e estudos fitossociológicos são importantes ferramentas para a geração de metadados, quantificação e caracterização da megadiversidade das florestas brasileiras. Neste sentido, o presente trabalho teve por objetivo descrever a composição e a estrutura da comunidade arbórea de um hectare de Floresta Ombrófila Densa Atlântica, na cota dos 800 m de altitude. Para tanto foram estabelecidas 100 subparcelas de 10 x 10 m, distribuídas em quatro blocos amostrais de 0,25 ha, onde foram amostrados todos os indivíduos arbóreos, incluindo palmeiras, fetos arborescentes e indivíduos mortos e em pé, com DAP (diâmetro à altura do peito) ≥ 4,8 cm. Após a identificação do material botânico e do cálculo dos parâmetros fitossociológicos usuais, foram calculados os índices de diversidade de Shannon (H') e de eqüabilidade de Pielou (J). Foram amostrados 1.791 indivíduos arbóreos, sendo 1.729 vivos pertencentes a 185 espécies, 100 gêneros e 46 famílias. Os resultados obtidos mostram forte similaridade de estrutura e composição florística com parcelas tanto de Floresta Ombrófila Densa Montana como Floresta Ombrófila Densa Submontana estudadas na mesma região, reforçando a hipótese que a transição entre as fitofisionomias da Floresta Ombrófila Densa Atlântica é gradual e que os limites entre elas não podem ser claramente estabelecidos.

Palavras-chave: *Conservação da biodiversidade, Megadiversidade, Floresta Atlântica, Parque Estadual da Serra do Mar, Sítio PELD/ILTER FGAF.*

Introduction

The Atlantic Forest, classified by Myers et al. (2000) among the five most threatened hotspots in the world, originally covered about 82% of the state of São Paulo (Joly et al. 1999), especially due to the coffee economic cycle, was reduced to about 5% of its original area (Kronka et al. 2005). This biome, which is classified as the second largest tropical rainforest on the American continent (Morellato & Haddad 2000), is composed of a mosaic of ecosystems with specific ecological processes (Campanili & Schaffer 2010).

The well-preserved stretches, which harbour great floristic diversity, are located mainly in the Serra do Mar region (SOS Mata Atlântica & INPE 2014), a set of scalloped escarpments that extend from

Rio de Janeiro to Santa Catarina (Almeida & Carneiro 1998). The largest continuous well-preserved stretch is protected by the Serra do Mar State Park (PESM).

During the last decade, the lack of large and intensive studies in the region of the north coast of São Paulo was greatly improved by work in the Picinguaba and Santa Virgínia Nucleus of the Serra do Mar State Park (Joly et al. 2012). However, between the elevations of 400 and 1000 m altitude, a large information gap persisted, because there was no floristic or phytosociological study with expressive representativeness of the arboreal vegetation in these intermediate levels.

Therefore, this work had the objective to describe the composition and structure of the tree community of one hectare of Montana Ombrophilous Dense Forest in the altitude of 800 m, an intermediate level between the altitudes of 400 and 1000 m. This proposal are of extreme importance for the generation of data that allow a better understanding of the full extent of the megadiversity of the forests that cover the Serra do Mar.

Material and Methods

1. *Location and characterization of the study area*

The Serra do Mar State Park (PESM) is about 332,000 ha, is predominantly covered by Ombrophilous Dense Forest (Veloso et al. 2012) and extends through 25 municipalities of São Paulo from sea level to around 1600 m altitude, including coastal plains, scarps and plateaus (Mattoso 2006). In the northern coastal region, in the plains area, the lowland forest presents a tropical climate without a dry season, with an average annual rainfall of 2200 mm, and even in the driest months (June to August) it is less than 60 mm (Setzer 1966, Rosado et al. 2012). However, the temperate tropical climate is present in the plateau, in the montane forest, with an average annual precipitation near 2000 mm and frequent occurrence of fogs that cover large areas of this forest (Rosado et al. 2010).

In the region, soils low in basic cations and rich in aluminum predominate in comparison to other tropical forests (Martins 2010, Joly et al. 2012). The study area (23º 21 ‘34-40 “S, 45º 06’ 31-40” W) is located in the cliffs of the Serra do Mar, presenting relief with strong slopes (Forest Institute 2010) and is within the PELD/ILTER Functional Gradient of Atlantic Forest/FGAF site, established in 2010 (http://peld-biotagradiente.net/). This was the portion denominated as P, following the sequence of areas previously studied. Although the area studied was administratively part of Picinguaba Nucleus, due to its proximity and logistics, the Santa Virgínia Nucleus was used as an operational base.

2. *Establishment of permanent plots and survey of vegetation*

In the studied area, 100 subplots of 10 x 10 m were established, distributed in four sample blocks of 0.25 ha (50 x 50 m) and a maximum distance between the blocks of around 40 m (Figure 1), between altitudes of 758 to 866 m (quota of 800 m). The allocation of subplots, georeferenced and delimited with PVC pipes of 1.5 m height and 5” diameter at the external vertices and 3/4” in the others, in order to allow long term studies and continuous monitoring in these areas was done by a team specialized in topography, as proposed by Joly et al. (2012).

The floristic survey was carried out from the collection of botanical material of the arboreal individuals, including palm trees, arborescent ferns and dead and standing individuals, with a diameter at breast height (DBH) of ≥ 4.8 cm. The collection trips were carried out from January 2011 to January 2012. The identifications were made with the aid of relevant literature and expert consultations, as well as comparisons with the IAC, UEC and HRCB herbarium collections. The species were grouped by families according to the classification system of Angiosperm Phylogeny Group (APG IV 2016) with indication of subfamilies in Fabaceae, according to the consensual proposal discussed by LPWG (2013). For the arborescent ferns, phylogenetic order was also followed (Christenhusz et al. 2011).

For the phytosociological analyses, the following parameters were represented: number of individuals; absolute frequency; absolute dominance and importance value, and the Shannon (H’) diversity indexes, using the natural basis, and Pielou (J) equation (Brower & Zar 1984) were calculated. In the multivariate analyses, the FITOPAC 2.1 program (Shepherd 2010) was used, from a quantitative matrix (absolute density of all morphospecies, identified at least up to the family level), and a dendrogram was constructed using the UPGMA method, using the coefficient of Bray Curtis, to verify the similarity among the four sample blocks.

Results

In the total area, 1.791 individuals were demarcated, of which 1.729 were alive, including 1610 trees (89.8% of individuals), 112 palm trees (6.2%) and 7 arborescent ferns (0.4%) belonging to 185 species, 100 genera and 46 families. Two individuals remained undetermined, not having been collected, due to the absence of branches and high height (Table 1). The individuals still dead and standing (62) represented 3.57% of the sample.

Myrtaceae was the richest family (48 species, 25.9%), followed by Rubiaceae (16 species, 8.6%), Fabaceae (11 species, 5.9% - being six Caesalpinioideae, three Faboideae and two Detarioideae), Lauraceae (9 species, 4.9%), Melastomataceae (8 species, 4.3%), Monimiaceae and Sapotaceae (7 species, 3.8% each), which together comprised 57.2% of the species found in the area. Four of these families were also among the most abundant: Rubiaceae (540 individuals.ha^{-1}, 31.3% of live trees), followed by Myrtaceae (233, 13.5%), Monimiaceae (122, 7.1%) and Melastomataceae (111, 6.4% – fifth position), together with Arecaceae (112, 6.5% – fourth position) and Nyctaginaceae (94, 5.4% – sixth position) added up to 70.2% of the live trees.

Most of the families that excelled in the richness and abundance parameters were eudicotiledonous, however, we also found representatives of magnoliids (Monimiaceae and Lauraceae) and monocotyledons (Arecaceae). Rubiaceae was the family with the highest

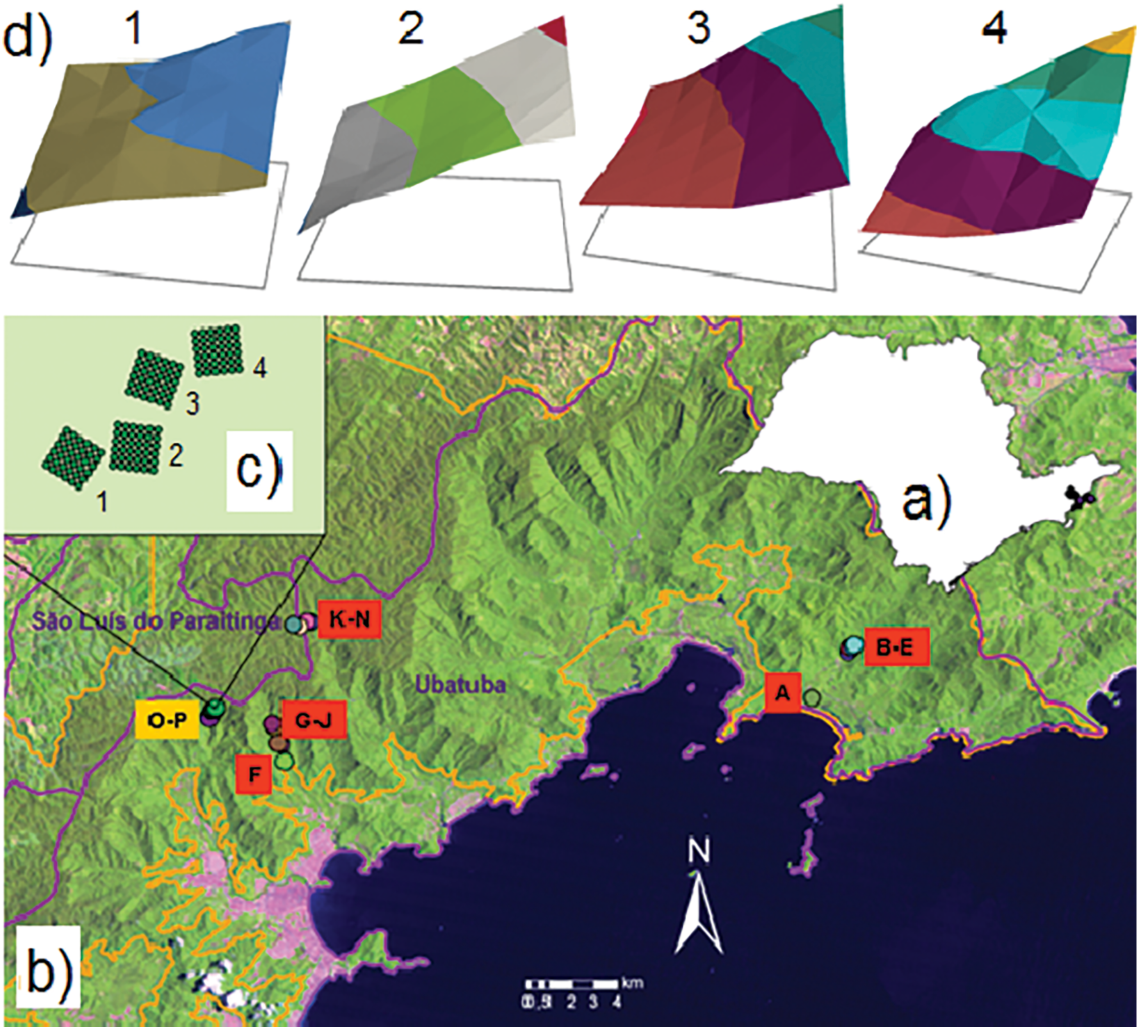

Figure 1. Location of the region and area of study. **a)** Núcleos Picinguaba and Santa Virgínia (PESM), in the region of the north coast (São Paulo - Brazil); **b)** Distribution of plots (1 ha each) with studies already carried out (A–J, 0–400 m; K–N 1000 m); **c)** Spatial arrangement of sample blocks of plot P (800 m); **d)** Topographic scheme in each sample block, where equal colour bands correspond to the same altitude (ranging from 758 m – block 1, to 866 m – block 4).

value of importance (60.9% of importance value index – IVI), followed by Myrtaceae (42.4%) and Monimiaceae (23.4%). In relation to genus, *Eugenia* (Myrtaceae) was the richest with 21 species (11.4% of the total), followed by *Mollinedia* (Monimiaceae) (seven species, 3.8%), *Marlierea* and *Myrcia* (Myrtaceae) and *Inga* (Fabaceae) (six species, 3.2% each), which added up to 24.8% of the species.

The estimated diversity of the community was H' = 4.3 and the equability was 0.82. *Rustia formosa* (151 individuals.ha^{-1}, 8.7% of live trees), *Euterpe edulis* (100, 5.8%), *Coussarea accedens* (89, 5.2%), *Psychotria suterella* and *Meriania calyptrata* (88, 5.1% each one of them), *Rudgea jasminoides* (75, 4.3%), *Ouratea multiflora* (55, 3.2%) and *Guapira opposita* (53, 3.1%) were the most abundant species (Table 2), accounting for 40.5% of living individuals.

With some variation of position, the eight most abundant species in the area were among the ten species with the highest IVI, standing out due to abundance and frequency. *Cryptocarya mandioccana* (24 individuals.ha^{-1}, 1.4% live individuals – eighth position) and *Mollinedia boracensis* (39, 2.3% – tenth position) completed the list of the ten species with the highest IVI, standing out due to dominance.

The majority (about 54.4%) of live trees (Figure 2) were 5-10 m in height. Emerging trees (≥ 20 m), which surpassed the regular forest canopy, included several species, such as *Copaifera trapezifolia*, *Syagrus pseudococos* and *Cryptocarya mandioccana*. Regarding the diameters, the majority of the individuals belonged to the class of 4.8 to 9.9 cm (Figure 3). The estimated basal area was 41.9 m^2 ha^{-1}.

The similarity analysis (Figure 4) that the floristic composition was similar between the blocks, with showed a high co-optic correlation (0.85) and grouped blocks 3 and 4 as the most similar (distance of 0.34). However, when we consider only the five species of the highest importance value per block (Figure 5), some differences between them stand out.

Rustia formosa (which had the highest IVI in the quota of 800 m) was the only species among the five species with the highest IVI in the four blocks. Other species that presented high IVI in more than one block were *Euterpe edulis* (blocks 1, 3 and 4), *Psychotria suterella* (blocks 1 and 3) and *Meriania calyptrata* (blocks 3 and 4). The other species stood out in only one of the blocks. *Coussarea meridionalis*, *Bathysia mendoncaei*, *Guapira hirsuta* and *Mollinedia argyrogyna* were

Table 1. Trees (in phylogenetic order by families and subfamilies and alphabetical by species) sampled in 1 ha of Montana Ombrophilous Dense Forest, at 800 m elevation, Serra do Mar State Park, Ubatuba – SP. Nº IAC = reference material in the herbarium of the Agronomic Institute; ①, ②, ③, ④ = sample blocks; X = occurrence of the species in the block and MS= material with specialist.

Family	**Species**	**Nº IAC**	**Occurrence**			
			1	**2**	**3**	**4**
Ferns						
CYATHEACEAE	*Alsophila sternbergii* (Sternb.) D.S.Conant	56501	X		X	X
	Cyathea dichromatolepis (Fée) Domin	56503			X	X
	Cyathea glaziovii (Fée) Domin	56502		X		
Basal angiosperms						
MYRISTICACEAE	*Virola bicuhyba* (Schott ex Spreng.) Warb.	50545	X	X	X	
ANNONACEAE	*Guatteria australis* A.St.Hil.	50510			X	
	Guatteria sp1	54021				X
	Guatteria sp2	53957		X	X	X
	Annona dolabripetala Raddi	48948				X
SIPARUNACEAE	*Siparuna brasiliensis* (Spreng.) A.DC.	49259				X
MONIMIACEAE	*Mollinedia argyrogyna* Perkins	56476	X	X	X	X
	Mollinedia boracensis Peixoto	48995	X	X	X	X
	Mollinedia aff. *oligantha* Perkins	56475	X	X		
	Mollinedia ovata Ruiz & Pav.	56494		X		
	Mollinedia schottiana (Spreng.) Perkins	50341	X	X	X	X
	Mollinedia triflora (Spreng.) Tul.	53991	X	X	X	X
	Mollinedia sp1	MS			X	X
LAURACEAE	*Cinnamomum triplinerve* (Ruiz & Pav.) Kosterm.	56464		X	X	X
	Cryptocarya mandioccana Meisn.	54073	X	X	X	X
	Cryptocarya saligna Mez	48981	X	X	X	
	Endlicheria paniculata (Spreng.) J.F. Macbr.	49790		X		
	Licaria armeniaca (Nees) Kosterm.	49789		X	X	
	Ocotea dispersa (Nees & Mart.) Mez	50547	X	X		
	Ocotea cf. *divaricata* (Nees) Mez		X	X		
	Ocotea frondosa (Meisn.) Mez	54019				X
	Ocotea teleiandra (Meisn.) Mez	53933	X	X		
CHLORANTHACEAE	*Hedyosmum brasiliense* Miq.	51161				X
Monocotyledon						
ARECACEAE	*Astrocaryum aculeatissimum* (Schott) Burret	51963	X	X		
	Attalea exigua Drude	44109		X		
	Euterpe edulis Mart.	49271	X	X	X	X
	Syagrus pseudococos (Raddi) Glassman	49191	X	X	X	X
Eudicots						
PROTEACEAE	*Euplassa* cf. *cantareirae* Sleumer	20054			X	
	Roupala montana var. *brasiliensis* (Klotzsch) K.S.Edwards	54007		X		
	Roupala paulensis Sleumer	53993		X		
FABACEAE Detarioideae	*Copaifera trapezifolia* Hayne	54009		X		
	Hymenaea courbaril L.	35618	X	X		
FABACEAE Caesalpinioideae	*Inga capitata* Desv.	50313	X	X		X
	Inga edulis Mart.	49311			X	
	Inga grazielae (Vinha) T.D.Penn.	49785				X

Continuation Table 1.

	Inga marginata Willd.	49339	X			X
	Inga schinifolia Benth.	54029	X			X
	Inga sessilis (Vell.) Mart.	48242			X	
FABACEAE Faboideae	*Dahlstedtia pinnata* (Benth.) Malme	54033	X		X	X
	Zollernia ilicifolia (Brongn.) Vogel	49308	X		X	X
	Fabaceae-Faboideae sp1	54006	X	X		
ROSACEAE	*Prunus myrtifolia* (L.) Urb.	49007		X		
MORACEAE	*Brosimum guianense* (Aubl.) Huber	56499	X			
URTICACEAE	*Cecropia glaziovi* Snethl.	49016	X			X
	Coussapoa microcarpa (Schott) Rizzini	49017	X	X	X	X
CELASTRACEAE	*Maytenus* sp1	53958	X		X	
	Maytenus sp2	56500				X
ELAEOCARPACEAE	*Sloanea guianensis* (Aubl.) Benth.	49778		X		
	Sloanea cf. *hirsuta* (Schott) Planch. ex Benth.	51299	X	X	X	X
ERYTHROXYLACEAE	*Erythroxylum* cf. *cuspidifolium* Mart.	54081		X		
OCHNACEAE	*Ouratea multiflora* (Pohl) Engl.	53932	X	X	X	X
	Quiina aff. *magalano-gomesi* Schwacke	56468	X	X	X	X
CLUSIACEAE	*Garcinia gardneriana* (Planch. & Triana) Zappi	49777	X	X	X	X
MALPIGHIACEAE	*Bunchosia maritima* (Vell.) J.F.Macbr.	54028			X	
CHRYSOBALANACEAE	*Couepia venosa* Prance	50297		X		X
	Hirtella hebeclada Moric. ex DC.	50298	X	X		X
	Licania cf. *hoehnei* Pilg.	56471	X	X		
	Licania cf. *kunthiana* Hook.f.	56470	X	X	X	X
	Parinari excelsa Sabine	56469		X		X
HUMIRIACEAE	*Vantanea* sp1	54027	X	X		X
	Humiriaceae sp1	56493	X	X	X	X
SALICACEAE	*Casearia decandra* Jacq.	33938	X		X	
	Casearia sylvestris Sw.	42118		X		
EUPHORBIACEAE	*Alchornea glandulosa* Poepp. & Endl.	49226	X			X
	Alchornea triplinervia (Spreng.) Müll.Arg.	48965	X		X	
	Sapium glandulosum (L.) Morong	48967	X			X
	Sebastiania sp1	54031		X		
PHYLLANTHACEAE	*Hieronyma alchorneoides* Allemão	54012	X	X	X	X
MYRTACEAE	*Calyptranthes grandifolia* O.Berg	56483				X
	Calyptranthes lucida Mart. ex DC.	49145	X			X
	Calyptranthes rufa O.Berg	49047	X			X
	Calyptranthes strigipes O.Berg	49877	X	X	X	
	Campomanesia guaviroba (DC.) Kiaersk.	50617	X			X
	Campomanesia phaea (O.Berg) Landrum	43144	X			
	Eugenia acutata Miq.	49854		X	X	X
	Eugenia batingabranca Sobral	49866	X	X		
	Eugenia burkartiana (D.Legrand) D.Legrand	56675				X
	Eugenia cerasiflora Miq.	49865		X		
	Eugenia cereja D.Legrand	56481	X	X	X	
	Eugenia copacabanensis Kiaersk.	45956	X		X	
	Eugenia cuprea (O.Berg) Nied.	50363			X	

Continuation Table 1.

MYRTACEAE	*Eugenia dodonaeifolia* Cambess.	51320				X
	Eugenia flamingensis O.Berg	56489				X
	Eugenia fusca O.Berg	50367	X			
	Eugenia involucrata DC.	50365	X			
	Eugenia melanogyna (D.Legrand) Sobral	50366		X		X
	Eugenia multicostata D.Legrand	56482	X	X	X	
	Eugenia neoverrucosa Sobral	49164		X		
	Eugenia oblongata O.Berg	53944		X		X
	Eugenia plicata Nied.	54004	X		X	X
	Eugenia pruinosa D.Legrand	37912		X		
	Eugenia pruniformis Cambess.	49057	X	X		
	Eugenia subavenia O.Berg	49058			X	X
	Eugenia verticillata (Vell.) Angely	56487	X	X		X
	Eugenia sp1	MS		X		
	Marlierea excoriata Mart.	46874		X	X	X
	Marlierea glazioviana Kiaersk.	50371		X		
	Marlierea obscura O.Berg	54000			X	X
	Marlierea racemosa (Vell.) Kiaersk.	54070	X		X	X
	Marlierea suaveolens Cambess.	56488	X	X		
	Marlierea tomentosa Cambess.	49887	X	X		X
	Myrceugenia cf. *campestris* (DC.) D.Legrand & Kausel	53947	X			
	Myrceugenia glaucescens (Cambess.) D.Legrand & Kausel	56477	X		X	
	Myrceugenia cf. *kleinii* D.Legrand & Kausel	53930				X
	Myrceugenia myrcioides (Cambess.) O.Berg	55770	X		X	X
	Myrceugenia sp1	MS	X			X
	Myrcia neoblanchetiana E.Lucas & Sobral	MS				X
	Myrcia pubipetala Miq.	50374	X		X	X
	Myrcia spectabilis DC.	53208	X	X		
	Myrcia tenuivenosa Kiaersk.	56485			X	
	Myrcia tijucensis Kiaersk.	54458	X			
	Myrcia ubatubana Mazine & Sobral	53476	X	X		
	Myrciaria floribunda (H.West ex Willd.) O.Berg	50377	X	X	X	X
	Myrciaria cf. *pallida* O.Berg	56498	X			X
	Neomitranthes glomerata (D.Legrand) D.Legrand	50515	X	X		
MELASTOMATACEAE	*Henriettea glabra* (Vell.) Penneys, F.A. Michelangeli, Judd et Almeda	50383			X	X
	Leandra acutiflora (Naudin) Cogn.	54077			X	
	Meriania calyptrata (Naudin) Triana	53959			X	X
	Miconia atlantica Caddah & R. Goldenb.	54041			X	X
	Miconia calvescens DC.	54040				X
	Miconia latecrenata (DC.) Naudin	54076	X	X		X
	Miconia tristis Spring	56480				X
	Mouriri chamissoana Cogn.	50381	X	X	X	

Continuation Table 1.

PICRAMNIACEAE	*Picramnia ciliata* Mart.	50401	X	X		
BURSERACEAE	*Protium* sp1	54001	X			
ANACARDIACEAE	*Tapirira guianensis* Aubl.	37240	X		X	
SAPINDACEAE	*Allophylus edulis* (A.St.-Hil. et al.) Hieron. ex Niederl.	56486			X	
	Allophylus cf. *membranifolius* Radlk.	56465	X		X	
	Allophylus petiolulatus Radlk.	56467	X	X	X	X
	Cupania furfuracea Radlk.	54071	X	X		X
	Cupania oblongifolia Mart.	50208	X			
	Matayba juglandifolia (Cambess.) Radlk.	50438	X			
MELIACEAE	*Cabralea canjerana* (Vell.) Mart.	48992	X		X	X
	Guarea cf. *guidonia* (L.) Sleumer	49486	X		X	X
	Guarea macrophylla Vahl	54035				X
	Trichilia elegans A.Juss. subsp.elegans	48052	X			
	Trichilia silvatica C.DC.	56490	X	X		
THYMELAEACEAE	*Daphnopsis schwackeana* Taub.	54075	X	X	X	X
OLACACEAE	*Heisteria silvianii* Schwacke	50396	X	X		
	Tetrastylidium grandifolium (Baill.) Sleumer	54013	X	X		
OPILIACEAE	*Agonandra excelsa* Griseb.	56496			X	
NYCTAGINACEAE	*Guapira hirsuta* (Choisy) Lundell	49894	X	X	X	
	Guapira nitida (Mart. ex J.A.Schmidt) Lundell	49002		X	X	
	Guapira opposita (Vell.) Reitz	56479	X	X	X	X
	Guapira venosa (Choisy) Lundell	56484	X	X		X
SAPOTACEAE	*Chrysophyllum flexuosum* Mart.	50440	X	X	X	X
	Chrysophyllum viride Mart. & Eichler	56474	X			
	Micropholis crassipedicellata (Mart. & Eichler) Pierre	55752	X	X		
	Pouteria caimito (Ruiz & Pav.) Radlk.	49927		X	X	X
	Pouteria psammophila (Mart.) Radlk.	56473		X		
	Pouteria venosa (Mart.) Baehni	50450	X		X	
	Sapotaceae sp1	54079		X		
PRIMULACEAE	*Ardisia martiana* Miq.	53956	X	X	X	X
RUBIACEAE	*Alibertia myrciifolia* Spruce ex K.Schum.	54030	X	X	X	X
	Amaioua intermedia Mart. ex Schult. & Schult.f.	51892		X		
	Bathysa mendoncaei K.Schum.	54037	X	X	X	
	Bathysa stipulata (Vell.) C.Presl	54076				X
	Choemelia cf. *pedunculosa* Benth.	56472				X
	Coussarea accedens Müll.Arg.	56495	X	X	X	X
	Coussarea cf. *hydrangeifolia* (Benth.) Müll.Arg.	54026			X	X
	Coussarea meridionalis (Vell.) M.Gomes	49942	X	X	X	
	Faramea hyacinthina Mart.	43943		X		
	Posoqueria latifolia (Rudge) Schult.	49907	X	X	X	X
	Psychotria leitana C.M.Taylor	50414		X		
	Psychotria pubigera Schltdl.	54038		X		
	Psychotria suterella Müll. Arg.	54022	X	X	X	X

Continuation Table 1.

	Randia armata (Sw.) DC.	54645		X		
	Rudgea jasminoides (Cham.) Müll. Arg.	54024	X	X	X	X
	Rustia formosa (Cham. & Schltdl. ex DC.) Klotzsch	56478	X	X	X	X
APOCYNACEAE	*Tabernaemontana* cf. *laeta* Mart.	53734		X		X
BORAGINACEAE	*Cordia sellowiana* Cham.	54084		X		
	Cordia trichoclada DC.	50682	X			
SOLANACEAE	*Cestrum schlechtendahlii* G.Don	54083			X	
	Solanum pseudoquina A.St.-Hil.	48202	X			
	Solanum rufescens Sendtn.	54072		X	X	X
LAMIACEAE	*Aegiphila integrifolia* (Jacq.) Moldenke	54036		X	X	
CARDIOPTERIDACEAE	*Citronella paniculata* (Mart.) R.A.Howard	53934	X	X	X	X
AQUIFOLIACEAE	*Ilex theezans* Mart. ex Reissek	56497		X		
ARALIACEAE	*Dendropanax denticulatus* Fiaschi	54025	X		X	X
	Dendropanax sp1	53992	X	X		
	Schefflera calva (Cham.) Frodin & Fiaschi	48950	X	X		
Undetermined	Undetermined sp1			X		
	Undetermined sp2					X

not among the 10 species with the highest IVI for the quota of 800 m, while *M. boracensis* was the only one among the 10 largest species with an IVI in the quota of 800 m that was not among the five species with the highest IVI per block (Figure 5).

Discussion

Most of the families that excelled in the richness and abundance parameters were eudicotiledonous; however, we also found representatives of magnoliids (Monimiaceae and Lauraceae) and monocotyledons (Arecaceae). Sequentially, the three richest families at 800 m (Myrtaceae, Rubiaceae and Fabaceae) were the same as those observed by Ramos et al. (2011) and Gomes et al. (2011) in stretches of Sub Montana Ombrophilous Dense Forest, respective to those of 200 and 350 m of altitude, located in the same region. However, in another area of the same region (350 m of altitude), Lauraceae occupied the second position among the richest families (Rochelle et al. 2011), as in the Montana Ombrophilous Dense (Montane) Forest (Padgurschi et al. 2011 - 1000 m altitude).

In terms of families higher values of importance (IVI), the first two sites (Rubiaceae and Myrtaceae) are usually cited in works from the north coast of São Paulo (Gomes et al. 2011, Rochelle et al. 2011, Joly et al. 2012). However, we highlight the importance of the Monimiaceae (third major VI) in the altitudinal range that corresponds to the Montane stretch of this forest, a pattern already observed by Padgurschi et al. (2011) in an area denominated plot K in the altitudinal elevation of 1000 m.

At the gender level, although *Eugenia* (21 species at 800 m) was the richest in all altitudinal heights, with no evidence of strong anthropic impact, it presented greater wealth in Sub Montana Ombrophilous Dense (Submontane) Forest (from 19 to 28 species – Rochelle et al. 2011, Gomes et al. 2011), than in the Montane FOD (12 species - Padgurschi et al. 2011).

Among the species with the highest IVI in the 800 m, *Euterpe edulis*, “palmito-jussara” (second position) was considered a characteristic species and the only one with importance in other studies carried out in Submontane and Montane Forests (Scudeller et al. 2001).

Rustia formosa (first position of IVI) occupies intermediate positions of importance in the Submontane Forests in the region (Gomes et al. 2011, Campos 2008, Lacerda 2001) and in an area in the municipality of Imbé in the north of Rio de Janeiro (Moreno et al. 2003), representing between 0.26 (Ramos et al. 2011) and 19.0% (Gomes et al. 2011) of the IVI, but it was not observed in the Montane Forest (Padgurschi et al. 2011). While *Coussarea accedens* (third position) occurs, it does not present significant importance (between 0.7 and 1.1% of IVI), in the Submontane Forests and was not founded in the Montane Forest.

The high percentage of species represented by only one individual, as in this study, has already been observed in other studies carried out in Atlantic forests in southeastern Brazil (Melo & Mantovani 1993, Guedes-Bruni & Mantovani 1999, Gomes et al. 2011, Rochelle et al. 2011, Padgurschi et al. 2011). Thus, the present work contributed to increase the knowledge of this parameter, being that the great percentage of species represented by a single individual is considered a standard for to the Atlantic Forest (Martins 1991, Valencia et al. 1994).

The present work contributed to the publication of a newly described species, *Myrcia ubatubana* Mazine & Sobral, for which the holotype was represented by one of our herbarium collections (A.C.O. Souza et al. s/no., IAC 53476), reinforcing that the Atlantic Forest is one of the largest centers of biodiversity in the world (Campanili & Prochnow 2006, Tabarelli et al. 2010), with new species still being recognized and described in the present.

The values of the Shannon diversity index and Pielou equability index reflect the high diversity and equitability of the area, being among the richest in the region. The greatest diversity value found in one hectare continues was 4.48 (Rochelle et al. 2011) and, in general for this region, mature areas of this type of forest has an index higher than four (Campos

Table 2. Number of individuals (**NInd**) and importance value index (**IVI**) of the species that comprise 70% of the IVI by sampling area (**1–4**) and total (**T**), and absolute frequency (**AFr**) and dominance (**ADo**) in decreasing order of the total IVI, sampled in 1 ha of Montana Ombrophilous Dense Forest, at an altitude of 800 m, Serra do Mar State Park, Ubatuba – SP.

Species			**NInd**			**AFr**	**ADo**			**IVI**		
	1	**2**	**3**	**4**	**T**			**1**	**2**	**3**	**4**	**T**
Rustia formosa	35	17	55	44	151	67	4.6	23.2	10.4	44.8	25.7	25.1
Euterpe edulis	33	21	19	27	100	56	0.7	15.2	9.9	11.4	11.8	12.0
Coussarea accedens	19	12	39	19	89	48	0.8	9.3	5.1	21.4	9.6	10.9
Guapira opposita	12	23	3	15	53	38	1.8	9.2	17.1	2.2	11.7	10.5
Psychotria suterella	20	20	26	22	88	53	0.4	10.0	8.3	12.9	10.3	10.3
Rudgea jasminoides	12	22	13	28	75	47	0.3	6.7	8.9	7.4	11.8	8.8
Meriania calyptrata			42	46	88	31	0.3			17.9	16.4	8.4
Cryptocarya mandioccana	4	4	6	10	24	19	1.9	5.9	3.8	6.7	12.9	7.4
Ouratea multiflora	19	20	8	8	55	35	0.4	10	8.8	4.3	4.4	6.9
Mollinedia boracensis	10	1	14	14	39	32	0.6	7.4	0.6	9.5	8.7	6.4
Bathysa mendoncaei	1	45	1		47	21	0.6	0.6	19.9	0.7		5.7
Mollinedia cf. *argyrogyna*	7	13	4	2	26	20	1.0	5.6	10.8	3.6	2.0	5.6
Eugenia subavenia	10	9	8	2	29	23	0.5	5.9	3.9	8.3	1.1	4.6
Hirtella hebeclada	7	6	1	7	21	16	0.8	7.3	4.4	0.8	4.8	4.4
Cabralea canjerana	1		3	7	11	10	1.1	3.3		7.6	6.4	4.2
Licania cf. *kunthiana*	1	5	3	2	11	11	1.0	0.6	5.7	5.6	3.9	4.0
Guapira hirsuta	1	31	1		33	12	0.4	1.1	12.8	0.7		3.9
Hieronyma alchorneoides	2	3	2	9	16	12	0.8	3.3	1.7	1.4	8.3	3.8
Mollinedia triflora	1	1	4	6	12	10	1.0	0.7	3.3	3.9	6.9	3.8
Coussarea meridionalis	23	6	1		30	18	0.2	10.9	3.1	0.7		3.6
Coussapoa microcarpa	2	3	2	9	16	15	0.6	1.9	2.5	2.3	7.2	3.5
Humiriaceae sp1	6	5	2	3	16	16	0.4	3.7	4.6	2.39	2.2	3.2
Citronella paniculata	9	1	2	3	15	13	0.5	7.9	0.6	2.61	2.1	3.1
Alibertia myrciifolia	2	8	2	10	22	18	0.1	1.2	3.8	1.33	5.1	2.9
Myrcia pubipetala	3		7	2	12	9	0.6	1.9		8.66	2.1	2.9
Chrysophyllum flexuosum	1	9	3	4	17	15	0.3	0.9	5.7	2.25	2.3	2.9
Calyptranthes lucida	5	4	2	2	13	13	0.4	4.4	3.2	2.9	1.2	2.8
Mollinedia schottiana	5	1	12	2	20	16	0.2	3.2	0.5	7.22	1.1	2.8
Campomanesia guaviroba	2			1	3	3	1.0	6.3			4.5	2.7
Alsophila sternbergii	3		7	4	14	10	0.4	1.8		5.53	3.5	2.6
Ocotea dispersa	1	2	4	2	9	9	0.6	1.7	4.3	2.97	1.3	2.0
Pouteria caimito		4	1	3	8	8	0.6		4.2	0.65	4.5	2.5
Eugenia cereja	7	4	2		13	10	0.3	3.5	2.6	3.22		2.2
Mollinedia sp1			5	8	13	12	0.2			3.67	5.4	2.2
Daphnopsis schwackeana	5	2	5	1	13	11	0.2	3.4	1.2	3.43	0.6	2.0
Cupania furfuracea	2	2		4	8	7	0.4	1.4	2.6		3.6	2.0
Calyptranthes grandifolia	2	4	1	3	10	10	0.3	1.3	3.1	0.77	2.5	2.0
Eugenia fusca	1				1	1	0.8	8.6				2.0
Eugenia batingabranca	2	8			10	9	0.3	3.3	4.3			2.0
Coussarea cf. *hydrangeifolia*			9	6	15	11	0.1			5.58	2.5	1.9
Marlierea tomentosa	2	4		2	8	8	0.3	2.9	2.3		1.6	1.8
Myrciaria floribunda	4	2	1	4	11	11	0.1	2.7	1.1	0.78	2.6	1.8
Sloanea cf. *hirsuta*	1	2	2	4	9	9	0.2	0.6	1.5	1.61	3.3	1.8
Marlierea excoriata		2	2	4	8	7	0.3		1.2	1.15	4.5	1.8
Syagrus pseudococos	4	1	3	1	9	9	0.2	3.1	0.6	2.69	0.7	1.7
Other 141 morphospecies			438			405	13.4			89.9		
Total live trees			1729			100	100			300		

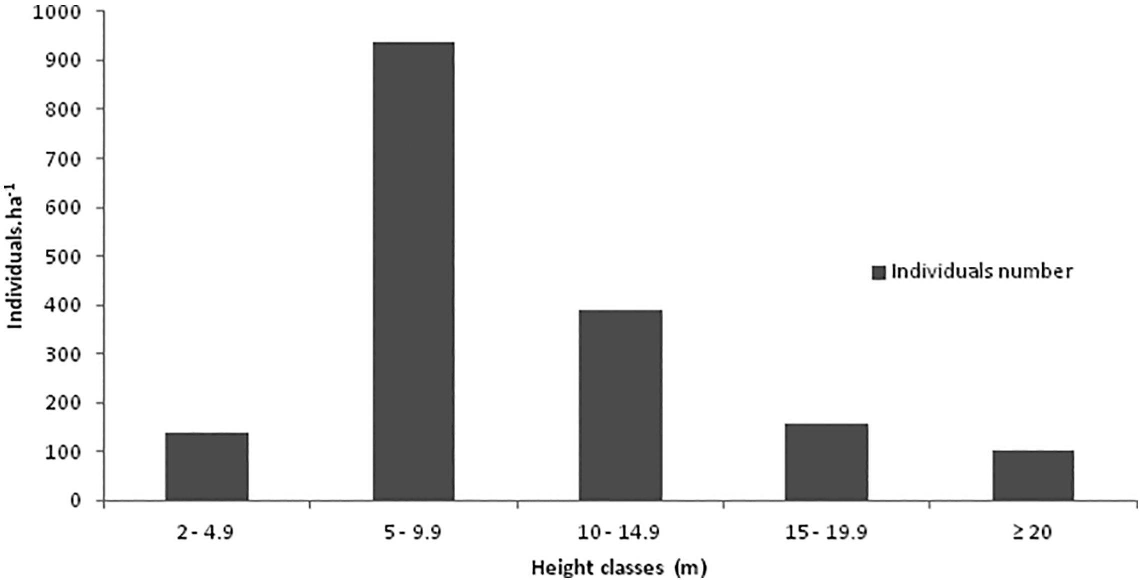

Figure 2. Distribution of the height classes of the tree component in 1 ha of the Ombrophilous Dense Montane Forest, at an altitude of 800 m, State Park of Serra do Mar, Ubatuba – SP.

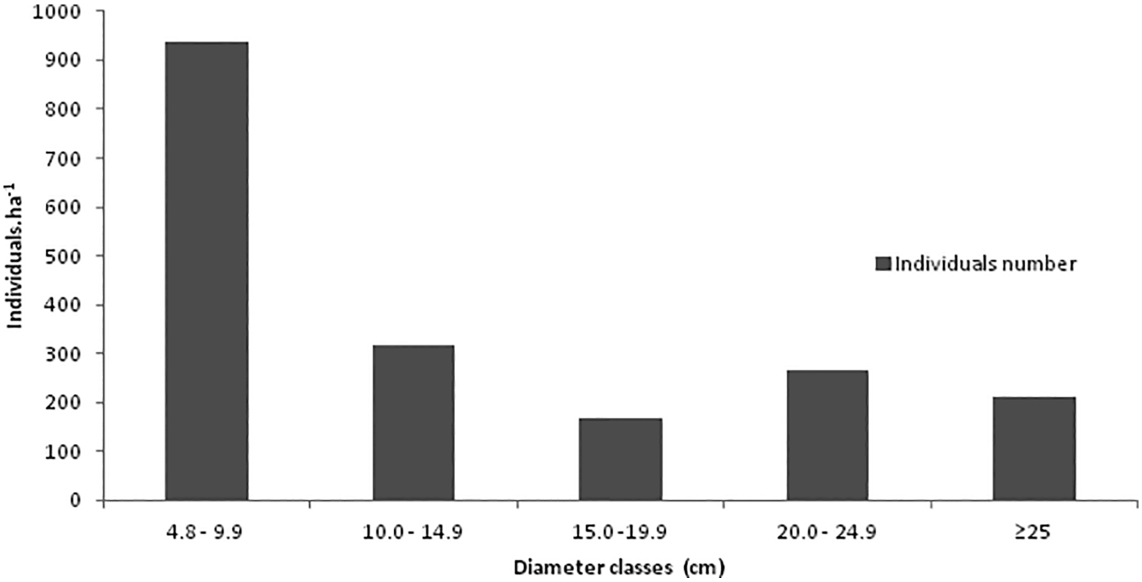

Figure 3. Distribution of the diameter classes of the tree component in 1 ha of Ombrophilous Dense Montane Forest, at an altitude of 800 m, State Park of Serra do Mar, Ubatuba – SP.

2008, Gomes et al. 2011, Padgurschi et al. 2011). With insufficient numbers of protected areas allied with other aggravating factors, the conservation of the Atlantic Forest is still insufficient (Tabarelli et al. 2005) and studies like this are fundamental to reinforce the need to preserve this biome and its valuable biodiversity.

It is widely known the proposed division of the Ombrophylous Dense Forest into phytophysiognomies that reflect the ecotypic variations of the altimetric bands and geomorphological situations (Veloso et al. 2012) however, recent studies have also highlighted the importance of local abiotic factors for floristic composition and structure of the vegetation (Joly et al. 2012). So although the proposed environmental continuum (Gleason 1926, Whittaker 1967) could consider this forest type as the only formation, there are peculiarities among the phytophysiognomies in the altitudinal gradient which are relevant and occur gradually.

Our results suggest that the 800 m altitude elevation is an environment of transition between the typical vegetation of the Submontane and Montane divisions of the Ombrophilous Dense Forest, already characterized by works carried out in the region. There are no abrupt transitions observed in this work, but it is noted that families of importance in the Montane areas begin to increase in the number of species and individuals (Monimiaceae). In this sense, the categorical division of altitude of the phytophysiognomies may not occur as proposed, and their limits are not clearly established.

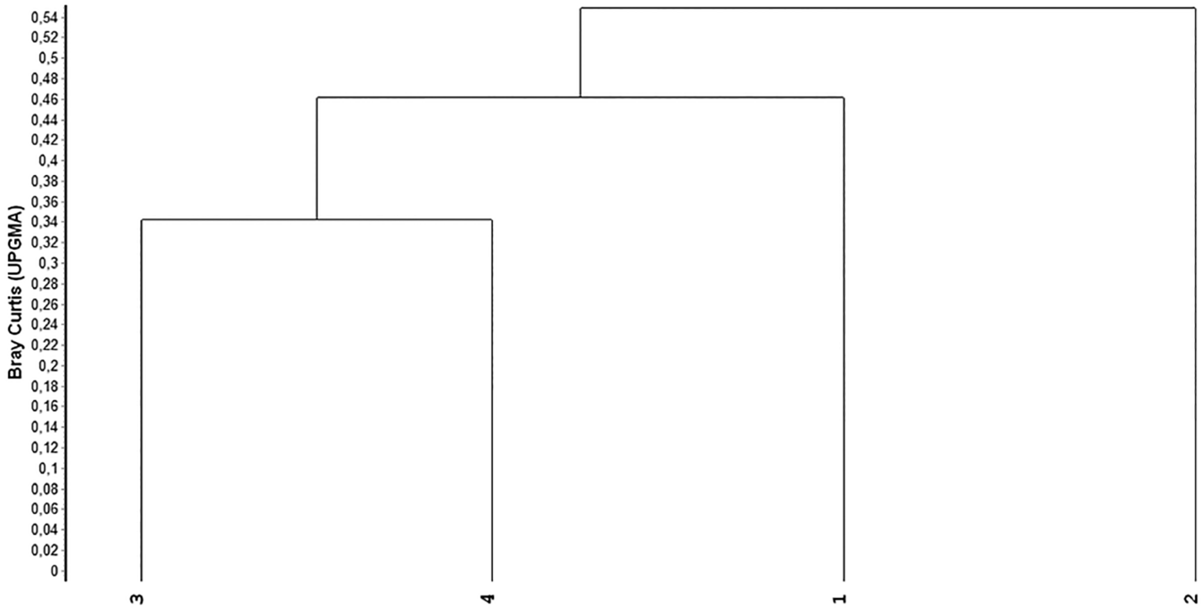

Figure 4. Dendrogram of similarity between the four sample blocks in 1 ha of Ombriphilous Dense Montane Forest, at 800 m elevation, Serra do Mar State Park, Ubatuba – SP.

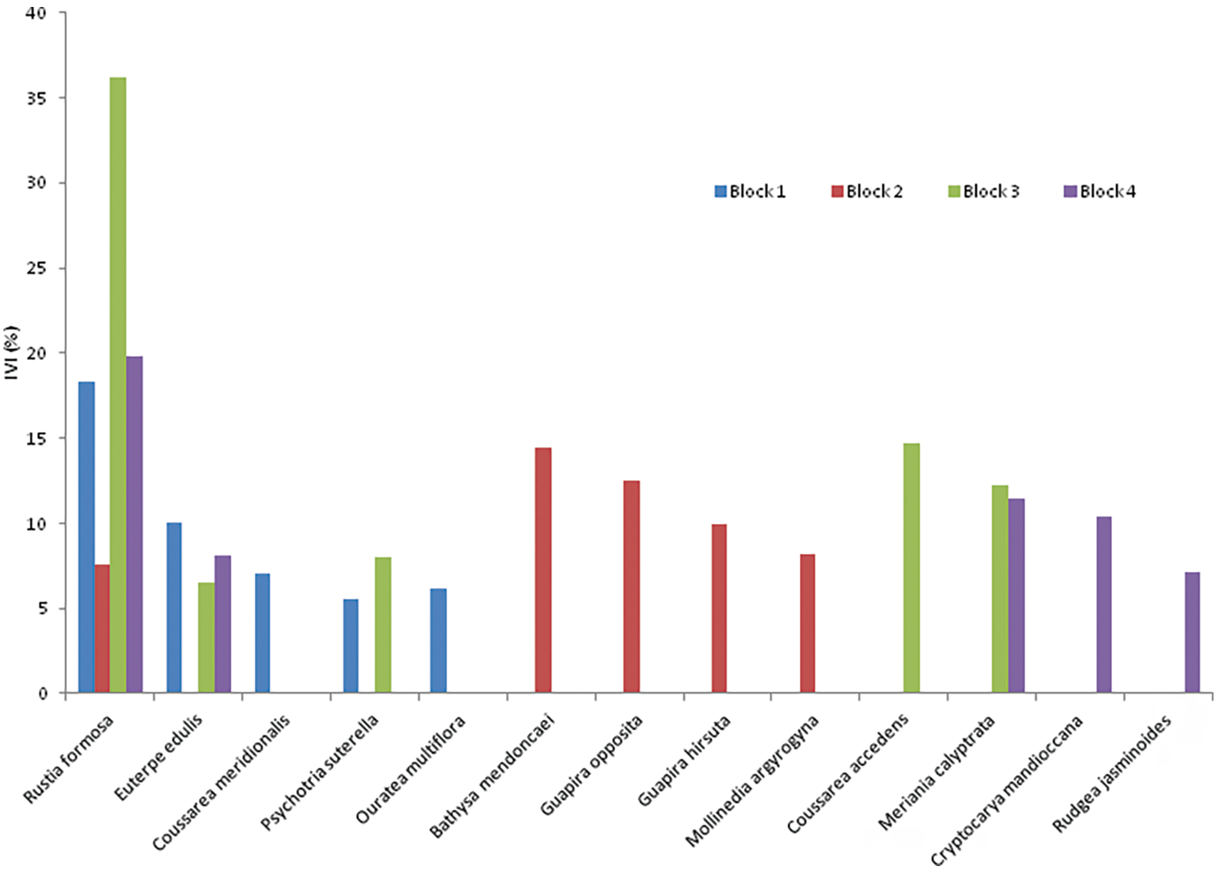

Figure 5. The five species that obtained the highest importance values per sampling area in 1 ha of Ombrophilous Dense Montane Forest, at an altitude of 800 m, Serra do Mar State Park, Ubatuba – SP.

In this way, these data corroborates with previous work which indicate that events of lower geographic scale, mainly related to the combination of relief and microclimate, could result in the phytophysiognomies stratification with smooth transitions (Scaramuzza et al. 2011, Joly et al. 2012). There are few vegetation studies developed in the Serra do Mar area at 800 m elevation, and therefore, the objective of describing the floristic composition and structure of this arborea community was essential to understanding the transition of this altitudinal gradient in the area, and new studies are desirable at the same quota altitudinal.

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Author Contributions

Ana Cláudia Oliveira de Souza: Contribution to data collection, data analysis and interpretation. Contribution to manuscript preparation.

Luís Benacci: Substantial contribution in the concept and design of the study. Contribution to data collection, data analysis and interpretation. Contribution to manuscript preparation.

Carlos Alfredo Joly: Substantial contribution in the concept and design of the study. Contribution to manuscript preparation.

Conflicts of interest

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

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Inventory

Checklist of Odonata (Insecta) in the state of Rio Grande do Sul, Brazil with seven new records

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Abstract: The regional knowledge of species diversity and distribution is important to support conservation strategies for species and their habitats. The main goal of this work is to present a checklist of Odonata species in the state of Rio Grande do Sul, as well as their known locations in the municipalities. The preparation of the list was based in data gathered from collections of Museu de Ciências da Universidade do Vale do Taquari (UNIVATES), Laboratório de Ecologia e Evolução da Universidade do Vale do Taquari (UNIVATES), Museu de Ciências Naturais da Fundação Zoootânica, Museu de Zoologia da Universidade do Vale do Rio dos Sinos (UNISINOS) plus data extracted from 65 publications and the sites *SpeciesLink*, All Odonata and Puget Sound University. A total of 182 Odonata species were recorded, spanning nine families and 57 genera. The most representative family was Libellulidae (80 species) followed by Coenagrionidae (41 species) and seven species are new records for Rio Grande do Sul. The list of species presented here is a significant advance compared to previous counts for Rio Grande do Sul, however, our list is by no means a final one. Some regions of the state remain poorly explored, such as the border to Uruguay also in the northernmost part of the state. Several families remain poorly sampled, especially those that inhabit small forested streams and probably there are many specimens which are not cataloged and identified yet in scientific collections, both in the state and in the country.

Keywords: *Anisoptera, Atlantic Forest, Neotropical Region, Pampa, Zygoptera.*

Lista de especies de Odonata (Insecta) no estado do Rio Grande do Sul, Brasil com sete novos registros

Resumo: O conhecimento regional da diversidade e da distribuição das espécies é importante para subsidiar estratégias de conservação tanto para as espécies e seus respectivos habitats. O principal objetivo deste trabalho é apresentar uma lista de espécies de Odonata que ocorrem no estado do Rio Grande do Sul, bem como suas localizações conhecidas nos municípios. Para a elaboração da lista, foram utilizados dados das coleções do Museu de Ciências da UNIVATES, do Laboratório de Ecologia e Evolução da Universidade do Vale do Taquari (UNIVATES), do Museu de Ciências Naturais da Fundação Zoo-Botânica e do Museu de Zoologia da Universidade do Vale do Rio dos Sinos (UNISINOS) somados à dados extraídos de 65 publicações e dos sites *SpeciesLink*, All Odonata e Puget Sound University. Um total de 182 espécies de Odonata foram registradas, abrangendo nove famílias e 57 gêneros. A família mais representativa foi Libellulidae (80 espécies) seguida por Coenagrionidae (41 espécies). Além do mais, sete espécies são novos registros para o Rio Grande do Sul. A lista de espécies aqui apresentada demonstra um avanço significativo em relação às contagens anteriores para o Rio Grande do Sul, no entanto, nossa lista não é de forma alguma final. Algumas regiões do estado continuam pouco exploradas, como aquelas que fazem fronteira com o Uruguai ou estão mais ao norte do Estado. Várias famílias permanecem mal amostradas, especialmente aquelas que habitam pequenos córregos em florestas e, provavelmente, existem muitos exemplares que ainda não estão catalogados e identificados em coleções científicas, tanto no estado quanto no país.

Palavras-chave: *Anisoptera, Mata Atlântica, Região Neotropical, Pampa, Zygoptera.*

Introduction

Damselflies and dragonflies are a small group of aquatic insects classified as Odonata, which includes about 6280 species (Schorr & Paulson 2018). This order is spread in temperate, tropical and subtropical zones, with more than 600 genera and 39 families belonging to three suborders: Anisoptera, Anisozygoptera and Zygoptera (Schorr & Paulson 2018). Neotropical region is one of the most diverse, presenting 1727 species (Von Ellenrieder 2009), of which 854 species, 146 genera and 15 families were recorded in Brazil (data available in Pinto 2018).

The regional knowledge of species diversity and distribution is important to provide numerical data, related closely to the actual species richness and abundance numbers (Costa et al. 2000), and support conservation strategies for species and their habitats. A quick survey on Brazilian studies concerning Odonata fauna reveals punctual works restricted to Southeastern and Mid-West regions, suggesting an unequal distribution of research regarding this group in Brazil (De Marco & Viana 2005). This lack of information is the main dead-lock in the elaboration of faunal status list, as the IUCN Red list, which represents a serious problem for conservation programs in Brazil (Rodrigues & Roque 2017). The states of Goiás, Mato Grosso do Sul, Minas Gerais, São Paulo and Rio de Janeiro already have a list of species.

The state of Rio Grande do Sul, located in southern Brazil, stands out due to its extensive agricultural areas and large industrial parks near to the metropolitan region, which causes the state to face serious environmental problems, resulting in a long list of endangered species. Two biomes are recognized in Rio Grande do Sul: Pampa Biome which cover about 66% of the state's area, and Atlantic Forest covering 29% of the total state area. The Atlantic Forest is among the five main nature *hotspots* on Earth, given its high biodiversity levels and endemisms. However, it has been subject to big rates of deforestation, what makes it even more vulnerable ecologically. The Pampa Biome which is restricted to Rio Grande do Sul, has the greatest endemism rates among the brazilian biomes (39%). It is also heavily threatened by the expansion of monocultures and the introduction of exotic species, which in turn, led to the complete change of its natural landscapes (Oliveira et al. 2017).

Until now, works have been dedicated to improve the knowledge about Odonata fauna in the state (Consatti et al. 2014, Hanauer et al. 2014, Kittel & Engel 2014, 2016, Renner et al. 2013, 2015, 2016, 2017), however, such studies are restricted and do not provide information on total number of species. A recent attempt to estimate the diversity of the state (Kittel & Engel 2014, 2016) recorded 108 species of Odonata, representing about 12% of the known species for Brazil (Pinto 2018), though the authors suggest that this number is much higher. Thus, the main goal of this work is to present the species diversity of Odonata (Insecta) in the state of Rio Grande do Sul, as well as their known locations in the municipalities.

Material and Methods

1. *Study area*

The state of Rio Grande do Sul, which is located in southernmost portion of Brazil, borders the state of Santa Catarina to the North, the Atlantic ocean to the East, Uruguay to the South and Argentina to the West. With a total area of 281,730 km², it covers the two subtropical biomes of Brazil: Atlantic Forest and Pampa. It presents, mostly, low relief, with 70% of its territory presenting less than 300m of altitude. The highest portion, of more than 600m altitude, is located in the Northeast, comprising 11% of the total surface. Two climatic types characterize Rio Grande do Sul: the humid subtropical climate (Cfa), with well distributed rainfall and hot summers, presenting average annual temperature between 18º and 20ºC, and the oceanic climate (Cfb), also with well distributed rains, but with mild summers, presenting an annual average temperature between 13º and 17ºC (Kuinchtner & Buriol, 2001).

The subtropical Atlantic Forest is located in the North and East half of the state, with an area of 103,505 km², which corresponds to 37% of the total area of the state, and 1.54% of the total area of Brazil. The dominance of the Atlantic Forest in Rio Grande do Sul consists of dense ombrophylous forest, mixed ombrophylous forest (Araucaria forest), semideciduous seasonal forest, altitude and 'restinga' fields (Marcuzzo et al. 1998). These subdivisions result from the great variation of altitude and climatic characteristics. In Rio Grande do Sul, there is an extremely rugged relief, between 0m and 1200m altitude. The climate presents well defined seasons of the year, and its vegetation is characterized by tall trees of broad crown in the upper stratum and by broad-leaved shrubs in the lower stratum.

The Pampa biome, located in the South and West half of the state (57° to 63° W and 34° to 30° S), presents 178,243 km² of total area, corresponding to 63% of the state territory and 2.07% of the Brazilian territory (Crawshaw et al. 2007). The relief in the southern fields is gently undulating, between 500m and 800m of altitude. Plains predominate, but some hills, in the region known as "coxilhas", may be found. Besides the coxilhas, there are also some plateaus. The climate presents the four seasons of the year well defined, and its vegetation is characterized by the presence of grasses, ground plants, shrubs and small trees.

2. *Elaboration of the list*

For the preparation of the list, we gathered data from collections of the Museu de Ciências da Universidade do Vale do Taquari (UNIVATES), Laboratório de Ecologia e Evolução da Universidade do Vale do Taquari (UNIVATES) concerning 39 municipalities. All these specimens were preserved in 96% ethanol, and later determined to species level according to Garrison et al. (2006, 2010), Heckman (2006, 2010) and Lencioni (2006). In addition, data were collected from 65 publications, from 1909 to 2018, of the material cataloged in the Museu de Ciências Naturais da Fundação Zootânica, Museu de Zoologia da Universidade do Vale do Rio dos Sinos (UNISINOS) and the sites *SpeciesLink*, All Odonata and Puget Sound University. In total, data were collected from 85 localities (Figure 1, Table 1) and 74 data sources. For systematic classification, we followed Dijkstra et al. (2013, 2014).

Results

The total number of Odonata species recorded for Rio Grande do Sul is 182 (Table 2). Additionally, there are five species cited by Kittel and Engels (2014) which are probably erroneous records: *Hetaerina laesa* Hagen *in* Selys, 1853 (Calopterygidae) known only from Northern Brazil; *Leptagrion acutum* Santos, 1961 and *Leptagrion capixabae* Santos, 1965 known only from Espirito Santo (Coenagrionidae);

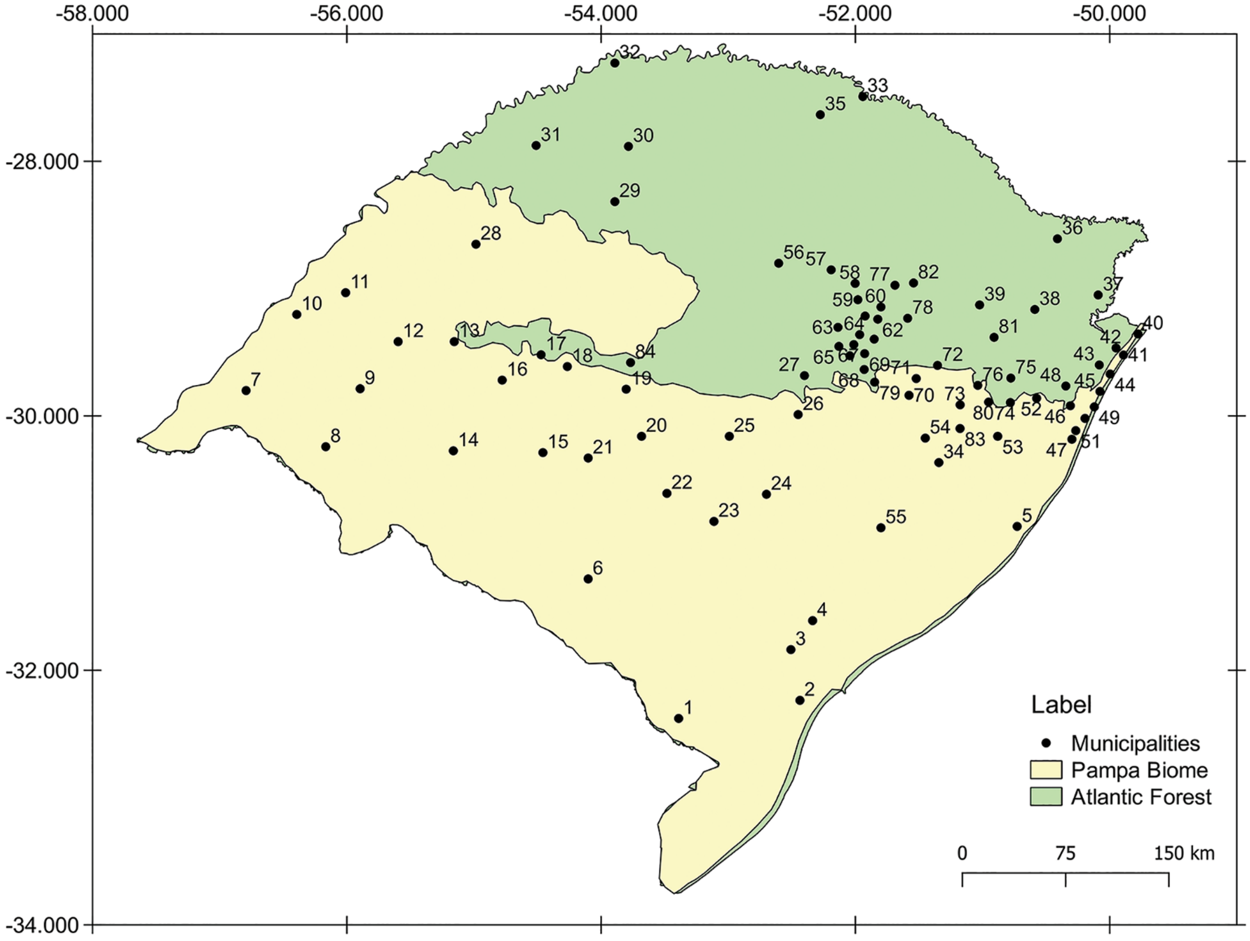

Figure 1. Municipalities with Odonata´s recorded in Rio Grande do Sul State. For code numbers see Table 1.

Heteragrion ictericum Williamson, 1919 (Heteragrionidae) known only from Amazonian Biome and *Teinopodagrion meridionale* De Marmels, 2001 (Megapodagrionidae) also cite by Marins et al. (2005), however this species is not recorded from Brazil, just from to mountainous and cloud forest of Andes (IUCN Red List 2018). Both studies didn't cite the source of the information, so we decide to not add these species to final checklist to avoid mistakes.

The total number of genera recorded for the state is 57, distributed in nine families. Libellulidae is the family with the highest number of records, with 22 genera and 80 species, followed by Coenagrionidae with 13 genera and 41 species. Aeshnidae with nine genera and 20 species, and Gomphidae with seven genera and 18 species. Other families recorded are: Corduliidae (one genera, three species), Calopterygidae (two genera, seven species), Heteragrionidae (one genera, three species), Lestidae (one genera, nine species), Megapodagrionidae (one genera, one species).

Seven species are new records for Rio Grande do Sul state: *Erythemis credula* (Hagen, 1861), *Erythrodiplax avittata* Borror, 1942, *Lestes dichrostigma* Calvert, 1909, *Nephepeltia berlai* Santos, 1950, *Oxyagrion chapadense* Costa, 1978, *Oxyagrion sulmatogrossense* Costa, Souza & Santos, 2000 and *Progomphus intricatus* Hagen in Selys, 1858.

Discussion

The 182 species listed from RS representing about 22% of the species known to occur in Brazil (Pinto 2018). This number increases the species records for the state by 73% (Kittel & Engels 2014, 2016). Odonata fauna of Rio Grande do Sul is similar to the known fauna, published in species lists, of other states. Rio de Janeiro and São Paulo are the states with the highest number of records, presenting 251 and 280 species respectively (Costa & Santos 2000, Costa et al. 2000). Minas Gerais recorded 218 species (Machado 1998), Mato Grosso do Sul recorded 199 species (Koroiva et al. 2017, Rodrigues & Roque 2017) and Goiás, 152 species (Nóbrega & De Marco 2011).

The list of species presented here represents a significant advance compared to previous counts for Rio Grande do Sul, however, our list is by no means a final one. Some regions of the state remain poorly explored, such as southernmost parts, in cities bordering Uruguay, such as Jaguarão or Santa Vitória do Palmar, or even in the northernmost part of the state (Figure 1). Several families remain poorly sampled, especially those that inhabit small forested streams, such as Corduliidae, Heteragrionidae or Megapodagrionidae, and probably there are many specimens which are still not cataloged and identified in scientific collections, both in the state and in the country.

Table 1. Numbers and codes for municipalities and references. *Municipalities sampled by Laboratório de Ecologia e Evolução – UNIVATES.

Municipalities	Code	Number	References	Number
Alegrete*	ALT	9	Calvert, 1909	1
Anta Gorda*	ANG	58	Ris, 1910	2
Arroio do Meio*	ARM	64	Ris, 1911	3
Arroio do Sal*	ART	41	Ris, 1913	4
Arvorezinha*	AVZ	57	Willianson, 1917	5
Bagé	BGE	6	Borror, 1931	6
Balneário Pinhal*	BLP	47	Navás, 1933	7
Barra do Ribeiro	BDR	34	Navás, 1934a	8
Bom Jesus	BMJ	36	Navás, 1934b	9
Bom Retiro do Sul*	BRS	68	Navás, 1935	10
Bossoroca	BSC	28	Montogomery, 1936	11
Caçapava do Sul*	CDS	22	Quentin, 1967	12
Cachoeira do Sul	CHS	25	Belle, 1970	13
Camaquã	CMQ	55	Costa, 1970	14
Cambará do Sul	CMS	37	Costa, 1971	15
Canoas	CNS	73	Teixeira, 1971	16
Capão da Canoa*	CDC	44	Belle, 1972	17
Capão do Leão	CDL	3	Quentin, 1973	18
Caraá	CRA	48	Leonard, 1977	19
Caxias do Sul	CXS	39	De Marmels & Rácenis, 1982	20
Cidreira*	CDR	51	Belle, 1984	21
Colinas*	CLN	66	Costa, 1986	22
Cruzeiro do Sul*	CZS	85	Wildermuth, 1991	23
Derrubadas	DER	32	Belle, 1992	24
Dr. Ricardo*	DRR	59	Watson, 1992	25
Encantado*	ENC	61	Assis & Costa, 1994	26
Encruzilhada do Sul*	EDS	24	von Ellenrieder & Muzón, 1999	27
Erechim	ECM	35	Costa et al., 2002	28
Estrela*	ETL	69	von Ellenrieder & Costa, 2002	29
Garibaldi	GRB	78	von Ellenrieder & Garrison, 2003	30
Glorinha	GLO	74	Pinto, 2003	31
Gramado	GMD	81	Neiss & Fiorentin, 2004	32
Gravataí	GVI	80	Lencioni, 2005	33
Guaíba	GIB	54	Machado, 2005	34
Ijuí	IJI	29	Pinto, 2005	35
Imbé*	IBE	49	Garrison, 2006	36
Itaara	ITA	84	Heckman, 2006	37
Itaqui*	IQI	10	Lencioni, 2006	38
Jaguarão	JGR	1	Costa & Machado, 2007	39
Lajeado*	LJD	67	von Ellenrieder, 2008	40
Maçambará	MÇB	11	Heckman, 2008	41
Manoel Viana*	MLV	12	Costa et al., 2009	42

Continuation Table 1.

Marcelino Ramos	MCR	33	Marins et al., 2005	43
Marques de Souza*	MDS	63	Pinto & Lamas, 2010	44
Mata*	MTA	17	Dalzochio, 2011	45
Montenegro	MTN	71	Neiss et al., 2011	46
Mostardas	MTD	5	Pinto & Lamas, 2011	47
Muçum*	MÇM	60	Costa et al., 2012	48
Novo Hamburgo	NVH	76	Dalzochio et al., 2012	49
Osório*	OSO	46	von Ellenrieder, 2012	50
Pelotas	PLT	4	Pessacq, 2012	51
Porto Alegre	POA	83	Pinto & Carvalho, 2012	52
Quaraí*	QRI	8	Almeida et al., 2013	53
Rio Grande	RGD	2	Renner et al., 2013	54
Rio Pardo	RPD	26	Consatti et al., 2014	55
Roca Sales*	RCS	62	von Ellenrieder, 2014	56
Rosário do Sul*	RDS	14	Haunaer et al., 2014	57
Santa Clara do Sul*	SCS	65	Kittel & Engels, 2014	58
Santa Cruz do Sul	SZS	27	Renner et al., 2015	59
Santa Margarida do Sul*	SMS	21	Kittel & Engels, 2016	60
Santa Maria	STM	19	Pinto & Almeida, 2016	61
Santa Rosa	STR	31	Renner et al., 2016	62
Santana da Boa Vista*	SBV	23	Garcia Junior, 2016	63
Santo Antônio da Patrulha	SAP	52	Acosta et al., 2017	64
Santo Augusto	STA	30	Renner et al., 2017	65
São Francisco de Assis*	SFA	13	Specis link (2017)	66
São Francisco de Paula	SFP	38	Pinto, 2018	67
São Gabriel*	SGL	15	FZB Collection	68
São Pedro do Sul*	SPS	18	Puget Sound University website	69
São Sebastião do Caí	SSC	72	All Odonata website	70
São Sepé*	SSE	20	Unisinos Collection	71
São Vicente do Sul*	SVS	16	This study	72
Soledade*	SLD	56	Pires et al., 2018	73
Taquara	TQR	75	Werneck-de-Carvalho, 2004	74
Taquari*	TQI	79		
Terra de Areia*	TEA	43		
Torres*	TRS	40		
Tramandaí*	TMD	50		
Três Cachoeiras	TCH	42		
Triunfo	TRF	70		
Uruguaiana	URG	7		
Veranópolis	VRP	82		
Viamão	VMA	53		
Vila Cotiporã	VCA	77		
Xangri-lá*	XGL	45		

Table 2. Species recorded for Rio Grande do Sul State, Brazil. For references and municipalities codes, please check Table 1.

Species	Municipalities	References
ZYGOPTERA		
Calopterygidae		
Hetaerina longipes Hagen in Selys, 1853	CDS; CXS; SFP	8; 62
Hetaerina rosea Selys, 1853	ALT; ANG; ARM; AVZ; CDS; CMQ; CDL; CLN; ENC; ETL; LJD; MLV; MDS; MÇM; PLT; POA; QRI; RGD; RCS; SCS; STM; SBV; SFA; SFP; SLD; TQR; TQI; VMA; URG	15; 16; 33; 41; 43; 55; 57; 58; 62; 63; 65; 71; 72
Mnesarete borchgravii (Selys, 1869)	SFP	58; 62
Mnesarete hyalina (Hagen in Selys, 1853)	POA	16
Mnesarete lencionii Garrison, 2006	CDS; MLV; SFA	65
Mnesarete pruinosa (Hagen in Selys, 1853)	ANG; CXS; DRR; PLT; POA; SFP; SLD	1; 8; 16; 33; 36; 41; 58; 62; 66; 72
Mnesarete pudica (Hagen in Selys, 1853)	SFA	22; 36; 38; 41; 58; 65
Coenagrionidae		
Acanthagrion apicale Selys, 1876	BMJ	66
Acanthagrion ascendens Calvert, 1909	CZS; LJD; MLV; MDS; MTA; SPS; SVS; SLD; VMA	19; 43; 54; 58; 59; 72
Acanthagrion cuyabae Calvert, 1909	ALT; BMJ; MLV; SZS; SBV; VMA	19; 33; 41; 43; 58; 65; 66
Acanthagrion gracile (Rambur, 1842)	ALT; ANG; ARM; AVZ; BMJ; BRS; CDS; CDC; CDL; CLN; CZS;DER; GVI; IJI; IQI; MLV; OSO; PLT; POA; QRI; RGD; SZS; STM; SBV; SFA; SFP; SSC; TRS; TMD; VRP; VMA; XGL; SSE	15; 16; 38; 41; 54; 55; 57; 58; 59; 62; 63; 65; 66; 72
Acanthagrion lancea Selys, 1876	ALT; ANG; AVZ; CDC; CDL; CZS; DRR; LJD; MLV; MTA; PLT; POA; QRI; RGD; RDS; SZS; SBV; SFA; SFP; SLD; TQI; SMS; SSE	16; 38; 54; 55; 58; 59; 62; 63; 65; 72
Argentagrion ambiguum (Ris, 1904)	ALT; BMJ; CDL; CLN; CZS; MLV; PLT; RGD; SBV; SFA; SFP	40; 54; 58; 59; 62; 63; 65; 66
Argia albistigma Hagen in Selys, 1865	ALT; ARM; BRS; CDS; CLN; CZS; DRR; ENC; MLV; MDS; QRI; RCS; SCS; SBV; SFA; TQI; URG	38; 41; 55; 57; 58; 65; 72
Argia croceipennis Selys, 1865	ARM; AVZ; BRS; CXS; CLN; CZS; ENC; ETL; LJD; MÇM; RCS; SFP; SLD; TQI	9; 38; 41; 54; 58; 59; 62
Argia cyathigera Navás, 1934	CXS	9; 41
Argia lilacina Selys, 1865	ALT; MLV; SFA; SSE	65; 72
Argia modesta Selys, 1865	ALT; CDS; MDS; QRI; SBV; SFA; URG	65; 72
Argia reclusa Selys, 1865	VMA	41
Cyanallagma corbeti Costa, Santos & I. de Souza, 2009	BMJ; SFP	42; 48; 66
Cyanallagma bonariense (Ris, 1918)	CDS; URG	73
Homeoura chelifera (Selys, 1876)	ALT; ART; BMJ; CDL; CDR; CZS; LJD; MLV; MDS; PLT; QRI; RGD; STM; SBV; SFA; SFP; TQI; TRS; VMA; URG	1; 15; 38; 40; 41 54; 55; 58; 59; 62; 63; 66; 68; 72
Ischnura capreolus (Hagen, 1861)	ALT; ANG; ART; AVZ; BMJ; BRS; CDS; CDC; CDL; CDR; CZS; MLV; MTA; PLT; POA; QRI; RGD; RDS; SBV; SFA; SFP; TRS; TMD; VMA; XGL; SGL; SMS; SSE; URG	16; 38; 41; 43; 54; 58; 59; 62; 63; 65; 66; 72
Ischnura fluviatilis Selys, 1876	ALT; ART; AVZ; BRS; CDS; CDC; CDL; CDR; CLN; CZS; GVI; LJD; MLV; MDS; MTA; MTD; GLO; PLT; POA; QRI; RGD; RPD; RCS; STM; SBV; SAP; SFP; SPS; SVS; TQI; TRS; TMD; VMA; XGL; SMS; SSE; URG	1; 15; 16; 38; 41; 54; 55; 57; 58; 59; 62; 63; 64; 65; 66; 68; 71; 72
Minagrion mecistogastrum (Selys, 1876)	BDR; BMJ; CMQ; GRB; IJI; ITA; MÇB; MTN; MTD; POA; SFP; TRF; VMA	38; 41; 58; 65; 66

Continuation Table 2.

Species	Municipalities	References
Minagrion waltheri (Selys, 1876)	SFA	65
Neoneura ethela Williamson, 1917	ALT; JGR; PEL; POA; RDS; STM	5; 33; 41; 51; 58
Neoneura leonardoi Machado, 2005	ALT; ANG; ARM; BRS; CDS; CXS; CLN; ENC; ECM; LJD; QRI	34; 38; 41; 51; 55; 57; 58; 59; 65
Oxyagrion basale Selys, 1876	CZS; LJD; MTA; STM; SPS; SVS; SLD	15; 38; 41; 55; 58; 59
Oxyagrion brevistigma Selys, 1876	RS	41
Oxyagrion chapadense Costa, 1978	CDS; MLV; MTA; SBV; SPS; SVS; URG	New Record
Oxyagrion hempeli Calvert, 1909	ALT; CDS; MLV; MDS; STM; SBV; SFA; SFP	15; 16; 38; 41; 58; 62; 65; 72
Oxyagrion microstigma Selys, 1876	BMJ; SFP	62; 66
Oxyagrion pavidum Hagen in Selys, 1876	CXS	8; 41
Oxyagrion rubidum (Rambur, 1842)	ALT; QRI; RDS; SBV; SFA; SSE; URG	65; 72
Oxyagrion santosi Martins, 1967	RS	38; 41
Oxyagrion simile Costa, 1978	CDL	38; 41; 63
Oxyagrion sulinum Costa, 1978	RS	38; 41
Oxyagrion sulmatogrossense Costa, Souza & Santos, 2000	ARV	New Record
Oxyagrion terminale Selys, 1876	ALT; ANG; AVZ; CDS; CZS; GMD; LJD; MLV; PLT; POA; RGD; STM; SBV; SFA; SFP; SLD	1; 15; 16; 38; 41; 54; 55; 58; 59; 62; 63; 65; 68; 72
Mecistogaster amalia (Burmeister, 1839)	CXS	45
Mecistogaster ornata Rambur, 1842	SFP	58
Peristicta janiceae Pessacq & Costa, 2007	SFP	51
Telagrion longum Selys, 1876	RS	38; 41
Telebasis carmesina Calvert, 1909	CZS; STR; VCA	38; 52; 54; 58; 59
Telebasis corallina (Selys, 1876)	ART; AVZ; CDS; CDC; STM; SBV; SFA; SVS; TRS; TMD; VMA; XGL; SMS; SSE	15; 38; 41; 52; 58; 65; 72
Telebasis theodori (Navás, 1934)	CXS; CZS; MLV; STR; SBV; SFA; SFP; SLD; VCA	9; 41; 52; 58; 59; 62; 65
Telebasis willinki Fraser, 1948	ALT; CDL; CZS; ENC; LJD; PLT; RGD; SFA; VMA	41; 43; 52; 54; 55; 57; 58; 59; 63; 65
Heteragrionidae		
Heteragrion consors Hagen in Selys, 1862	SFP	58
Heteragrion luisfelipei Machado, 2006	SFP	62
Heteragrion triangulare Hagen in Selys, 1862	AVZ; SFA	59; 65
Lestidae		
Lestes auritus Hagen in Selys, 1862	SFP	58; 62
Lestes bipupillatus Calvert, 1909	CDS; CZS; ETL; MLV; STM; SBV; SFA; SFP	15; 33; 41; 54; 58; 59; 62; 65
Lestes dichrostigma Calvert, 1909	AVZ	New Record
Lestes forficula Rambur, 1842	BMJ	66
Lestes minutus Selys, 1862	RGD	63
Lestes pictus Hagen in Selys, 1862	AVZ; CLN; CZS; MÇM; SFP; SLD; TQI	54; 58; 59; 62; 72
Lestes paulistus Calvert, 1909	ARM; AVZ; CDS	59
Lestes tricolor Hoffmansegg in Shomburgk, 1848	PLT; POA; RGD	16; 55; 57; 58; 63
Lestes undulatus Say, 1840	BMJ; CDC; CDL; CDR; PLT; POA; RGD; SFP; TRS	1; 33; 41; 58; 62; 63; 66; 72
Megapodagrionidae		
Allopodagrion brachyurum De Marmels, 2001	CRA	46

Continuation Table 2.

Species	Municipalities	References
ANISOPTERA		
Aeshnidae		
Anax amazili (Burmeister, 1839)	BGE; EDS; MTD; POA; STM; SAP	15; 16; 32; 49
Anax concolor Brauer, 1865	CZS; LJD; NVH	32; 54; 59; 60
Castoraeschna decurvata Dunkle & Cook, 1984	CRA, NVH, SFP	32; 74
Castoraeschna januaria (Hagen, 1867)	SFP, STA	16; 74
Coryphaeschna perrensi (McLachlan, 1887)	NVH	32
Coryphaeschna adnexa (Hagen, 1861)	RS	67
Gynacantha bifida Rambur, 1842	NVH	32
Limnetron debile (Karsch, 1891)	NVH; SBV	32; 65
Remartinia luteipennis (Burmeister, 1839)	CDS; CDL; NVH; PLT; RGD; SFA; SVS	32; 37; 60; 63; 65; 72
Rhionaeschna cornigera (Brauer, 1865)	CDL; CXS; NVH	32; 63; 68
Rhionaeschna bonariensis (Rambur, 1842)	ANG; CNS; CDL; MLV; MTN; MTD; NVH; PLT; POA; QRI; RGD; STM; SBV; SFA; SFP; TRS; VMA	15; 16; 32; 37; 43; 6; 62; 63; 65; 66; 68; 71; 72
Rhionaeschna brasiliensis (von Ellenrieder & Martins Costa, 2002)	SFP	29; 57; 60; 62
Rhionaeschna confusa (Rambur, 1842)	PLT	25; 37; 60
Rhionaeschna diffinis (Rambur, 1842)	RS	37
Rhionaeschna eduardoi (Machado, 1984)	SFP	60
Rhionaeschna planaltica (Calvert, 1952)	ALT; AVZ; CZS; LJD; MTA; NVH; POA; SFA; SFP; SVS; SLD	16; 32; 37; 54; 59; 60; 62; 65; 72
Rhionaeschna punctata (Martin, 1908)	NVH	32
Staurophlebia reticulata (Burmeister, 1839)	NVH; SCS; SFA	32; 65; 72
Triacanthagyna nympha (Navás, 1933)	NVH; PLT; POA	30; 32
Triacanthagyna ditzleri Williamson, 1923	LJD	37; 59; 60
Corduliidae		
Neocordulia androgynis (Selys, 1871)	RS	44
Neocordulia fiorentini Costa & Machado, 2007	SFP	39; 44; 60
Neocordulia gaucha Costa & Machado, 2007	SLD	39; 44; 60
Gomphidae		
Aphylla molossus Selys, 1869	MLV	65
Aphylla producta Selys, 1854	CZS; POA	12;
Aphylla theodorina (Navás, 1933)	BSC; PLT; POA; SBV; SFA; SMS	7; 12; 13; 17; 24; 65; 66; 71; 72
Archaeogomphus densus Belle, 1982	SBV; URG	65
Cyanogomphus waltheri Selys, 1873	SAP	61
Phyllocycla argentina (Hagen in Selys, 1878)	CDR; POA	17; 18
Phyllocycla propinqua Belle, 1972	ARM; BRS; ENC	59; 60
Phyllocycla viridipleuris (Calvert, 1909)	POA	12
Phyllogomphoides annectens (Selys, 1869)	RS	12
Phyllogomphoides regularis (Selys, 1873)	QRI; SFP	21; 37; 53; 60; 62; 72
Progomphus aberrans Belle, 1973	RS	37
Progomphus basistictus Ris, 1911	MLV; QRI; STM; SBV; SFA	15; 65
Progomphus complicatus Selys, 1854	PLT	63
Progomphus costalis Hagen in Selys, 1854	POA	16

Continuation Table 2.

Species	Municipalities	References
Progomphus gracilis Hagen in Selys, 1854	SFP	60
Progomphus intricatus Hagen in Selys, 1858	CDR	New Record
Progomphus lepidus Ris, 1911	CDR; CLN; CZS; QRI	18; 37; 54; 59; 60
Tibiagomphus noval (Rodrigues, 1985)	CDR; GIB; JGR; PLT	18; 53
Libellulidae		
Brachymesia furcata (Hagen, 1861)	ALT; SZS; SBV; SFA; XGL	3; 65; 72
Brechmorhoga nubecula (Rambur, 1842)	SFA	65
Dasythemis mincki (Karsch, 1890)	ANG; CXS; CZS; POA; SZS; SBV; SFA; SFP	1; 2; 8; 16; 31; 37; 59; 60; 62; 65; 66; 72
Dasythemis venosa (Burmeister, 1839)	SFA	65
Diastatops intensa Montgomery, 1940	ALT; CDS; CNS; CDC; CDL; LJD; MLV; MCR; PLT; POA; RGD; STM; SBV; SFA	11; 15; 16; 7; 59; 60; 63; 65; 68; 70
Diastatops obscura (Fabricius, 1775)	SZS; ALT	2; 65
Dythemis velox Hagen, 1861	SZS	1
Dythemis nigra Martin, 1897	MTA; SFA; SVS	65; 72
Erythemis attala (Selys in Sagra, 1857)	CDL; PLT; POA; RGD	16; 63; 66
Erythemis credula (Hagen, 1861)	ART; CDC; TRS	New Record
Erythemis peruviana (Rambur, 1842)	ALT; CDC; CDL; CZS; MLV; PLT; QRI; RGD; RDS; SZS; SCS; SFA	3; 55; 59; 63; 65; 66; 72
Erythemis plebeja (Burmeister, 1839)	BMJ; CDL; LJD; MDS; PLT; RGD; SCS; SFA; TQI	55; 9; 60; 63; 65; 66; 72
Erythemis vesiculosa (Fabricius, 1775)	ALT; CDL; MLV; POA; QRI; RGD; SBV; SFA	16; 63; 65
Erythrodiplax atroterminata Ris, 1911	ALT; ANG; AVZ; CDS; CDL; CDR; CZS; MLV; MDS; MTA; PLT; POA; QRI; RGD; RCS; RDS; STM; SBV; SFA; SFP; SPS; SVS; SLD; TMD; VMA; SMS; SZS; URG	4; 15;16; 31; 37; 54; 55; 57; 59; 60; 62; 63; 65; 66; 72
Erythrodiplax anomala (Brauer, 1865)	CXS; SZS	8
Erythrodiplax avittata Borror, 1942	CDC; TMD; XGL	New Record
Erythrodiplax basalis (Kirby, 1897)	POA	16
Erythrodiplax chromoptera Borror, 1942	CHS; CDL; POA; RGD; STM	15; 16; 37; 60; 63
Erythrodiplax connata (Burmeister, 1839)	CDC; SFP; TCH	3; 37; 60; 66
Erythrodiplax diversa (Navás, 1916)	SFP	60
Erythrodiplax fusca (Rambur, 1842)	ARM; AVZ; CDL; CXS; CLN; CZS; ENC; ETL; LJD; PLT; RGD; STM; SFP; SLD; SZS; TQI	8; 15; 37; 54; 57; 59; 60; 62; 63; 66
Erythrodiplax hyalina Förster, 1907	ALT; ARM; CDL; CLN; CZS; ENC; ETL; LJD; MLV; PLT; RGD; SBV; SFA; SFP; SLD; TQI; URG	6; 37; 57; 59; 60; 62; 65; 72
Erythrodiplax juliana Ris, 1911	BMJ; STM; SGL; SZS	3; 15; 66
Erythrodiplax latimaculata Ris, 1911	MLV; MTA; POA; SFA; SVS; SZS	16; 72
Erythrodiplax lygaea Ris, 1911	SFA	65
Erythrodiplax melanorubra Borror, 1942	ALT; ANG; MLV; SBV; SFA; SSE; URG	16; 65; 72
Erythrodiplax media Borror, 1942	ALT; ARM; ART; AVZ; BRS; CDS; CDC; CDL; CDR; CLN; CZS; ENC; ETL; MLV; MDS; MTA; PLT; POA; QRI; RGD; RCS; RDS; STM; SBV; SFA; SFP; SVS; SLD; TQI; TRS; TMD; XGL; SGL; SMS; SSE; URG	15; 16; 37; 55; 57; 59; 60; 62; 63; 65; 72
Erythrodiplax minuscula (Rambur, 1842)	GVI; SAP	1
Erythrodiplax nigricans (Rambur, 1842)	ALT; ART; CDS; CDL; GIB; MLV; PLT; POA; RGD; STM; SBV; SFA; SVS; SZS; URG	3; 15; 16; 37; 60; 63; 65; 66; 68; 72
Erythrodiplax ochracea (Burmeister, 1839)	PLT; SFP	60; 66

Continuation Table 2.

Species	Municipalities	References
Erythrodiplax paraguayensis (Förster, 1905)	ALT; ANG; ART; AVZ; BMJ; CDS; CDC; CDL; CDR; MLV; GLO; PLT; QRI; RGD; SBV; SFA; SVS; TRS; TMD; VMA; XGL; SGL; SMS	37; 60; 63; 64; 65; 66; 72
Erythrodiplax umbrata (Linnaeus, 1758)	ALT; BMJ; CDC; URG	37; 60; 66; 72
Gynothemis venipunctata Calvert, 1909	SFA	65
Idiataphe longipes (Hagen, 1861)	SBV; SFA	65
Libellula herculea Karsch, 1889	SFP	65
Macrothemis heteronycha (Calvert, 1909)	ALT; MLV; QRI; SBV; SFA	65; 72
Macrothemis imitans Karsch, 1890	ANG; CDS; DRR; MLV; MDS; SCS; STM; SBV; SFA; URG	15; 65; 72
Macrothemis lutea Calvert, 1909	MLV	65
Macrothemis marmorata Hagen, 1868	ALT; CDS; CXS; MLV; PLT; SBV; SFA; SFP; SZS	1; 4; 8; 62; 65; 69
Macrothemis musiva Calvert, 1898	SZS	1; 4
Macrothemis hemichlora (Burmeister, 1839)	SZS	4
Miathyria marcella (Selys in Sagra, 1857)	ALT; CDL; MLV; MDS; PLT; POA; RGD; SCS; SBV, SZS	1; 4; 31; 35; 63; 65; 66; 72
Miathyria simplex (Rambur, 1842)	RS	35
Micrathyria artemis Ris, 1911	SFP	62
Micrathyria catenata Calvert, 1909	CDL; PLT; RGD	63
Micrathyria hesperis Ris, 1911	ALT; ANG; AVZ; CDR; JGR; MLV; PLT; POA; RGD; SFA	26; 28; 37; 60; 65
Micrathyria hypodidyma Calvert, 1906	CDL; PLT; POA; RGD; SZS	1; 3; 16; 28; 37; 60; 63; 66
Micrathyria laevigata Calvert, 1909	RS	60
Micrathyria longifasciata Calvert, 1909	MLV; POA; SFA	16; 65; 72
Micrathyria ocellata Martin, 1897	ART; AVZ; BRS; CDC; CLN; CZS; LJD; MLV; MÇM; RDS; SCS; SBV; SFA; SVS; SLD; TQI	54; 55; 57; 59; 60; 72
Micrathyria pseudeximia Westfall, 1992	CDL; PLT	63
Micrathyria pseudhypodidyma Costa, Lourenço & Viera, 2002	RGD	37; 63
Micrathyria spinifera Calvert, 1909	RS	28
Micrathyria spuria (Selys, 1900)	RDS; SBV; SFA; SVS; SMS	65; 72
Micrathyria stawiarskii Santos, 1953	ALT; AVZ; CDL; JGR; PEL; RGD; RDS; STA; SVS	26; 28; 37; 60; 63; 72
Micrathyria tibialis Kirby, 1897	ALT; CDS; CDL; CZS; LJD; MLV; MÇM; RGD; SBV; SFA; SLD; TQI; URG	1; 3; 37; 54; 55; 59; 60; 63; 65; 72
Micrathyria ungulata Förster, 1907	RS	28
Nephepeltia berlai Santos, 1950	ANG	New Record
Nephepeltia flavifrons (Karsch, 1889)	ALT; ART; GIB; IBE; MLV; MDS; PLT; QRI; RDS; SFA; TRS; TMD; XGL; SMS	1; 3; 7; 56; 60; 65; 72
Oligoclada laetitia Ris, 1911	CZS; POA; STM; SFP; SLD; VMA	6; 15; 37; 44; 54; 59; 60; 62
Orthemis aequilibris Calvert, 1909	ALT; MLV; SFA	65
Orthemis ambinigra Calvert, 1909	CDS; CDL; MLV; PLT; POA; QRI; RGD; SFA	37; 50; 60; 63; 65
Orthemis attenuata (Erichson in Schomburgk, 1848)	ALT; MLV;	65
Orthemis cultriformis Calvert, 1899	CNS	8
Orthemis discolor (Burmeister, 1839)	ALT; ANG; AVZ; CDS; CZS; DRR; LJD; MLV; QRI; SCS; SBV; SFA; SFP; SLD; SMS; URG	54; 55; 59; 60; 62; 65; 72
Orthemis ferruginea (Fabricius, 1775)	CZS; ENC; POA; STM; SFP; TQI	15; 16; 54; 57; 59
Orthemis nodiplaga Karsch, 1891	CDL; MTN; PLT; POA; RGD; STM; SZS; TMD; VMA	2; 15; 31; 37; 60; 63; 66; 68; 69

Continuation Table 2.

Species	Municipalities	References
Pantala flavescens (Fabricius, 1798)	ALT; BRS; CDS; CDC; CDL; CDR; CZS; ETL; LJD; MLV; MDS; MTD; PLT; POA; RGD; RCS; SCS; SBV; SFA; TQI; TEA; TMD; URG	1;4; 16; 31; 35; 54; 55; 57; 59; 60; 63; 65; 66; 71; 72
Perithemis icteroptera (Selys in Sagra, 1857)	ALT; JGR; MLV; POA; QRI; RPD; SCS; SFP	16; 27; 54; 55; 59; 60; 62; 65; 66; 71; 72
Perithemis mooma Kirby, 1889	ALT; ANG; AVZ; CDS; CDC; CDL; CZS; JGR; MLV; MDS; MTD; PLT; POA; QRI; RGD; RPD; RDS; STM; SBV; SFA; SFP; SPS; SVS; TRS; XGL; SMS; SSE; SZS; URG	2; 15; 23; 27; 54; 59; 62; 63; 65; 66; 71; 72
Perithemis domitia (Drury, 1773)	SZS	2
Planiplax erythropyga (Karsch, 1891)	QRI; SZS; URG	3; 37; 60; 72
Tauriphila argo (Hagen, 1869)	CZS; MLV; RDS	35; 54; 59; 60; 65; 72
Tauriphila risi Martin, 1896	CDL; PLT; RGD; SZS	1; 4; 63; 65
Tauriphila xiphea Ris, 1913	CDL; PLT; RGD	63
Tholymis citrina Hagen, 1867	MLV	65
Tramea abdominalis (Rambur, 1842)	LJD; MLV	59; 60; 65
Tramea binotata (Rambur, 1842)	ALT; AVZ; CDS; MLV; SBV; SFA; XGL	1; 35; 65; 72
Tramea calverti Muttkowski, 1910	CHS	20
Tramea cophysa Hagen, 1867	ALT; ART; CDS; CDC; CDL; CZS; ENC; LJD; MLV; PLT; POA; QRI; RGD; RPD; SCS; SZS; STM; SFA; SVS; SLD; VMA; URG	4; 10; 15; 16; 20; 31; 35; 43; 54; 59; 60; 63; 65; 66; 71; 72

We found seven species that had not yet been recorded for Rio Grande do Sul. *Erythemis credula*, *Erythrodiplax avittata* and *Progomphus intricatus* were recorded for native areas of coastal plain of RS. The coastal plain is the region which suffers the most due to the advance of urbanization, and protected areas are scarce in this region too. All species were recorded in places with high degree of preservation, as *Erythrodiplax avittata* sampled in Itapeva Conservation Unit. *Lestes dichrostigma, Oxyagrion sulmatogrossense* and *Nephepeltia berlai* were recorded on montane forest (600 m), in the center-east portion of state (Perau de Janeiro, Arvorezinha, Anta Gorda). Lastly, *Oxyagrion chapadense* was recorded for seven municipalities of the Pampa bioma (see Table 2). These new records emphasize the need of faunal studies for the state and remark the risk of species loss due to the reduction of natural areas and the lack of areas under protection.

In the list, we verified the presence of one species mentioned as endemic to the Pampa biome *Cyanallagma bonariensis* (Ris, 1918). Until recently, there was no record of this species for Brazil, only for localities in Argentina and Uruguay. However, Pires et al. (2018), in a recent work about future climate changes on species distribution in Pampa region, also made its first record, which confirms its endemism for the Pampa region. *Cyanallagma corbeti* described in 2009 by Costa, Santos & Souza occurred only in high altitude fields of São Francisco de Paula and Bom Jesus, being probably endemic from this region. We detected also that some species mentioned as endemic of Pampa biome, were found in Atlantic forest as well, such as *Tibiagomphus noval* (Rodrigues, 1985) and *Castoraeschna decurvata* Dunkle & Cook, 1984 (Pires et al., 2018).

In conclusion, the order Odonata in Rio Grande do Sul State still needs special attention, mostly in non-explored parts of the Pampa Biome and Seasonal forest. These biomes are under intense pressure and species may quickly disappear. Additionally, for most species, the conservation status is not known (IUCN Red List 2018), which makes it difficult to adopt environmental policies. Thus, we highlight the need of information regarding diversity, distribution, biogeography and ecology of species since biodiversity inventories play a key role in the development of conservation strategies (Renner et al. 2017).

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Authors’ Contributions

Marina Schmidt Dalzochio: Contribution to data collection and species id. Substantial contribution in the concept and design of the study. Contribution to critical revision, adding intelectual content.

Samuel Renner: Contribution to data collection and species id. Contribution to manuscript preparation. Contribution to critical revision, adding intelectual content.

Cleber Sganzerla: Contribution to data collection and species id.

Gabriel Prass: Contribution to data collection and species id.

Gerson Junior Ely: Contribution to data collection and species id.

Luana Carla Salvi: Contribution to data collection and species id. Contribution to manuscript preparation. Contribution to critical revision, adding intelectual content.

Norton Dametto: Contribution to data collection and species id.

Eduardo Périco: Contribution to data collection and species id. Substantial contribution in the concept and design of the study. 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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Movement patterns of humpback whales (*Megaptera novaeangliae*) reoccupying a Brazilian breeding ground

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Abstract: The population of humpback whales from breeding stock A is increasing, and little is known about the routes used by humpbacks that move north of the main calving area of Brazil, the Abrolhos Bank. The aim of this study was to describe the movements of humpback whales in a reoccupation wintering area (Serra Grande, Bahia state, Brazil) based on land-based surveys to test if movement patterns change during the season and between years, due to group composition, behavioral state, and distance to the coast. The mean leg speed of the groups sighted was 6.88 (±2.92) km/h, and leg speed was positively correlated with distance to the coast. There was an increase in leg speed and distance to the coast with increasing number of escorts in the groups with calves. The mean linearity value for group trajectory was 0.81 (±0.19) and the mean reorientation rate was 25.72 (±19.09) °/min. We observed a predominance of trajectories heading south throughout the study. Groups exhibiting more erratic movements early in the season, and groups moving south showed more linear trajectories than groups moving north, indicating the beginning of their migration back to the feeding grounds. Energy conserving strategies and social context affect the movements of humpback whales in Serra Grande, resulting in the observed patterns of the reoccupation of available and suitable habitat north of Abrolhos. Thereby, special attention should be given managing activities with the potential to disturb or displace whales using the region to calve and breed.
Keywords: *calving area, distance to the coast, land-based station, linearity, speed.*

Padrões de movimento das baleias-jubarte (*Megaptera novaeangliae*) reocupando uma área reprodutiva brasileira

Resumo: A população das baleias-jubarte do estoque reprodutivo A está aumentando, e pouco se sabe sobre as rotas usadas pelas baleias que se movem a norte da maior área de reprodução do Brasil, o Banco dos Abrolhos. O objetivo deste estudo foi descrever os movimentos das baleias-jubarte em uma área de reocupação (Serra Grande, estado da Bahia, Brasil) através do monitoramento por ponto fixo para testar se os padrões de movimento mudam ao longo da temporada e entre os anos, devido à composição de grupos, estado comportamental, e distância à costa. A média da velocidade da pernada dos grupos observados foi de 6,88 (±2,92) km/h, apresentando uma correlação positiva com a distância à costa. Houve um aumento da velocidade da pernada e da distância à costa com o aumento do número de escortes nos grupos com filhotes. A linearidade média das trajetórias dos grupos foi de 0,81 (±0,19) e a taxa média de reorientação foi de 25,72 (±19,09) °/min. Observamos uma predominância das rotas com rumo para sul ao longo do estudo. No início da temporada, os grupos apresentaram movimentos mais erráticos, e os grupos se deslocando para sul apresentaram trajetórias mais lineares que grupos se movendo para norte ou para outras direções, indicando o início da migração de volta ao seu sítio alimentar. Estratégias para economizar energia e o contexto social afetam o movimento das baleias-jubarte em Serra Grande, resultando nos padrões observados de reocupação de habitat disponível e adequado a norte de Abrolhos. Desse modo, uma atenção especial deve ser dada para a gestão de atividades com o potencial para perturbar ou deslocar as baleias que usam a região para se reproduzir.
Palavras-chave: *berçário, distância à costa, linearidade, ponto fixo, velocidade.*

Introduction

Humpback whales (*Megaptera novaeangliae* Borowski, 1781) are migratory except for the Arabian Sea population (Mikhalev 1997), reproducing in warmer waters (Rasmussen et al. 2007) of low latitudes during the winter and feeding in cooler waters during the summer (Dawbin 1966; Clapham 2000). In calving areas, humpback whales often concentrate near the coast, islands and reef banks (Dawbin 1966; Herman 1979; Clapham 2009). Proximity to the coastline ensures shallow water (Ersts & Rosenbaum 2003; Cartwright et al. 2012) and protection from predators (Corkeron & Connor 1999), providing ideal conditions for whales to calve and nurse (Whitehead & Moore 1982; Craig et al. 2014).

Humpbacks have different movement patterns depending on their location and the phase of their life cycle (breeding, migrating or feeding). They tend to move at a lower speed in both feeding and breeding areas than in migratory corridors, even for mother and calf groups (Lagerquist et al. 2008; Kennedy et al. 2014), and also more erratically in feeding areas than in migratory corridors (Zerbini et al. 2006). This may be related to search/encounter patterns when mating or feeding. However, Dalla Rosa et al. (2008) suggested that variation in speed of humpback whales may occur due to individual behavioral patterns than to common patterns that differ among the areas or life stages. Tyack & Whitehead (1983) observed increased speed and straighter tracks with increasing group size. Coastline orientation seems to be a key factor that influences the trajectories of humpback whales at least for same populations (Dawbin 1956; Findlay et al. 2011), where whales move following its orientation.

Humpback whales from the breeding stock A (BSA) (IWC 2005) migrate from their feeding area off South Georgia and South Sandwich Islands (Engel & Martin 2009; Zerbini et al. 2011) to breed along the Brazilian coast between June and November (Martins et al. 2001). The main breeding area of humpback whales in Brazil is the Abrolhos Bank (Andriolo et al. 2010). Nevertheless, the population is increasing (Bortolotto et al. 2016; Pavanato et al. 2017) and areas used before the whaling period are being reoccupied (Rossi-Santos et al. 2008).

While migration routes used by humpback whales leaving the Brazilian breeding ground towards the feeding areas are almost a straight course of 170º (Zerbini et al. 2006; Horton et al. 2011; Zerbini et al. 2011), information on the small-scale movement patterns of humpbacks in the wintering grounds is still scarce. The understanding of the influence of environmental features and social context on the behavior of humpback whales could explain their fine-scale movement variability (Kavanagh et al. 2016). It may shed light on questions related to individual/group small-scale movement, and on within-season movement, especially with the expansion of the Brazilian breeding ground. The availability of appropriate habitat may influence the distribution of humpback whales that sometimes can only be verified at local and fine-scales (Rasmussen et al. 2007).

Serra Grande still has low anthropic activity and exhibits similar geomorphological characteristics to the remaining northeast and east coast north of Abrolhos, where the increased density of humpback whales has been observed (Bortolotto et al. 2017), contrasting with the extensive continental shelf of the main breeding area. This study will allow us to verify the plasticity of the population from BSA to the availability of habitat with different characteristics as the number of humpback whales is increasing.

Land-based surveys offer methods that allow the tracking of humpback whales without interfering with their behavior (Würsig et al. 1991; Sagnol & Reitsma 2014). This methodology has been used to study habitat use (*e.g.* Smultea 1994; Danilewicz et al. 2016), the effect of whale watching (*e.g.* Williams et al. 2002; Schaffar et al. 2009), and group movement patterns (*e.g.* Best et al. 1995; Bailey & Thompson 2006). It has been noted that movement parameters such as speed and net course may be measured in land-based surveys with the same accuracy as boat surveys (Godwin et al. 2016).

The aim of this study was to characterize the movements of humpback whales in the Serra Grande region. It will provide a baseline information for movement patterns in the reoccupation areas that present the main features of the Brazilian coast north of the Abrolhos Bank, in order to test the hypothesis if spatio-temporal and behavioral factors influence on the movement patterns of the whales in this wintering area.

Material and methods

1. *Study area*

Data were collected from a land-based observation station in Serra Grande (14°28'30" S; 39°01'50" W), Bahia state, Northeastern Brazil. The platform is 93 m above the sea level and is 315 m from the shoreline. The radius of observation from the land-based station was 15 km, between azimuths 70 and 184º, covering a surface area of 224.5 km^2 (Figure 1). The orientation of the coastline, the presence of rocky coast and vegetation reduced the monitoring of northern areas.

The study area is on the narrowest continental shelf of the Brazilian coast (Amorim et al. 2011; IBGE 2011), reaching 100 m depth at approximately 14 km from the coastline (Figure 1). Depth increases with increasing distance to the coast (Gonçalves et al. 2018). The dominant southward Brazil Current occurs from October to March, and the northward North Brazil Undercurrent occurs from April. However, there is an inversion of the predominant current from north to south from August (Rezende et al. 2011). These flows close to the shelf break are expected to interact with the shelf currents due to its narrow width (Amorim et al. 2011).

2. *Visual surveys*

The visual monitoring of humpback whales was conducted during the breeding season from July to October in the years 2014 and 2015. Data were collected during daytime between 07:22 am and 04:40 pm when weather conditions were favorable ensuring visibility and during sea state below or equal to 4 on the Beaufort scale. The focal animal sampling methods were used when solitary individuals were sighted, and a focal group approach was used when 2 or more individuals were sighted (Mann 1999). Focal follows lasted until the whales moved out of the study area or if visibility became an issue, affecting the quality of observation (Morete et al. 2003). In the presence of more than one group in the area, the one chosen was based on the surface and active behavior of the individuals and then by the proximity of the groups to the coast. A total station TOPCON ES105 with 5' of precision and 30-power monocular magnification was used to track the groups. Group size and composition, time, angles and behavioral states were recorded. Events of individuals merging and/or splitting of the groups

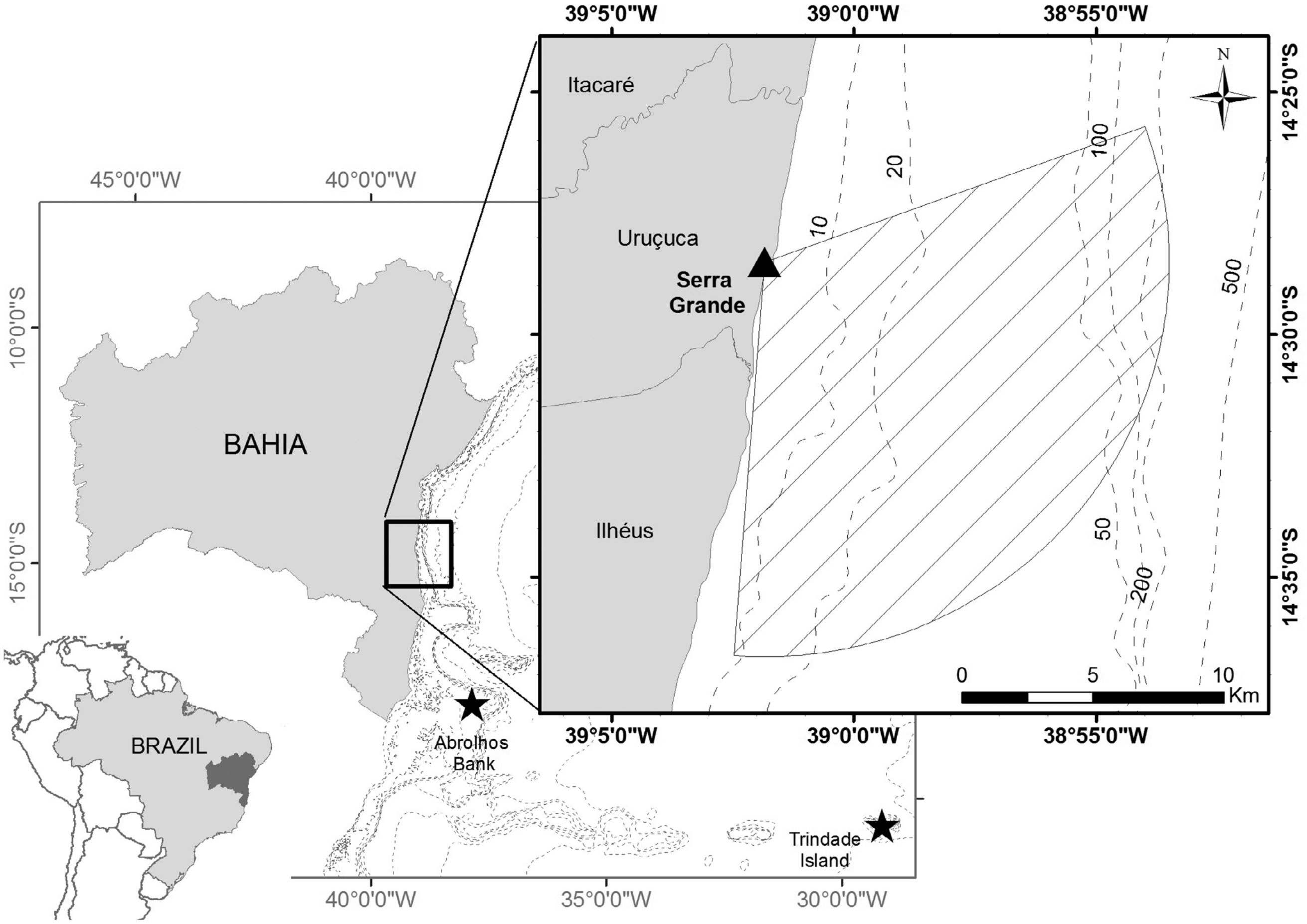

Figure 1. Serra Grande study area located in North-eastern Brazil where surveys occurred from a land-based observation station at a height of 93 m with 15 km radius, covering 224.5 km^2 (shaded area).

were also recorded. In those cases, focal sampling continued with the group selected for tracking which was considered a new group (Best et al. 1995; Barendse et al. 2010). In the presence of more than one individual, the angles were taken from the leading individual (directing the movement) or from the calf when present.

The land-based team was comprised of two or three observers: the principal observer (the same person throughout the study) who operated the total station; a second observer who registered data; and a third observer, who followed the group with the aid of 7x50 binoculars, checking if any other group appeared in the area or approached the focal group. Wind speed and direction, cloud cover and sea state on the Beaufort scale were registered every 30 minutes or when weather conditions changed. The presence of boats in the region is very low so its occurrence was disregarded.

3. *Definitions*

A group was classified either as a single individual or an association of individuals based on a distance of up to 100 m from each other and if all individuals were moving in the same general direction with a coordinated behavior (Whitehead 1983; Morete et al. 2008).

We considered group composition as 1AD, when a solitary adult was observed; dyad, a group of two adults; multiple group, three or more adults, MOC, a group of mother and calf; MOCE, when mother and calf were escorted by another adult; and MOCE/+, a group of mother, calf and two or more escorts (Morete et al. 2007a). Due to the observational distance from the groups, we considered two age classes: adult and calf.

Behavioral states were classified as resting - when the whale(s) remained on the surface of the water, exposing the dorsal fin and with no apparent movement; swimming - when the whales(s) alternated from being submerged and on the surface; and; active - when aerial displays occurred, as breaching, tail and pectoral slaps (Morete et al. 2003; Morete 2007).

4. *Spatial analyses*

A total station provides horizontal and vertical angles to an object, where the first angle is between the object and a known reference point, and the second between the observer and the object. These angles allowed the estimation of the distance between the observer and the object (Gailey & Ortega-Ortiz 2002). Universal Transverse Mercator (UTM) coordinates from the total station and the reference point were measured with millimetric precision by Global Navigation Satellite System positioning. The orthometric altitudes of these points were determined by Geoidal MAPGEO 2010 model (Monico 2008). UTM coordinates (E, N) of all the measured points were calculated with the height of the total station and tidal variation through trigonometric equations (Gonçalves 2017). The horizontal distances (calculated from data collected by the total station) were transformed for spherical distances considering the curvature of the earth (Vanicek & Krakiwsky 1996).

Distances between the group and coastline were calculated using the distances to the meridians, taking into consideration the first position sampled of the group using Google Earth to have more precise values due to the higher resolution of the maps.

5. *Movement analyses*

We only considered those tracks for which four positions were sampled for at least 10 min, and with group composition identified. The following parameters were calculated:

Leg speed: mean of speeds calculated between two consecutive positions divided by the distance between two positions and the time taken to travel between them (Barendse et al. 2010). Speeds above 30 km/h were not considered since the maximum speeds recorded for humpback whale vary from 18 km/h (Findlay & Best 1996) to 27 km/h (Zenkovich 1937 apud Winn & Reichley 1985).

Net speed: calculated by dividing the linear distance between the first and last positions (net distance) and the total time of the track (Barendse et al. 2010; Findlay et al. 2011).

Linearity: index calculated by dividing the total distance between the first and last positions (net distance) by the sum of the distances between each position (cumulative distance) of the track (Williams et al. 2002; Gailey et al. 2007; Barendse et al. 2010). The values range from 0 to 1 with values closer to 0 representing more circular paths and values closer to 1 represent more direct trajectories (Schaffar et al. 2009; Burns 2010).

Reorientation rate: calculated as the sum of all absolute values of change of bearing between two consecutive positions, dividing by the total time of the track (Smultea & Würsig 1995; Gailey et al. 2007). It represents the pattern of bearing that changes along a track line, in which higher values of reorientation indicate more erratic paths (Burns 2010).

Net course: the true bearing in degrees considering the first and last positions of the track (Best et al. 1995; Barendse et al. 2010; Findlay et al. 2011). We divided the net course into three classes: north (from 320º to 50º), south (from 140º to 230º) and other directions (between 50º and 140º and between 230º and 320º), considering the coastline orientation.

Martins (2012) equations were used to calculate all parameters cited above. When we observed more than one behavioral state during the same track, leg speed and reorientation rate were calculated for each behavioral state separately.

6. *Statistical analyses*

Active groups with more than one individual were excluded (n=16), except for analyses involving exclusively net course. The only exception to include active groups with more than one individual in the analyses was mother and calf groups when it was possible to follow the calf during the entire sighting. The reason to exclude the other groups was to avoid confusion in groups of several individuals performing aerial activities simultaneously. We checked whether assumptions of normality and equal variance were met before performing statistical tests. The distribution of variables in groups for t-Tests and errors in groups for Analyses of Variances (ANOVA) were visually inspected and moderate deviations were tolerated given the robustness of these tests. Differences in variances between groups were tested using Levene tests and the size of the ratio between the highest and the smallest variance.

Groups: To test whether the distances where groups were observed varied with the group composition and the period of the season an ANOVA followed a Tukey honest significant difference (HSD) test were used for each variable.

We considered three periods of the season (initial, middle and final, Table 1) due to differences in migratory timing of sexes and reproductive status of the groups and their behavioral differences associated with each period (Morete et al. 2007b). As the number of individuals varies throughout the season and the years, we calculated the day when the number of individuals would be higher for each season using a segmented regression model for the number of individuals observed throughout the season and considered it as the center of the middle period (Gonçalves et al. 2018).

Movement parameters: To test whether there was a difference in parameter values between the two years we used t-Tests. To test whether the movement parameter values were influenced by group composition, the season period, behavioral state, and net course class, we used ANOVAs followed by Tukey honest significant difference (HSD) tests for each variable analyzed. For net course, we used a circular variance analysis, and to check if the movement of humpback whale groups presented circular uniformity throughout the study we used the Rayleigh's test (Zar 1974). Statistical analyses were run in R 3.0.2 (R Development Core Team) and in PAST 3.10 (Paleontological Statistics).

Results

1. *Research effort*

We monitored 125 humpback whale tracks with a total of 2240 positions during 67 days in the field. Minimum and maximum distances tracked were 0.21 km and 10.59 km respectively, with a mean tracked distance of 2.88±2.20 km per focal.

The total time of group tracking was 61.4 h for both years and varied throughout the season due to the lower number of whales observed and bad weather conditions in the initial period. Total tracking time was 10.3 h in the initial period, 25.7 h in the middle period and 25.4 h in the final period. Focal time ranged from 0.16 h to 1.93 h (x= 0.49±0.34 h). The focal time for each group composition was: 2.5 h for solitary animals, 6.2 h for dyads, 17.3 h for multiple groups, 21.5 h for MOCs, 5.7 h for MOCE, and 8.2 h for MOCE/+ groups (Table 2).

2. *Groups*

The most frequently tracked group types were those with calves (60.80%), followed by multiple groups (19.20%), dyads (12.80%) and solitary individuals (7.20%). Within groups with calf, MOC represents 60.52%, MOCE 22.37% and MOCE/+ 17.11%. A single group of 2 adults and 2 calves was observed but not considered for the analyses because it was not possible to distinguish which calf would be used for tracking.

Mean distance from the coast of groups without calf was significantly greater than for groups with a calf (t=-6.4417, df=114.07, p<0.001, Table 3). Within groups with a calf, we found significant variation in the distances from the coast of the groups sighted (F=11.32, df=73, p<0.001). Mean distance from the coast of MOC was significantly lower than MOCE (p<0.05) and MOCE/+ (p<0.001) groups, and no

Table 1. Beginning and end dates of initial, middle and final periods considered for each year in the study in Serra Grande (Bahia state, Brazil). The middle period has 41 days.

Year	Initial	Middle	Final
2014	11 July-2 August	3 August-12 September	13 September-31 October
2015	20 July-14 August	15 August-24 September	25 September-25 October

Table 2. Number of effort days and duration of the focal follows of groups along the periods of the season in the study area in Serra Grande (Bahia state, Brazil). Total duration of focal follows in hours and number in parentheses for each group type. 1AD = one adult, dyad = two adults, multiple = three or more adults, MOC = mother and calf, MOCE = mother and calf and one escort, MOCE/+ = mother and calf and two or more escorts.

Year		Period of the season			Total
		Initial	Middle	Final	
2014	Field effort	11	10	16	37
	1AD tracks	-	-	0.6 (2)	0.6 (2)
	Dyad tracks	0.2 (1)	0.9 (2)	0.8 (2)	1.9 (5)
	Multiple tracks	2.7 (5)	2.5 (2)	2.1 (3)	7.3 (10)
	MOC tracks	-	1.6 (3)	9.1 (15)	10.7 (18)
	MOCE tracks	-	0.7 (2)	1.1 (3)	1.8 (5)
	MOCE/+ tracks	0.4 (1)	0.4 (1)	1.1 (3)	1.9 (5)
2015	Field effort	7	13	10	30
	1AD tracks	0.9 (3)	0.5 (2)	0.5 (2)	1.9 (7)
	Dyad tracks	1.9 (4)	1.8 (6)	0.6 (1)	4.3 (11)
	Multiple tracks	1.9 (3)	5.2 (7)	2.9 (4)	10.0 (14)
	MOC tracks	-	6.1 (17)	4.7 (11)	10.8 (28)
	MOCE tracks	-	3.4 (8)	0.5 (1)	3.9 (9)
	MOCE/+ tracks	2.3 (3)	2.6 (5)	1.4 (3)	6.3 (11)

significant difference in distance from the coast was found between MOCE and MOCE/+ (p=0.17, Table 3). We observed significant variation in the distances from the coast of the groups among periods of the season (F=21.25, df=122, p<0.001), with a decrease in distance as the season progressed (Table 3). There was a significant difference in group sighting distances from the coast between the initial and middle periods of the season (p<0.001) and between the initial and final periods of the season (p<0.001). However, there were no significant differences in the distance of groups from the coast between the middle and final periods of the season (p=0.35).

Table 3. Mean values and standard deviation of distances to coast (km) where groups of humpback whales were initially observed from a land-based observation station in Serra Grande (Bahia state, Brazil) during 2014 and 2015: groups with calves and all groups between periods of the season. MOC = mother and calf, MOCE = mother and calf and one escort, MOCE/+ = mother and calf and two or more escorts.

	Mean (±SD) km
Without calf	8.38 (±2.95)
With calf	5.21 (±2.50)
MOC	4.14 (±2.53)
MOCE	6.07 (±3.30)
MOCE/+	7.84 (±1.75)
Initial period	10.05 (±1.16)
Middle period	6.12 (±3.14)
Final period	5.37 (±2.76)

MOC groups spent more time resting (46.22%) and MOCE and MOCE/+ swimming (66.46% and 80.29% respectively). Time spent resting decreased with increasing numbers of escorts. Time spent swimming increased with increasing number of escorts (Figure 2).

3. *Movement parameters*

Leg and net speed: Mean leg speed of groups sighted in Serra Grande was 6.88±2.92 km/h and mean net speed was 4.92±2.46 km/h (Table 4). Net and leg speed showed a positive correlation (r=0.72;

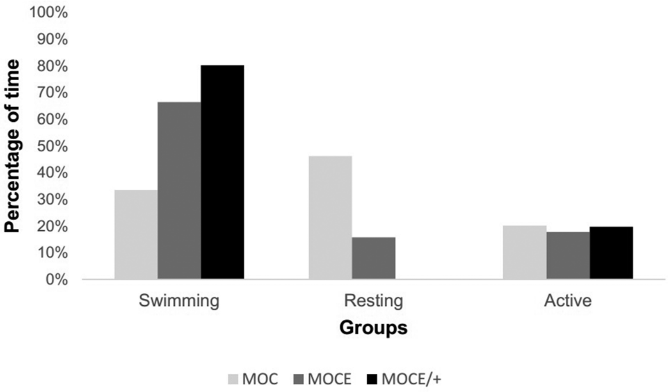

Figure 2. Percentage of time spent in the different behavioral states by humpback whale groups with a calf observed from a land-based observation station in Serra Grande (Bahia, Brazil) in 2014 and 2015. MOC = mother and calf, MOCE = mother and calf and one escort, MOCE/+ = mother and calf and two or more escorts.

$p < 0.001$). Therefore, for the remaining analyses we used the leg speed only considering the full path travelled by the group to avoid underestimating the speed values. Leg speed and distance from the coast showed a positive correlation (r=0.51; $p < 0.001$), with an increase in leg speed with increasing distance from the coast. No difference in leg speed was found between years 2014 and 2015 (t=-0.79263, df=68.834, p=0.4307).

We found significant differences in mean values of leg speed between group compositions (F=8.764, df=104, p<0.001, Figure 3a): between MOC and MOCE/+ (p<0.001), MOCE and MOCE/+ (p<0.05), and MOC and multiple groups (p<0.001). Leg speed of groups with calf increased with increasing number of escorts (Figure 3a). The decrease of leg speed across the periods of the season did not imply significant differences between them (F=2.438, df=107, p=0.092) or between net course classes (F=0.8447, df =107, p=0.4326). Mean values of leg speed varied between behavioral states (F=10.16, df=89, p<0.001) with significantly lower values in resting groups than active (p<0.05) and swimming groups (p<0.001, Figure 3b). Within groups with calf which were swimming, there was a significant difference in leg speed (F=9.357, df=42, p<0.001, Table 5): MOCE/+ moved significantly faster than MOC (p<0.001) and than MOCE (p<0.05). When resting, no difference was found in mean values of leg speed between MOC and MOCE groups (F=0.557, df=22, p=0.463, Table 5).

Linearity: Linearity mean value of groups tracked was 0.81±0.19 (Table 4) and 69.09% of humpback whale tracks showed values above 0.80. No significant difference was found between years (t=0.3466, df=78.581, p=0.7298) nor between group composition (F=0.6478. df=104, p=0.6638). Linearity values varied (F=6.44, df=107, p<0.05) and increased during periods of the season (Figure 4a): being significantly lower in the initial period than in final period (p<0.05). There was a greater linearity variation in the initial and middle periods than in the final (Figure 4a). No significant difference was found between behavioral states (F=1.031, df=89, p=0.3608). Linearity values showed a significant difference depending on net course classes (F=14.87, df=107, p<0.001) between south and north (p<0.05) and between south and other directions (p<0.001). Groups moving south presented more linear trajectories (x=0.87±0.13) than groups moving to north (x=0.73±0.25) and towards another direction (x=0.62±0.21, Figure 5).

Table 4. Descriptive statistics (mean, standard deviation, minimum, and maximum values) of movement parameters of humpback whale groups tracked from a land-based observation station in Serra Grande (Bahia state, Brazil) during 2014 and 2015.

	Mean	SD	Minimum	Maximum
Net speed (km/h)	4.92	±2.46	0.20	11.34
Leg speed (km/h)	6.88	±2.92	0.70	15.73
Linearity	0.81	±0.19	0.19	1.00
Reorientation rate (°/min)	25.72	±19.09	0.45	93.09
Net course (°)	178.22	65.34	2.48	359.59

Table 5. Mean and standard deviation values of leg speed (km/h) of mother and calf groups in swimming and resting behavior observed from a land-based observation station in Serra Grande (Bahia state, Brazil) during 2014 and 2015. MOC = mother and calf, MOCE = mother and calf and one escort, MOCE/+ = mother and calf and two or more escorts.

	Swimming	Resting
MOC	6.21 (±2.58)	3.69 (±2.37)
MOCE	6.88 (±2.22)	4.62 (±1.46)
MOCE/+	9.81 (±2.04)	-

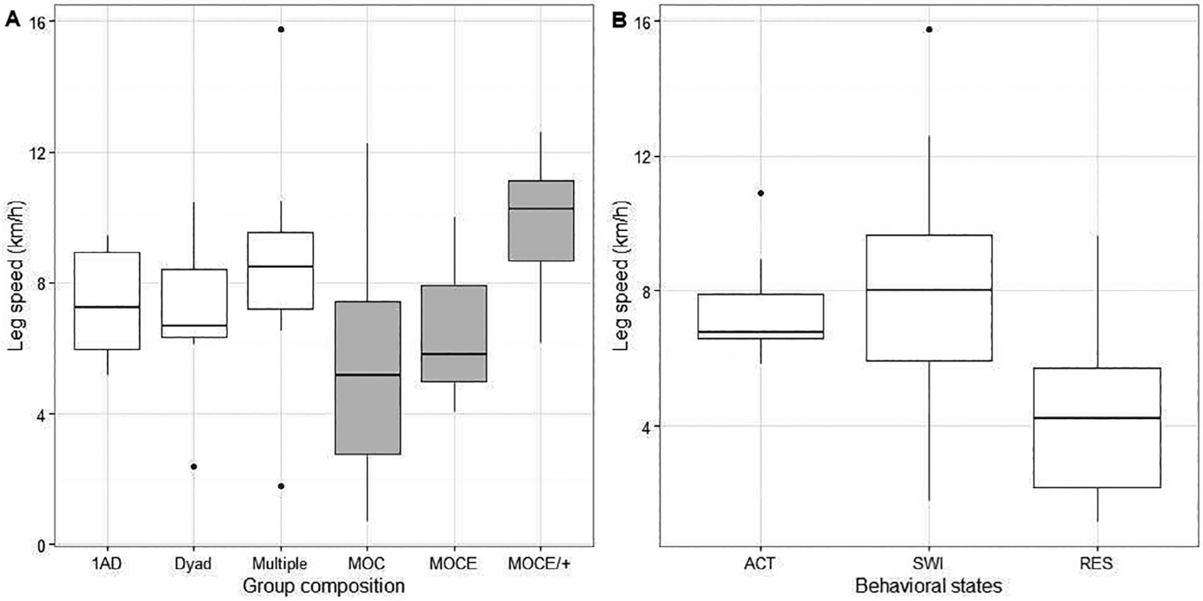

Figure 3. Box plot of leg speed among group composition (A) and among behavioral states (B) of humpback whale groups sighted from a land-based observation station in Serra Grande (Bahia, Brazil) in 2014 and 2015. 1AD = solitary individual, Dyad = two adults, Multiple = three or more adults, MOC = mother and calf, MOCE = mother and calf and one adult, MOCE/+ = mother and calf and two or more adults. ACT = active groups, SWI = swimming groups, RES = resting groups. The minimum and maximum values are represented at the extremities, the center line represents the median, the bottom line of the box is the first quartile and the upper line is the third quartile.

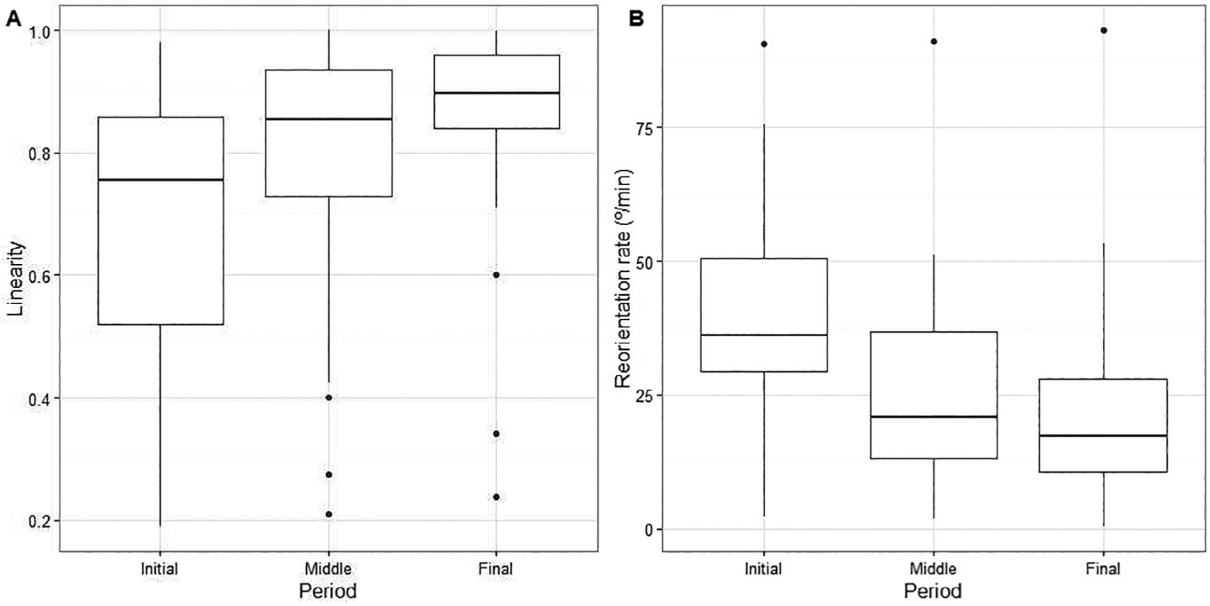

Figure 4. Boxplot of linearity (A) and of reorientation rate (B) of humpback whale groups observed from a land-based observation station in Serra Grande (Bahia state, Brazil) in 2014 and 2015 between periods of the season. The minimum and maximum values are represented at the extremities, the center line represents the median, the bottom line of the box is the first quartile and the upper line is the third quartile.

Reorientation rate: The mean reorientation rate was 25.72±19.09 °/min (Table 4). There was no significant difference between reorientation rates between 2014 and 2015 ($t=-0.4145$, $df=81.524$, $p=0.6796$) or among group composition ($F=0.641$, $df=104$, $p=0.0669$). Reorientation rate varied significantly between periods of the season ($F=6.71$, $df=107$, $p<0.05$), being higher in the initial period of the season than in the final period ($p<0.05$, Figure 4b). Reorientation rate did not change with behavioral state ($F=0.1443$, $df=89$, $p=0.8658$) nor within classes of net course ($F=2.682$, $df=107$, $p=0.073$).

Net course: The mean value of net course of observed groups in Serra Grande was 178.22±65.34º (Table 4). The null hypothesis for uniformity of movement of the groups was rejected (Rayleigh's $R=65.277$, Rayleigh's $Z = 34.054$, $r=0.522$, $p<0.001$) with south direction predominating (Figure 6) throughout the season (Figure 7). We did not find significant difference in mean values of net course between the years ($F=0.065$, $df=1$, $p=0.799$), among group compositions ($F=0.7204$, $df=5$, $p=0.7204$), behavioral states ($F=1.6782$, $df=2$, $p=0.5101$) or season periods ($F=0.9558$, $df=2$, $p=0.3873$).

Discussion

Movement patterns and information about behavioral states of humpback whales in the Brazilian wintering ground are poorly known and required to understand habitat use off Brazil (Zerbini et al. 2006; Bortolotto et al. 2017). This study showed that the movement of the groups from BSA that were observed in Serra Grande, representing similar geomorphological characteristics with the Brazilian coast northern of Abrolhos, are influenced by the orientation of the coast which guides most trajectories parallel to it. Our hypothesis of the influence of spatio-temporal and behavioral aspects on the movement patterns was supported by our results.

Most groups were sighted heading south, and as one of the limitations of studies performed through land-based stations is the geographic range of the study area (Morete et al. 2017), most whales moving north maybe were not sampled during our effort. This may be a result of variation in habitat use in this area, with whales moving north using offshore waters, out of our monitoring range, as suggested in a previous work (Gonçalves et al. 2018). Humpback whale's sightings in July and August off Trindade Island (approximately 1100 km away from the coast) (Siciliano et al. 2012) support the hypothesis that groups moving northward early in the season could travel further offshore as also observed in Australia (Jenner et al. 2001). Another explanation for the low number of groups heading north could be that they are moving north before July, but because the visual surveys were only initiated in July and the weather conditions were not always ideal, this might have affected the number of observed groups towards that direction. However, whales moving south seem to follow the coastline more closely.

Humpback whales may use the South Equatorial Current Bifurcation which reaches approximately 17ºS in July (Rodrigues et al. 2007) to travel closer to the Brazilian coast. Whales might be taking advantage of the predominant current in the region to save energy. The North Brazil Undercurrent flows north until July and is stronger further away from shore (Rezende et al. 2011). From August, the Brazilian Current predominates (Rezende et al. 2011) and it could help the whales going south. The net course of whale groups in oceanic habitats in Madagascar is influenced by the prevailing currents (Trudelle et al. 2016). Studies in larger spatial scales are needed from whales travelling from Southern feeding grounds towards the Brazilian coast, helping to understand their preferred routes.

We observed a decrease in the distance to the coast of the groups as the season progressed which should be justified by the increase in the number of calves throughout the season, which remain closer to the coast. Segregation of groups with calves close to shore in shallower waters in Serra Grande is consistent with the findings from other breeding areas (Smultea 1994; Ersts & Rosenbaum 2003; Guidino et al. 2014). The increase in distance from the coast of the groups

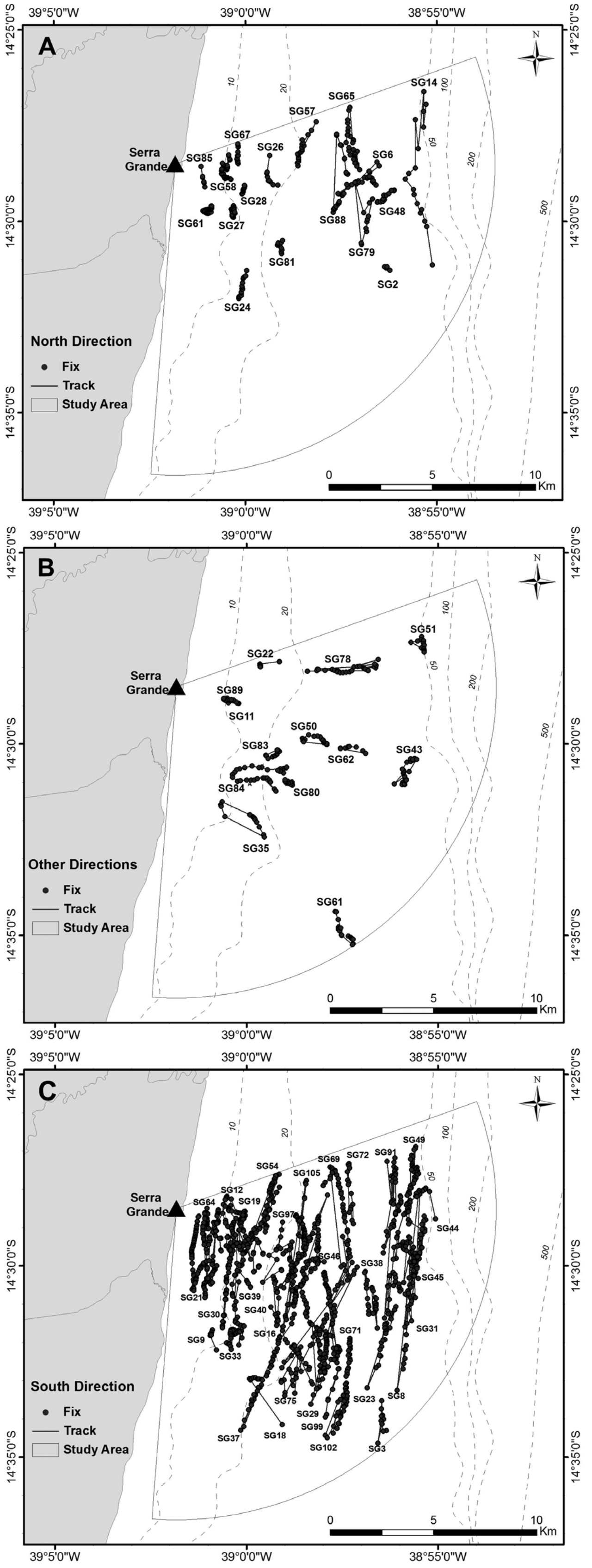

Figure 5. Tracks of humpback whale groups sighted from a land-based observation station in Serra Grande (Bahia state, Brazil) in 2014 and 2015 by the different classes of net course. North = from 320º to 50º (A), Other directions = between 50º and 140º and between 230º and 320º (B), South = from 140º to 230º (C).

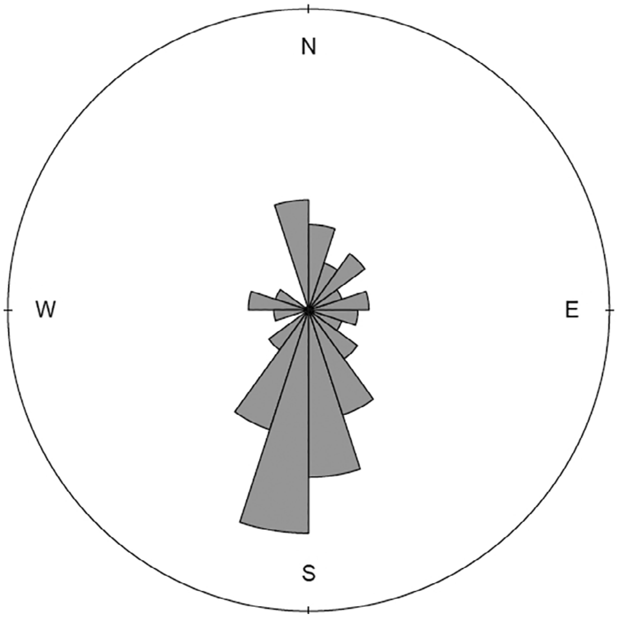

Figure 6. Frequencies of net course followed by humpback whale groups sighted from a land-based observation station in Serra Grande (Bahia, Brazil) in 2014 and 2015.

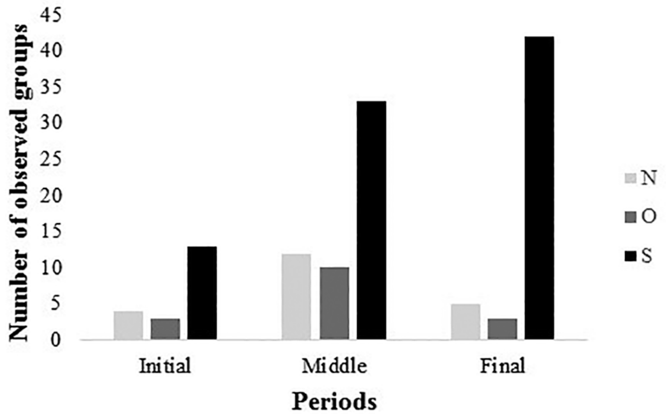

Figure 7. Frequencies of number of humpback whale groups observed from a land-based observation station in Serra Grande (Bahia, Brazil) among the periods of the season (initial, middle and final) by the different classes of net course. N = from 320º to 50º, O = between 50º and 140º and between 230º and 320º, S = from 140º to 230º.

with calves with increasing number of escorts was also observed by Félix & Botero-Acosta (2011) in Ecuador and by Craig et al. (2014) in Hawaii using two different platforms of observation, opportunistic boat sightings and from a land-based station, respectively. Concentration of mothers with calves in shallower waters may be a strategy to avoid male harassment, maternal care disturbance, and calf injury during male courting behavior (Smultea 1994; Ersts & Rosenbaum 2003; Craig et al. 2014). Calves spent a long time in sucking behavior, so mother and calf groups become more vulnerable to disturbance, and it is advantageous to stay in calmer waters (Videsen et al. 2017). Zoidis & Lomac-MacNair (2017) registered interruption of lactation in the presence of an escort. Pack et al. (2017) also observed that mothers adjust their habitat preferences according to calves age and grow in Hawaiian breeding grounds. Mother and calf groups stayed closer to shore when the calves are younger, presenting a gradual movement to deeper water with the maturation of the calves, maybe preparing them to migration (Pack et al. 2017).

The number of escorts of a mother and calf group, besides increasing their distance from the coastline, also resulted in an increase movement speed. Faster movements can lead to an increase in energy expenditure (Craig et al. 2014) for both mother and calf, and maybe a decrease in the growth rate of the calf (Braithwaite et al. 2015). In fact, we found the same general pattern of increased speeds with greater distance from shore for all group compositions as observed in Madagascar (Trudelle et al. 2016). Cartwright & Sullivan (2009) found that, despite the increase of speed related to the number of escorts, there was no significant difference in movement patterns between MOC and MOCE groups, and the same pattern was observed in Serra Grande. Those authors suggested that females choose to have a single male escort close by, as proposed by the bodyguard hypothesis (Mesnick 1996). A single escort may protect females with calves from harassment by other males when the mother-calf pair becomes more vulnerable in open waters further away from the coast (Cartwright & Sullivan 2009).

Two factors contributing to energy expenditure in humpback whales are the mean movement speed and the time spent in resting versus swimming (Braithwaite et al. 2015). Our data suggest that time spent resting behavior by MOCE compared to MOC groups was reduced to less than half, and it was not observed in MOCE/+. The opposite was observed in time spent swimming, which doubles for mothers and calves that are escorted by one male and becomes the dominant activity of MOCE/+ groups. Additionally, resting is a strategy that benefits the mother-calf pair allowing more time for nursing (Braithwaite et al. 2015). Therefore, mother and calf groups seem to adjust their movement patterns in response to social factors, such as the presence of escorts.

The mean leg speed of 6.88 km/h observed in Serra Grande was higher compared to other studies conducted from land-based stations. The mean leg speeds off the migratory corridor in west coast of Africa was 4.6 km/h (Barendse et al. 2010), and during the Southern migration on the east coast of Australia was 4.7 km/h (Burns 2010). Differences in local current speeds could be one of the reasons to explain those differences. The highest current speed in the area can reach 3.6 km/h with the mean value of 0.72 km/h (BAMIN 2011), being one of the possible reasons for the high-speed value observed, as for example of the groups in resting behavior, that could be drifting with the current. Nevertheless, the mean net speed obtained in our study was close to that found from other breeding areas (Frankel et al. 1995; Findlay et al. 2011) but higher than the ones registered in an area next to a sheltered bay, with possibly quieter waters (Barendse et al. 2010) than Serra Grande, which is an open ocean area. Environmental variables may influence the behavior of humpback whales (Kavanagh et al. 2016), and more protected waters could allow moving at lower speeds. During satellite-monitoring studies of humpback whales of BSA, Horton et al. (2017) reported speeds between 4.3 and 5.0 km/h during south-directed movements. Speed data may show variation depending on how they were obtained (Findlay et al. 2011), using land-based surveys or satellite telemetry, with more detailed data being obtained from land platforms. For example, Chittleborough (1953) reported a mean speed of 8.0 km/h during aerial surveys in Australia on short-term observations during migration.

As observed by Dalla Rosa et al. (2008), speed was dependent on the behavioral state. However, no variation in the reorientation rate and linearity was observed among behavioral states: whales in swimming behavior did not show more directional paths as initially expected. Travelling individuals could follow more direct paths than individuals resting or searching for mates, but this was not observed. Nevertheless, reorientation rate and linearity were influenced by the period of the season. Groups at the beginning of the season had more erratic movements and as the season progressed they were gradually showing more linear movements, which may be related to the approaching migration to feeding areas. We found that the groups going south showed higher linearity than other groups. Groups with less linear paths might be still searching for a mate or may be pregnant females arriving to give birth at the calving grounds. However, despite the differences in linearity, no differences were found in the speed of groups moving north or south. Individuals may maintain a similar speed regardless of the direction they follow, for example, while they are in the breeding area, some individuals may be trying to mate, and mothers may take advantage of the time before the migratory period to feed and nurse their calves.

The mean linearity value obtained here suggests limited straight movement in the study area (Barendse et al. 2010), which highlights that in spite of Serra Grande is in a low-density area (Andriolo et al. 2010), the humpback whales do not use it only as a travelling corridor. The erratic movements and the high percentage of groups with calves in resting behavior reinforce the idea that the area is a calving ground (Gonçalves et al. 2018), where mother and calves stay to nurse, and is indeed reoccupying areas previously affected by whaling (Rossi-Santos et al. 2008). The linearity values were similar to those observed in other breeding areas (Schaffar et al. 2009), lower than in migratory areas (Burns 2010) but higher than observed in some feeding areas (Stanistreet et al. 2013). Groups of humpback whales in Serra Grande showed erratic movements with great change of direction per minute and reorientation rates higher than other breeding areas such as at the calm waters of New Caledonia (Schaffar et al. 2009). There were no differences in the reorientation rate between group composition. In contrast, Noad & Cato (2007) reported that groups with calves in Australia changed direction more often during migration. Linearity patterns of groups with calves in Serra Grande were similar to those observed in the Abrolhos Bank (Bisi 2006). However, the higher speed observed for groups with calves in Serra Grande could be due to less shallow and protected waters compared to the Abrolhos Bank.

Movement patterns of humpback whales can vary between breeding and feeding areas and migratory corridors (Lagerquist et al. 2008). These patterns can also vary within breeding areas, where humpback whales can adjust their behavior depending on the characteristics of the area and energy demands. Serra Grande is an area that is used during the breeding season by mothers with calves to rest. Consequently, with the increase of the population (Bortolotto et al. 2016; Pavanato et al. 2017) and extension of the breeding areas, special attention should be given to the planned human activities in the region, such as the construction of a new offshore port approximately 10km from the study area (BAMIN 2011). Anthropogenic activities may impact humpback whales because of the lack of reinforcement of protected measures. Resting areas are particularly sensitive to disturbance because the interruption of resting behavior may lead to decreased lactation time and growth of calves (Braithwaite et al. 2015). Monitoring any changes in movement patterns, such as an increase in reorientation rate to avoid vessels and/or adoption of more linear paths and higher speeds to move away from the region would raise concerns about the potential impacts on the humpback whales in the Serra Grande region.

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Author Contributions

Maria Isabel Carvalho Gonçalves: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Renata Santoro de Sousa-Lima: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Niel Nascimento Teixeira: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Gustavo Henrique Carvalho: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Daniel Danilewicz: Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision, adding intelectual content.

Júlio Ernesto Baumgarten: 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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Inventory

Mammals of the Saracá-Taquera National Forest, northwestern Pará, Brazil

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Abstract: The Amazonian rainforest harbors one of the most diverse mammal faunas found anywhere in the world, although this fauna is still poorly known. Inventories are essential for the understanding of the biology and ecology of species, and provide basic data for conservation. Over 15 years of sampling in the Saracá-Taquera National Forest, we recorded 72 species of small (nonvolant), medium, and large mammals belonging to 30 families in 10 orders. These taxa included 29 species endemic to the Amazon biome, and 14 classified as threatened with extinction. Overall, the mammalian species richness recorded in the present study was equal to or greater than that recorded in other Amazonian studies, reflecting high levels of diversity on a biogeographic scale. This reinforces the importance of this national forest for the maintenance of the region's mammalian fauna. Some of the species were recorded in the region for the first time, thus extending their known geographic distribution.
Keywords: *Trombetas, Oriximiná, Amazonia, species richness, environmental monitoring, species list.*

Mamíferos da Floresta Nacional de Saracá-Taquera, Pará, Brasil

Resumo: A floresta amazônica abriga uma das maiores riquezas de mamíferos do mundo. Entretanto, existe pouco conhecimento sobre a fauna local. Estudos de levantamentos podem contribuir para o incremento no conhecimento sobre a biologia e a ecologia das espécies e são a base para a conservação. Ao longo de 15 anos de amostragem, nós registramos 72 espécies de pequenos, médios e grandes mamíferos pertencentes a 10 ordens e 30 famílias, das quais 29 espécies são endêmicas da Amazônia e 14 são também consideradas ameaçadas de extinção. No geral, a riqueza de mamíferos foi igual ou superior ao de outros estudos conduzidos no bioma, indicando uma alta diversidade em escala biogeográfica. Os resultados mostram a importância desses ambientes para a manutenção das espécies. Algumas tiveram seu primeiro registro para a área e outras a ampliação da sua área de distribuição.
Palavras-chave: *Trombetas, Oriximiná, Amazônia, riqueza, monitoramento ambiental, lista de espécies.*

Introduction

Recent studies (Wilson and Reeder 2005; Paglia et al. 2012) have estimated that approximately 700 mammal species occur in Brazil, of which, at least 399 are present in the Amazon biome. This is the most diverse mammalian fauna of any equivalent region in South America. Even so, the number of species found in the Amazon continues to grow, as new species are described, including primarily primates, bats and small rodents (Gualda-Barros et al. 2012; Mittermeier et al. 2013; Dalponte et al. 2014; Oliveira et al. 2016; Pavan et al., 2017; Voss et al. 2018).

Mammals play an great variety of roles in the ecosystems in which they are present (Keuroghlian and Eaton 2009; Haugassen et al. 2010; Desbiez et al. 2013). Marsupials and rodents, in particular, influence the ecological dynamics of Neotropical forests through seed predation and dispersal, as well as the dispersion of mycorrhizal fungi (Brewer and Rejmánek 1999, Mangan and Adler 1999, 2000). Larger-bodied mammals disperse over longer distances and are important in the acquisition and redistribution of nutrients and seeds within the landscape, as well as participating in the control of populations through predation (Asquith et al. 1999; Fragoso et al. 2006; Stoner et al. 2007). Given this, some mammals are considered to be bioindicator species, given their specific habitat and microhabitat preferences or requirements (Vieira and Monteiro-Filho 2003; Cullen-Jr. et al. 2005; Pardini and Umetsu 2006; Morrison et al. 2007).

Overall, 15.7% of Brazilian mammals are classified as threatened, and at least 10% of these species are found in the Amazon (Costa et al. 2005; Paglia et al. 2012; MMA 2014). The Amazon is the largest forest formation in Brazil, and one of the most biodiverse ecosystems in the world (Mittermeier et al. 2003). However, its unique and complex environments and biota are being increasingly impacted by a range of anthropogenic disturbances, which affect the populations of large numbers of species (Peres and Lake 2003; Benchimol and Venticinque 2014; Antunes et al. 2016).

The mammalian fauna of the Amazon has only been well documented at a few localities, and most inventories are preliminary and incomplete (Voss and Emmons 1996; Peres 2005). This deficiency is related primarily to the vast size of the biome, and its many remote and inaccessible areas, which are hard to reach, and extremely difficult to survey, even in a minimally adequate fashion (Silveira et al. 2003; Peres and Lake 2013). Given this, many areas should be considered to be of high priority for surveys, especially given the importance of occurrence data for other types of ecological study, and in particular conservation planning (Margules and Pressey 2000; Peres 2005).

Saracá-Taquera National Forest (STNF), located in northwestern Pará, Brazil, was created by federal decree number 98,704 of December 27th, 1989, and has an area of 429,600 ha. Most studies of the mammalian populations of this protected areas have been conducted since early 2000, with the primary aim of monitoring and evaluating the response of local populations to environmental disturbance, in particular, the loss of habitat, and assessing species resilience (Calaça 2014). The management plan of STNF (STCP 2001) listed 58 species of terrestrial, aquatic, and semiaquatic mammals. In subsequent years, however, a number of additional species have been recorded, and the inventory was revised and the list of management plan updated (Gomes et al. 2014). Given their ecological and morphological differences, mammals can be allocated to three principal groups: “small flying mammals”, “small non-volant mammals” and “medium and large mammals”, with the latter two groups being the focus of the present study. The main objective of the present study was to update the list of mammalian species known to occur in the Saracá-Taquera National Forest, based on an ecological and conservationist approach, based on three long-term surveys conducted between 2009 and 2015.

Materials and Methods

1. *Study area*

The study area is located within the Saracá-Taquera National Forest (STNF), which is distributed among the neighboring municipalities of Oriximiná, Faro, and Terra Santa, in northwestern Pará (01°40’ S, 56°00’ W), a state in northern Brazil. STNF is formed predominantly by dense rainforest, interspersed with areas of alluvial forest, blackwater swamp (igapó), and, to a lesser extent, campinarana scrub (Gomes et al. 2014). The region’s climate is of Köppen’s *Am* type, with rainy summers and dry winters, mean annual precipitation of approximately 2200 mm, and temperatures of 20–35°C (Parrota et al. 1997). STNF area is rich in bauxite, which is being mined by a consortium of companies known as Mineração Rio do Norte (MRN), which operates on a small number of the plateaus found within the area of the national forest. In the present study, we sampled 12 plateaus, known locally as: Almeidas, Aviso, Aramã, Bacaba, Bela Cruz, Cipó, Greig, Monte Branco, Papagaio, Periquito, Saracá, and Teófilo (Figure 1).

2. *Data collection and analysis*

To compile the species list, we used primarily data from three surveys, conducted in 2009–2010, 2010–2012, and 2015. During these surveys, different approaches were used to sample small rodents and marsupials, and medium-and large-sized mammals (Silveira et al. 2003; Ribeiro and Melo 2013; Bovendorp et al. 2017) and, given this, the results are presented separately. The species richness is presented as the number of species recorded during any given period, independently of indices of biodiversity.

3. *Small non-volant small mammals*

During the three study periods, from from 2009 to 2015, we trapped small mammals in both rainy and dry seasons, focusing on the tropical rainforest in three topographies, plateau, slope, and valley bottom. Two types of trap were used, metal live traps (Tomahawk and Sherman) and pitfall traps. Captured specimens were identified using Patton et al. (2000, 2015), Bonvicino et al. (2008), and Rossi et al. (2010). Specimens were allocated to either the order Didelphimorphia (family Didelphidae) or the Rodentia (Cricetidae, Echimydae and Sciuridae). The sampling effort is presented in Table 1.

4. *Medium and large mammals*

Medium and large mammals, were surveyed by line transect, with individual transects varying in length from 500 m to 4000 m. In all three study periods, surveys were conducted during the day, in the morning (7:00–11:00 h) and afternoon (13:00–17:00 h), and at night, between 19:00 h and 22:00 h. Whenever an animal was visualized or some other evidence was detected, a standard set of data was compiled, including the identification of the species, the type of record, the number of individuals, the time and the location on the transect, and the geographic coordinates. Whenever possible, the animal or vestige were photographed.

To complement these data, we installed two to eight camera traps on each transect, which remained active for six to 20 days, depending on the duration of the fieldwork. During the first period, a trap was installed at each end of the trail on the 500-m transects. During the second period, four traps were installed at 50 m, 100 m, 250 m, and 500 m along the transect, to evaluate the influence of edge effects, while in the third period, the traps were installed at 1000-m intervals along the 4000 m transect. The camera traps were installed on tree trunks, 30 cm above the ground, and were baited with fruit, bacon and/or sardines, to maximize the chances of animals visiting the sites. The sampling effort for each period is shown in Table 1. Species were identified based on Paglia et al. (2012), with the nomenclature updated to include Cingulata and Cetardiodactyla (Montgelard et al. 1997; Delsuc et al. 2016).

To guarantee as complete an inventory as possible, in addition to the field surveys, records of mammals were gleaned from published studies and other records obtained during fieldwork at STNF, including interviews, although the interview data should obviously be treated with caution. These data were not included in the analyses. The trapping and transect data were grouped for the plotting of species accumulation curves using the first-order Jackknife method, run in EstimateS 8.20 (Heltshe and Forrester 1983; Colwell 2009).

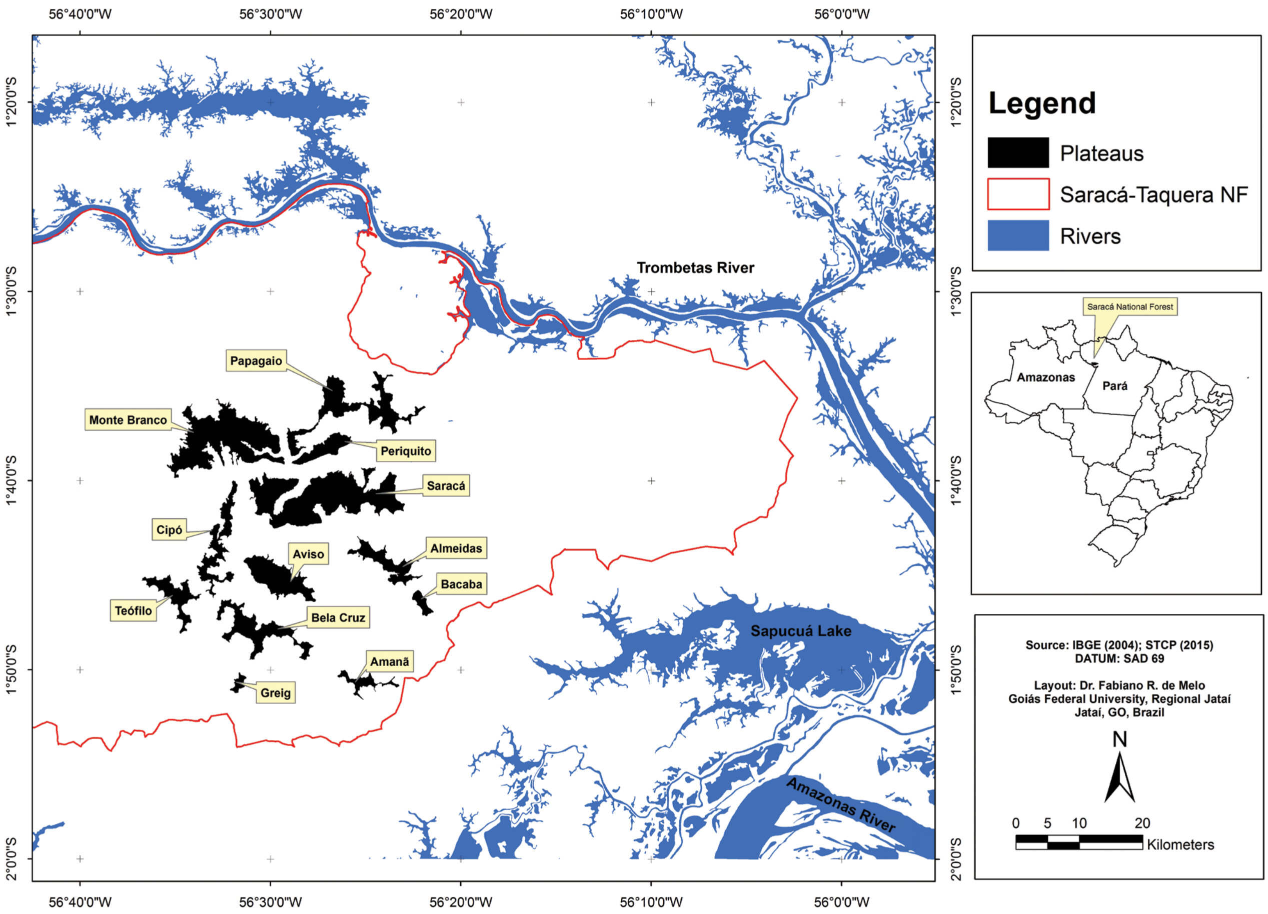

Figure 1. Location of the Saracá-Taquera National Forest and the sample sites, in the state of Pará, Brazil.

Table 1. Sampling effort employed in the three periods of studies to search for small, medium and large mammals at Saracá-Taquera National Forest, Pará.

	Sampling		
	2009-2010	**2010-2012**	**2015**
Effort (trap/night)	24.960	63.168	20.160
Census (km)	642	1.176	384
Cameras/day	4.388	5.232	2.304

Results

Over the 15 years of the study period, we recorded 72 species of small, medium and large mammals in the Saracá-Taquera National Forest (Table 2, Figures 2, 3), of which, five were recorded during interviews with local residents. The composition of the mammalian community was extremely diverse, and included representatives of 30 families and 10 orders. The most diverse orders were the rodents, with 16 species, followed by the carnivores, with 14 species, and the primates and marsupials, with 10 species each (Table 2).

Species accumulation curves for the trapping data, i.e., small non-volant mammals (Figure 4A), and the line transect data, i.e., medium-large mammals (Figure 4B) both reached the asymptote, indicating that the sampling effort employed was sufficient for the registration of the majority of the species that occur in the study area. The accumulation curve for the cameras trap data (Figura 4C) was still following an upward trend, however, indicating that additional species would likely be added, with increased sample effort.

Discussion

Five of the 72 species recorded in the present study were confirmed only by interviews with local residents. While the data are important as complementary records, they must be treated with extreme caution, especially as some of the species cited may not actually occur in the region. In a review of the records of the Pilosa and Cingulata from the Saracá-Taquera National Forest, for example, Oliveira et al. (2006) found that some of the species cited, such as the Brazilian three-banded armadillo (*Tolypeutes tricinctus* (Linnaeus, 1758)) are typical of other biomes, such as the semi-arid Caatinga, and are unlikely to be found in the northern Amazon basin. Similarly, while the six-banded armadillo (*Euphractus sexcinctus* (Linnaeus, 1758)) has been recorded in interviews, and listed in technical reports, it has never been recorded during fieldwork (Oliveira et al. 2006). Also, the occurrence of two primates, *Cebus olivaceus* (Schomburgk, 1848) and *Aotus trivirgatus* (Humboldt, 1812), in the STNF has been confirmed

Table 2. List of mammal species recorded in Saracá-Taquera National Forest, northwest of State Pará, Brazil. Type of record: VO = visual observation, V = vocalization, T = track, F = feces, B = burrow, C = carcass, CT = camera trap, LT = live trap, PT = pitfall trap, I = interview. Global (IUCN 2016), National (MMA 2014) and Regional (Pará 2006) threat category. EN = Endangered, VU = Vulnerable; NT = Near Threatened and DD = Data Deficient.

TAXON	COMMON NAME	TYPE OF RECORD	CONSERVATION STATUS		
			Global	National	Regional
Order Didelphimorphia					
Family Didelphidae					
Caluromys philander (Linnaeus, 1758)	Bare-tailed Woolly Opossum	LT			
Didelphis imperfecta (Mondoli & Pérez-Hernández, 1984)	Guianan White-eared Opossum	LT			
Didelphis marsupialis (Linnaeus, 1758)	Common Opossum	LT, PT			
Gracilinanus emiliae (Thomas, 1909)	Emilia's Gracile Opossum	PT	DD		
Marmosa murina (Linnaeus, 1758)	Linnaeus's Mouse Opossum	LT, PT			
Marmosops parvidens (Tate, 1931)	Delicate Slender Mouse Opossum	LT, PT			
Metachirus nudicaudatus (E. Geofroy, 1803)	Brown Four-eyed Opossum	LT, PT			
Marmosa demerarae (Thomas, 1905)	Woolly Mouse Opossum	LT, PT			
Monodelphis arlindoi (Pavan, Rossi & Schneider, 2012 Erxleben, 1777)	Short-tailed opossums	LT, PT			
Philander opossum (Linnaeus, 1758)	Gray Four-eyed Opossum	LT			
Order Cingulata					
Family Chlamyphoridae					
Cabassous unicinctus (Linnaeus, 1758)	Southern Naked-tailed Armadillo	VO, CT, T, B, C, PT			
Euphractus sexcinctus (Linnaeus, 1758)[1]	Yellow Armadillo	I			
Priodontes maximus (Kerr, 1792)	Giant Armadillo	VO, CT, T, B	VU	VU	VU
Family Dasypodidae					
Dasypus novemcinctus Linnaeus, 1758	Nine-banded Armadillo	VO, CT, T, B, C			
Dasypus kappleri Kraus, 1862	Greater Long-nosed Armadillo	VO, CT, T, B			
Dasypus septemcinctus Linnaeus, 1758	Brazilian Lesser Long-nosed Armadillo	VO			
Order Pilosa					
Family Bradypodidae					
Bradypus tridactylus Linnaeus, 1758	Pale-throated Three-toed Sloth	VO, C			
Family Megalonychidae					
Choloepus didactylus (Linnaeus, 1758)	Two-toed Sloth	VO			
Family Cyclopedidae					
Cyclopes didactylus (Linnaeus, 1758)	Silky Anteater	VO, PT			
Family Myrmecophagidae					
Myrmecophaga tridactyla Linnaeus, 1758	Giant Anteater	VO, CT, T	VU	VU	VU
Tamandua tetradactyla (Linnaeus, 1758)	Southern Tamandua	VO,CT, T			
Order Primates					
Family Aotidae					
Aotus trivirgatus (Humboldt, 1811)[2]	Northern Night Monkey	I			
Family Atelidae					
Allouatta macconnelli Elliot, 1910	Guianan Red Howler Monkey	VO, V, F, C			
Ateles paniscus (Linnaeus, 1758)	Guiana Spider Monkey	VO, V, F, C	VU	EN	

Continuation Table 2.

TAXON	COMMON NAME	TYPE OF RECORD	CONSERVATION STATUS		
			Global	National	Regional
Family Callitrichidae					
Saguinus martinsi (Thomas, 1912)	Martin's Ochraceous Bare-face Tamarin	VO, V			
Saguinus midas (Linnaeus, 1758)	Golden-handed Tamarin	VO			
Family Cebidae					
Cebus olivaceus Schomburgk, 1848[2]	Weeper Capuchin	I			
Saimiri sciureus (Linnaeus, 1758)	Common Squirrel Monkey	VO, CT, V			
Sapajus apella (Linnaeus, 1758)	Bearded Capuchin	VO, CT, V			
Family Pitheciidae					
Chiropotes sagulatus (Traill, 1821)	Bearded Saki	VO, V			
Pithecia pithecia (Linnaeus, 1766)	White-faced Saki	VO			
Order Carnivora					
Family Canidae					
Speothos venaticus (Lund, 1842)*	Bush Dog	VO	NT	VU	
Family Felidae					
Leopardus pardalis (Linnaeus, 1758)	Ocelot	VO, CT, T			
Leopardus wiedii (Schinz, 1821)	Margay	VO, CT	NT	VU	
Leopardus tigrinus (Schreber, 1775)[3]	Northern Tiger Cat	I	VU	EN	
Panthera onca (Linnaeus, 1758)	Jaguar	VO, CT, T, F, C	NT	VU	VU
Puma concolor (Linnaeus, 1771)	Puma	VO, CT, T, F		VU	VU
Puma yagouaroundi (É. Geoffroy, 1803)	Jaguarundi	VO, CT		VU	
Family Mustelidae					
Eira barbara (Linnaeus, 1758)	Tayra	VO, CT			
Galicitis vittata (Schreber, 1776)*	Greater Grison	VO, C			
Lontra longicaudis (Olfers, 1818)	Neotropical Otter	VO, F, T, B	NT		
Pteronura brasiliensis (Gmelin, 1788)	Giant Otter	VO, F, T, B	EN	VU	VU
Family Procyonidae					
Nasua nasua (Linnaeus, 1766)	South American Coati	VO, CT, T			
Potus flavus (Schereber, 1774)	Kinkajou	VO, CT			
Procyon cancrivorus (G. [Baron] Cuvier, 1798)[3]	Crab-eating Raccoon	VO, I			
Order Cetartiodactyla					
Family Delphinidae					
Sotalia fluviatilis (Gervais & Deville, 1853)	Tucuxi	VO	DD		
Family Iniidae					
Inia geoffrensis (Blainville, 1817)	Amazon River Dolphin	VO	DD	EN	
Family Tayassuidae					
Pecari tajacu (Linnaeus, 1758)	Collared Peccary	VO, CT, T			
Tayassu pecari (Link, 1795)	White-lipped Peccary	VO, CT, T	VU	VU	
Family Cervidae					
Mazama americana (Erxleben, 1777)	Red Brocket Deer	VO, CT, T, F	DD		
Mazama gouazoubira (G. Fisher, 1814)	Gray Brocket Deer	VO, CT, T, F			

Continuation Table 2.

TAXON	COMMON NAME	TYPE OF RECORD	CONSERVATION STATUS		
			Global	National	Regional
Mazama nemorivaga (F. Cuvier, 1817)	Amazonian Brown Brocket Deer	VO, CT			
Odocoileus virginianus (Zimmermann, 1780)[3]	White-tailed Deer	I, T			
Order Perissodactyla					
Family Tapiridae					
Tapirus terrestris (Linnaeus, 1758)	Lowland Tapir	VO, CT, F, T	VU	VU	
Order Sirenia					
Family Trichechidae					
Trichechus inunguis (Natterer, 1883)	Amazonian Manatee	VO, F	VU	VU	EN
Order Rodentia					
Family Caviidae					
Hydrochoerus hydrochaeris (Linnaeus, 1766)	Capybara	VO, F			
Family Cricetidae					
Euryoryzomys macconnelli (Thomas, 1910)	Macconnell's Rice Rat	LT, PT			
Hylaeamys megacephalus (Fischer, 1814)	Large-headed rice rat	LT, PT			
Nectomys rattus (Pelzen, 1883)	Small-footed Bristly Mouse	PT			
Oecomys bicolor (Thomas, 1860)	Bicolored Arboreal Rice Rat	LT, PT			
Rhipidomys nitela (Thomas, 1901)	Splendid Climbing Mouse	LT, PT			
Zygodontomys brevicauda (Allen & Chapman, 1893)	Short-tailed Cane Mouse	LT, PT			
Family Cuniculidae					
Cuniculus paca (Linnaeus, 1766)	Spotted Paca	VO, CT, T, F			
Family Dasyproctidae					
Dasyprocta leporina (Linnaeus, 1758)	Red-rumped Agouti	VO, CT, T, F, V			
Myoprocta acouchy (Erxleben, 1777)	Red Acouchi	VO, CT, T, V			
Family Erethizontidae					
Coendou prehensilis (Linnaeus, 1758)	Brazilian Porcupine	VO, CT, C			
Family Echimyidae					
Echimys chrysurus (Zimmermann, 1780)	White-faced Tree Rat	C			
Isothrix pagurus Wagner, 1845	Plain Brush-tailed Rat				
Mesomys hispidus (Desmarest, 1817)	Spiny Tree Rat	PT			
Proechimys cuvieri (Petter, 1978)	Cuvier's Spiny Rat	LT, PT			
Family Sciuridae					
Guerlinguetus aestuans (Linnaeus, 1766)	Gianan Squirrel	VO, CT, LT			
Order Lagomorpha					
Family Leporidae					
Sylvilagus brasiliensis (Linnaeus, 1758)[3]	Tapeti	I			

* Random Record; [1] From Oliveira et al. 2006; [2] From Oliveira et al. 2009; [3] From STCP, 2008.

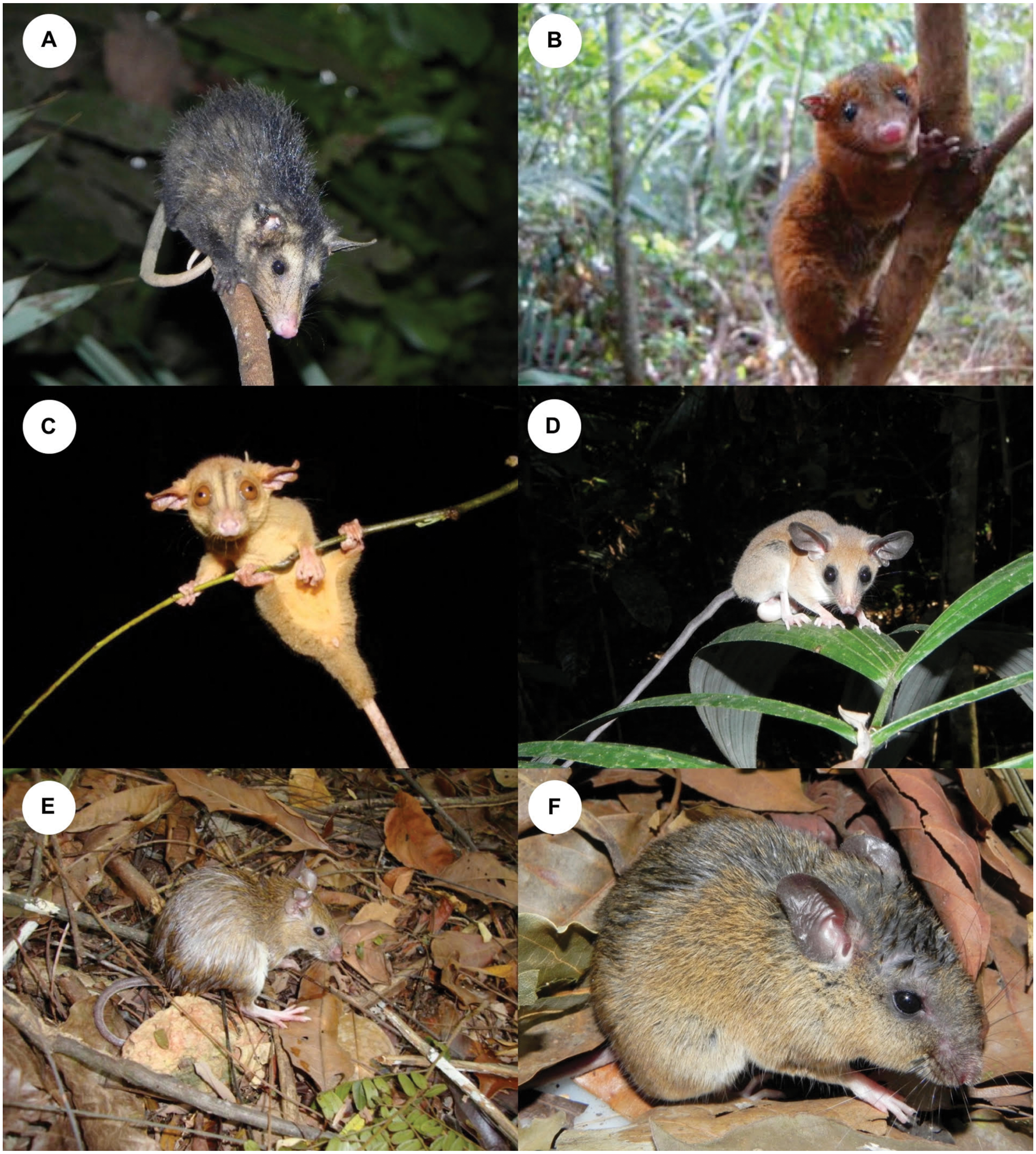

Figure 2. Small non-volant mammals recorded in the Saracá-Taquera National Forest, Pará, Brazil: A) *Didelphis marsupialis*; B) *Monodelphis arlindoi*; C) *Marmosops parvidens*; D) *Caluromys philander*; E) *Marmosa demerarae*; F) *Hylaeamys megacephalus*; G) *Proechimys cuvieri*.

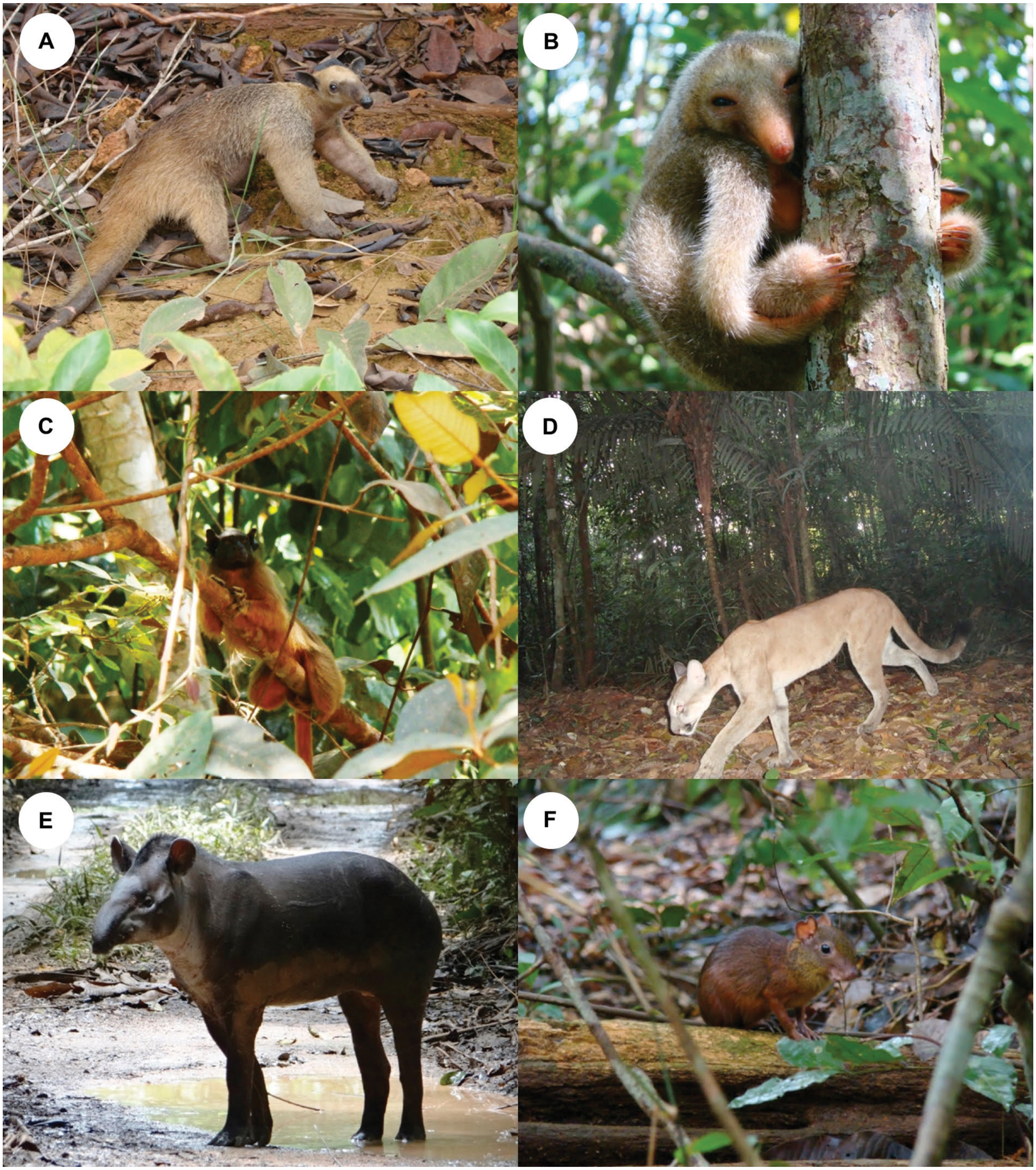

Figure 3. Medium and large mammals recorded in the Saracá-Taquera National Forest, Pará, Brazil: A) *Tamandua tetradactyla*; B) *Cyclopes didactylus*; C) *Saguinus martinsi*; D) *Puma concolor*; E) *Tapirus terrestris*; F) *Myoprocta acouchy*.

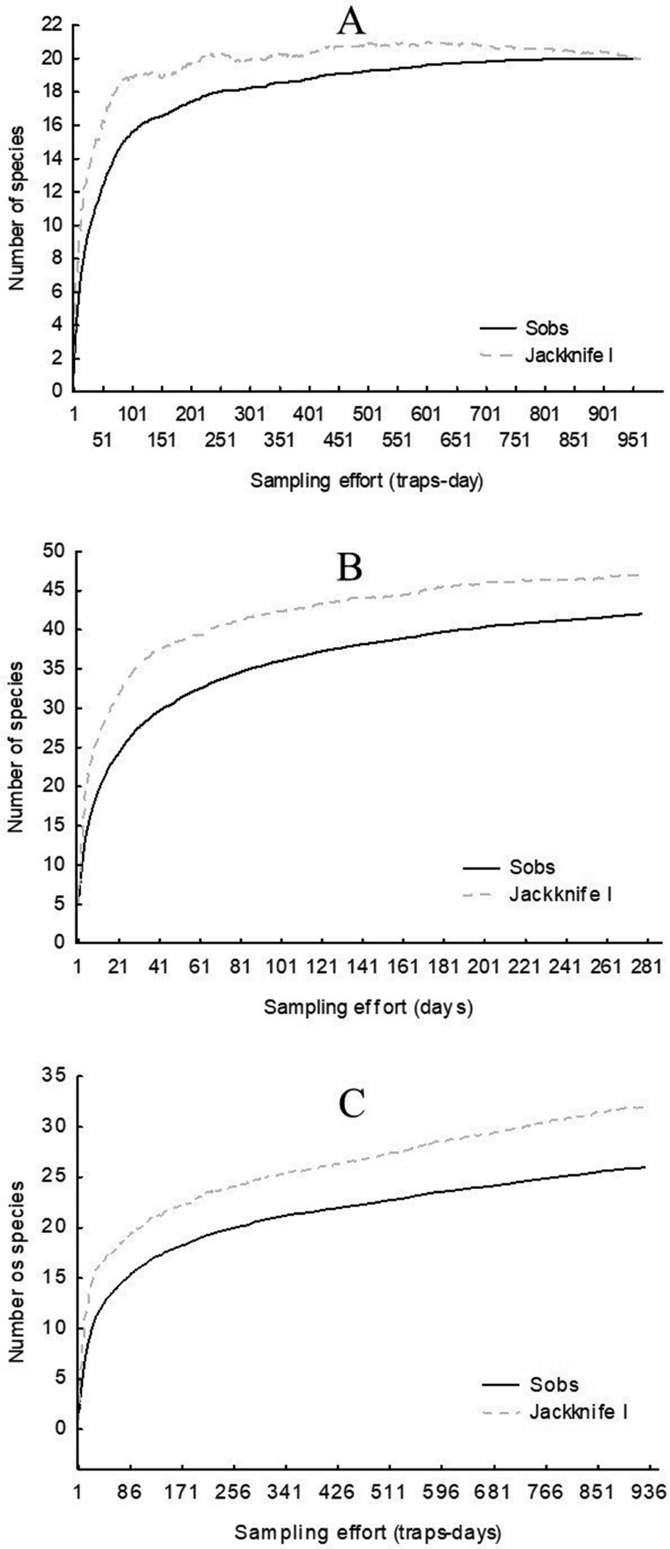

Figure 4. Species accumulation curve for small mammals (A), and medium and large mammals recorded by linear transect (B) and cameras trapping (C) in the Saracá-Taquera National Forest, Pará, Brazil.

only by interviews and published records (Oliveira et al. 2009). The wedge-capped capuchin, *C. olivaceus*, has been recorded from the left margin of the Trombetas River (Oliveira et al. 2009), although the STNF is located on the right margin. In the Amazon, rivers typically act as geographic barriers to primates (Peres et al. 1996; Boubli et al. 2015). In other cases, such as that of the northern tiger cat (*Leopardus tigrinus*), the species can easily be confused with other small-medium felines, especially by non-expert observers, and Payan & Oliveira (2016) concluded that this species is rare or absent from large areas of the Amazon basin. In addition to these four species, the tapeti (*Sylvilagus brasiliensis* (Linnaeus, 1758)), while widely-distributed in South America, has never been recorded in the current study area. While the lack of records of these five species may reflect their local rarity, we believe that continued sampling may eventually confirm their presence in the STNF and, as such, while they are included in the list of species that occur in this national forest, their presence in this protected area cannot yet be confirmed definitively.

Overall, 29 of the species recorded in the present study are considered to be endemic to the Amazon, including the marsupials (*Didelphis imperfecta* (Mondolfi & Pérez-Hernández, 1984)*, Gracilinanus emiliae* (Thomas, 1909)*, Marmosops parvidens* (Tate, 1931), and *Monodelphis arlindoi* (Pavan et al., 2012)*,* the greater long-nosed armadillo (*Dasypus kappleri* (Krauss, 1982), two species of sloths (*Bradypus tridactylus* (Linnaeus, 1758) and *Choloepus didactylus* Linnaeus, 1758)), all the ten primate species recorded in the present study (see Table 1), the deer *Mazama nemorivaga* and *Odoicoleus virginianus*, the Amazonian manatee (*Trichechus inunguis* (Natterer, 1883)), and the rodents *Euryoryzomys macconnelli* (Thomas, 1910), *Rhipidomys nitela* (Thomas, 1901), *Zygodontomys brevicauda* (Allen, 1897)*, Myoprocta acouchy* (Thomas, 1903)*, Echimys chrysurus* (Zimmermann, 1780)*, Isothrix pagurus* (Wagner, 1845)*, Mesomys hispidus* (Desmarest, 1817)*, Proechimys cuvieri* (Petter, 1978), and *Guerlinguetus aestuans* (Linnaeus, 1766) (Bonvicino et al. 2008; Paglia et al. 2012; Patton et al. 2015). *Monodelphis arlindoi* was recently described by Pavan et al. (2012), and is endemic to the study region (see Figure 2B). No other species of mammal recorded in the present study is endemic to the Saracá-Taquera National Forest, Martin's bare-face tamarin (*Saguinus martinsi* (Thomas, 1912)) is protected only by this conservation unit.

Fourteen of the species recorded here are also included in one or more of the lists of species threatened with extinction published by the International Union for Conservation of Nature (IUCN 2017), the Brazilian Ministry of the Environment (MMA 2014), and Pará state resolution 054/2007 (Table 1). A number of these threatened species are extremely sensitive and have undergone population decline throughout most of their geographic ranges, due primarily to habitat loss and fragmentation, hunting pressure, wildfires, and roadkill (Chiarello et al. 2008; Naveda et al. 2008; Medici et al. 2012), although some, such as the tapir, the white-lipped peccary, and the jaguar, may be relatively tolerant of disturbed environments (Naveda et al. 2008; Keuroghlian et al. 2013; Quigley et al. 2017).

The most abundant of the ten marsupial species recorded in the Saracá-Taquera National Forest were *Marmosa demerarae* (Thomas, 1905) and *Marmosops parvidens* (Tate, 1931), which were both widely distributed in all the environments sampled. *Marmosa demerarae* (Thomas, 1905) was captured primarily in Sherman traps and, despite being arboreal, it was commonly captured on the ground and in the pitfall traps, as observed by Voss (2001). By contrast, the rarest marsupials were *Philander opossum* (Linnaeus, 1758) (N = 1), *Gracilinanus emiliae* (N = 2) and *Didelphis imperfecta* (Mondolfi & Pérez-Hernández, 1984) (N = 3). *Philander opossum* is recorded commonly in the Amazon (Patton et al. 2000), although only one individual was trapped in the present study. While this may reflect the local rarity of the species, limitations of the sampling effort cannot be ruled out. Two marsupials, *D. imperfecta* and *Echimys chrysurus* (Zimmermann, 1780), were recorded in the region for the first time, extending their known geographical distributions (Faria and Melo 2017).

A majority of the species of the orders Cingulata and Pilosa were distributed homogeneously among the areas surveyed, with the nine-banded armadillo (*Dasypus novemcinctus* (Linnaeus, 1758)) and the greater long-nosed armadillo (*Dasypus kappleri (*Krauss, 1862) being the most frequent. The silky anteater (*Cyclopes didactylus* (Linnaeus, 1758)) was the rarest member of the Pilosa, which is probably related to the fact that this species is extremely difficult to locate, due to its cryptic, nocturnal and arboreal behavior, and the fact that it rarely descends to the ground (Medri et al. 2011). These characteristics, together with the dense vegetation of most of the STNF, may have limited visibility in the extreme, although the species was encountered in riverside habitats (Moura and Sapucuá), where much of the forest is regenerating.

Sloths were also recorded rarely, being found primarily during wildlife rescue operations, prior to planned deforestation for mining. Some sloths have also been rescued from the railroad track that crosses part of the natural forest. This scarcity of records reflects the cryptic coloration and behavior of the sloths, which may be difficult to spot in the high canopy of STNF, despite their relatively large size (for an arboreal mammal).

The diversity of Amazonian primates is among the greatest in the world (Rylands and Mittermeier 2009; Mittermeier et al. 2013), and more than half the biome's primate genera are found in the Saracá-Taquera National Forest. The bearded saki (*Chiropotes sagulatus*), bearded capuchin (*Sapajus apella*), Guianan red howler monkey (*Alouatta macconnelli* (Elliot, 1910)) and the Guianan spider monkey (*Ateles paniscus* (Linnaeus, 1758)) were the species encountered most frequently. The ecology of the bearded saki and Martin's bare-face tamarin (*Saguinus martinsi* (Thomas, 1912)) has been monitored in STNF since 2009, and a number of important findings in the area have already been published (Barnett et al. 2012; Melo et al. 2013; Boyle et al. 2015; Shaffer et al. 2015).

The order Carnivora had the largest number of endangered species. The bush dog (*Speothos venaticus* (Lund, 1842)) was the rarest carnivore in the region, being visualized on only a few occasions by other research teams working in STNF. While the bush dog has an ample distribution and is considered to be a habitat generalist, it appears to be naturally rare in most areas in which it is found (Dematteo et al. 2011). The top predators, the jaguar (*Panthera onca* (Linnaeues, 1758)) and the cougar (*Puma concolor* (Linnaeus, 1771)), were recorded on most of the plateaus surveyed, including areas of habitat recuperation. The other carnivore species had a more irregular distribution within the study area.

Aquatic mammals of the orders Cetardiodactyla and Sirenia were recorded mainly during transit to the sampling points at Moura and Sapucuá, during the 2009–2010 survey. In 2011, in addition, the Amazonian manatee was monitored more directly at these sites, in particular Sapucuá Lake. At the same time, conflicts were observed between members of the local riverside communities and the dolphins, due to their attempts to retrieve fish caught in fishing nets, a behavior also observed in other regions (Loch et al. 2009).

Ungulates (orders Perissodactyla and Cetartiodactyla) were common in the study area, and were abundant in areas of habitat recuperation. The Amazonian brown brocket deer (*Mazama nemorivaga* (Cuvier, 1817)) was recorded recently in the region, with its presence being confirmed by experts, indicating the need for a taxonomic review of this and a number of other species (mainly rodents) recorded in STNF. Other ungulates, such as the peccaries (*Pecari tajacu* (Linnaeus, 1758) and *Tayassu pecari* (Link, 1795)), were recorded on a number of different plateaus and, together with the tapir (*Tapirus terrestris* (Linnaeus, 1758)), may play a unique, and vitally important functional role in the local ecosystem (Calaça 2014).

The order Rodentia was represented by some of the most abundant species found in the study area, such as the red-rumped agouti (*Dasyprocta leporina* (Linnaeus, 1758)) and the red acouchi (*Myoprocta acouchy* (Erxleben, 1777)). Both these species were common, and were recorded frequently within the study area, especially as, when they detect the presence of researchers, these animals emit a characteristic alarm vocalization before fleeing (Eisenberg and Thorington 1973; De Thoisy et al. 2008). The most common small rodents were Cuvier's spiny rat, *Proechimys cuvieri* (Petter, 1978) and the large-headed rice rat (*Hylaeamys megacephalus* (Fischer, 1814)), which were both common and widespread (Patton, et al. 2015). Other rodents were distributed more irregularly within the study area.

The order Lagomorpha, represented by a single species in Brazil, the tapeti (*Sylvilagus brasiliensis* (Linnaeus, 1758)), was only recorded in the study region through interviews, and most local residents, in particular the younger individuals, are unfamiliar with the species. Although the tapeti is common and widely distributed (Diersing 1981), it is possible that it may be very rare in the study area, or has become locally extinct.

The mammalian fauna of the Saracá-Taquera National Forest is rich and diverse, with at least as many species as most other, similar areas that have been surveyed in the Amazon biome (George et al. 1988; Calouro 1999; Marques-Aguiar et al. 2003; Pontes et al. 2008; Bergallo et al. 2012; Santos and Mendes-Oliveira 2012). While some other regions in the Amazon may have higher species richness, reinforcing their priority for conservation (Oliveira et al., 2016), the relatively high species richness recorded in the present study emphasizes the importance of this environment for the maintenance of local and regional diversity. The ecological diversity found in the present study, in terms of habitat use and feeding adaptations, is vitally important for complementary coexistence at the community level. This diversity is essential for balanced community structure and efficient ecosystem functioning (Walker 1992).

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Author Contributions

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

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

Diego Afonso Silva: Contribution to data collection; Contribution to manuscript preparation.

Áquila Oliveira Fialho: Contribution to data collection; Contribution to manuscript preparation.

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

Conflicts of interest

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

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Article

New species of *Temnocephala* (Platyhelminthes, Temnocephalida) ectosymbiont on vulnerable species of aeglids (Crustacea, Anomura) from the Neotropical Region

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SEIXAS, S.A., DAMETTO, N., PÉRICO, E. **New species of *Temnocephala* (Platyhelminthes, Temnocephalida) ectosymbiont on vulnerable species of aeglids (Crustacea, Anomura) from the Neotropical Region**. Biota Neotropica. 18(4): e201704725. http://dx.doi.org/10.1590/1676-0611-BN-2017-0475

Abstract: A new species of the genus *Temnocephala* Blanchard, 1849 from southern Brazil was found on two species of anomuran crustaceans, *Aegla spinipalma* Bond-Buckup & Buckup, 1994 and *Aegla grisella* Bond-Buckup & Buckup, 1994, the latter classified as a vulnerable species by the "Lista de Referência da Fauna Ameaçada de Extinção no Rio Grande do Sul. Decreto no 41.672, de 11 junho de 2002". The crustaceans were collected from a tributary creek of the Forqueta river, Perau de Janeiro, Arvorezinha and a tributary creek of the Fão river, Pouso Novo, Rio Grande do Sul, Brazil; both localities belong to the Sub-Basin of Forqueta River. The new species differs from seven other temnocephalans epibionts on *Aegla* Leach, 1820, by having the following characters: 1. a long and slightly curved cirrus, 2. two vaginal sphincters, one proximal, big and asymmetric, and one distal, smaller and symmetric, and; 3. longer than wide, elongated epidermal 'excretory' syncytial plates (EPs), with a almost horizontally central excretory pore, displaced to the anterior portion of the plate. The new species' EP is the largest in total length among epibionts temnocephalans in crustaceans already registered. Regarding the similarities with the male reproductive system of *Temnocephala axenos* Monticelli, 1898, the new species has important differences in the female reproductive system. It has a larger proximal vaginal sphincter, located in the middle of the vagina, while the smaller distal one is at the extreme end of the organ. Besides that, the vaginal portion between the proximal and distal sphincters is conspicuous, with a strong muscular wall. This is the first record of a species of *Temnocephala* in the Taquari Valley, as well in the 'Perau de Janeiro', which is an area with a rich endemic fauna.
Keywords: *crustacean, ectosymbiont, South America, symbiosis, Taxonomy.*

Nova espécie de *Temnocephala* (Platyhelminthes, Temnocephalida) ectosimbionte sobre espécies vulneráveis de eglídeos (Crustacea, Anomura) da Região Neotropical

Resumo: Uma nova espécie do gênero *Temnocephala* Blanchard, 1849 da região sul do Brasil foi encontrada sobre duas espécies de crustáceos anomuros, *Aegla spinipalma* Bond-Buckup & Buckup, 1994 e *Aegla grisella* Bond-Buckup & Buckup, 1994, a última classificada como uma espécie vulnerável pela Lista de Referência da Fauna Ameaçada de Extinção no Rio Grande do Sul. Decreto no 41.672, de 11 junho de 2002. Os crustáceos foram coletados em um arroio tributário do Rio Forqueta, Perau de Janeiro, Arvorezinha e em um arroio tributário do Rio Fão, Pouso Novo, Rio Grande do Sul, Brazil; ambas localidades pertencem a Sub-Bacia do Rio Forqueta. A nova espécie se diferencia dos outros sete temnocefalídeos epibiontes sobre *Aegla* Leach, 1820 pelos caracteres a seguir: 1. cirro longo e levemente curvo, 2. dois esfíncteres vaginais, um proximal, grande e assimétrico e um distal, menor e simétrico, e, 3. placas sinciciais epidérmicas 'excretoras' (PEs) alongadas, mais longas do que largas, com poro excretor quase central horizontalmente e deslocado para a porção anterior da placa. A PE da nova espécie é a maior em comprimento total entre os temnocefalídeos epibiontes sobre crustáceos registrados até o momento. Embora haja similaridades com o sistema reprodutor masculino de *Temnocephala axenos* Monticelli, 1898, a nova espécie apresenta diferenças importantes no sistema reprodutor feminino. O esfíncter vaginal proximal é maior, localizado no meio da vagina, enquanto o distal é menor e se localiza no final do órgão. Além disso, a porção da vagina entre os esfíncters proximal e distal é conspícua, com uma forte parede muscular. Esta é a primeira espécie de *Temnocephala* registrada para o Vale do Taquari, assim como para o Perau de Janeiro, área com uma fauna endêmica rica.
Palavras-chave: *América do Sul, crustáceos, ectosimbionte, simbiose, Taxonomia.*

Introduction

The first host taxon of temnocephalans was recorded on Crustacea Brünnich, 1772. This group also has the largest number of epibiont species of the genus *Temnocephala* Blanchard, 1849. From the 21 ectosymbiont species on crustaceans, seven were recorded from the species of the genus *Aegla* Leach, 1820: *Temnocephala chilensis* (Moquin-Tandon, 1846), *Temnocephala axenos* Monticelli, 1898, *Temnocephala mexicana* Vayssière, 1898, *Temnocephala talicei* Dioni, 1967, *Temnocephala cyanoglandula* Amato, Amato & Daudt, 2003, *Temnocephala mertoni* Volonterio, 2007, and *Temnocephala dionii* Ponce de León, Berón Vera & Volonterio, 2015.

Temnocephala chilensis was the first species of the genus to be described and was recorded consistently after that (Dioni 1967a, Damborenea 1992). However, the authors have not updated the species description using more recent techniques.

Dioni (1972) recorded *T. mexicana* in *Aegla* sp. and *Parastacus* sp. from Argentina. The species was described by Vayssière (1898) and re-described by Lamothe-Argumedo (1968). Both publications were based on specimens collected from *Procambarus digueti* (Bouvier, 1897) and *Pseudothelphusa jouyi* Rathbun, 1893 (added by Lamothe-Argumedo in 1968) from Mexico.

Temnocephala talicei has also been recorded a few times (Dioni 1968, Damborenea 1992, et al. 1997), being subsequently re-described and having a neotype designated by Volonterio (2009).

Temnocephala axenos is the most well-studied species (Baer 1931, Dioni 1967b, 1968, Damborenea 1992, et al. 1997), but it has substantial taxonomic problems, such as a misidentification of the host type (Amato et al. 2003) and the loss of the holotype at the Berlin Natural History Museum because of war damage. The superficial description of *T. axenos* (Monticelli 1898) lead to the consideration of this species as a senior synonym of *Temnocephala brasiliensis* Merton, 1922 by Baer (1931) and *Temnocephala bresslaui* Pérez-González, 1949 by Dioni (1967c). Volonterio (2007) stated that *T. bresslaui* was erroneously synonymized by Dioni (1967c) and it is, probably, still a valid species. The author re-described *T. axenos*, solving some of these issues. The incomplete description of *T. cyanoglandula*, with only data of the male reproductive system, has led Volonterio (2007) to suggest a synonymy for this species with *T. axenos* or *T. bresslaui.* However, a recent study of the female reproductive system (Seixas et al. 2015a) has confirmed *T. cyanoglandula* as a valid species.

While describing *T. mertoni,* an epibiont species on anomuran crabs, Volonterio (2007) pointed out the difficulties of distinguishing temnocephalans species on crustaceans given the similarities in the males' reproductive system. Among other features, the author highlighted the importance of describing in detail the female reproductive system of ectosymbionts hosted by crustaceans.

Temnocephala dionii was the last species described as an ectosymbiont on *Aegla neuquensis* Schmitt, 1942 from Argentina (Ponce de León et al. 2015).

There are no records of temnocephalans at the Forqueta River Sub-Basin (Fig. 1), where the crustaceans fauna is less well investigated. The Forqueta and Fão Rivers, localized at the municipalities of Arvorezinha and Pouso Novo, respectively, represent the two main rivers of the sub-basin. The present study aims to describe a new species of *Temnocephala* ectosymbiont on *Aegla spinipalma* Bond-Buckup & Buckup, 1994 and *Aegla grisella* Bond-Buckup & Buckup, 1994 (Fig. 2a), which is classified as a vulnerable species by Rio Grande do Sul State Law (Marques et al. 2002). Both are being registered as new host species for neotropical temnocephalans.

Material and Methods

One hundred and six specimens of *A. grisella* were collected from a tributary creek of the Forqueta river (28º51'9.85"S; 52º17'55.02"W), Perau de Janeiro, Arvorezinha, Rio Grande do Sul, Brazil; and eighty-two specimens of *A. spinipalma* were collected from a tributary creek of the Fão river (29°12'2.81"S; 52°11'31.80"W), Pouso Novo, Rio Grande do Sul, Brazil. Both localities belong to Forqueta River Sub-Basin.

The collections occurred monthly between August 2014 and April 2015 as part of a larger project for taxonomic and ecological studies. All crustaceans were collected with dip nets, sexed, measured, and returned to their natural habitat. Only a few specimens (≅ 10) were taken alive to the "Setor de Evolução e Ecologia, Univates" to be examined for temnocephalans.

The temnocephalans were studied through a series of techniques focusing especially on the morphology of the vagina and other female reproductive organs, as well as the morphology of the cirrus structure and the epidermal 'excretory' syncytial plates (EPs).

For general measurements, the helminths were fixed with AFA, under slight cover slip pressure, following the protocols established by Amato et al. (2007) and Seixas et al. (2010). The specimens were stained with Delafield's hematoxylin or aceto-carmine/fast green, cleared in cedar oil, and mounted as permanent slides on Canada balsam.

For the EPs studies, the specimens were dehydrated according to a protocol adapted from Kashi et al. (2014) for Scanning Electron Microscopy (SEM). The SEM preparations and photomicrographs were made at the 'Laboratório MEV (Microscopia Eletrônica de Varredura)' at Tecnovates, Univates. The images of the EPs were measured, according to Seixas et al. (2015b), using the AxioVision Zeiss LE 4.7.2 software.

Cirrus measurements were taken from extracted cirri mounted on Faure´s mounting medium (F). The terminology used to describe the male reproductive structures followed Seixas et al. (2010).

Photomicrographs of the temnocephalans were taken with the microscope Zeiss Axiolab. The line drawings and photographic images were prepared using Adobe's Fireworks® CS6 and Adobe's Photoshop® CC 2017. Measurements are in micrometers (µm) unless otherwise indicated, ranges are followed (between parentheses) by the mean, the standard deviation values, and the number of specimens measured for a given character (when different than 25). The ecological concepts applied to the symbiotic organisms follow Bush et al. (1997).

The whole mounts of adult and juvenile specimens, as well as slides containing individual cirri mounted on F were deposited in the following scientific collections: 1. 'Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC)', Rio de Janeiro, RJ, Brazil; 2. 'Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata (MLP)', La Plata, Argentina; and 3. 'Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia (INPA)', Manaus, AM, Brazil. Some host specimens were deposited in the 'Coleção de Crustáceos, Departamento de Zoologia, UFRGS', Porto Alegre, RS, Brazil, and 'Coleção Zoológica, Museu de Ciências Naturais da Univates (MCN/UNIVATES)', Lajeado, RS, Brazil. The remaining

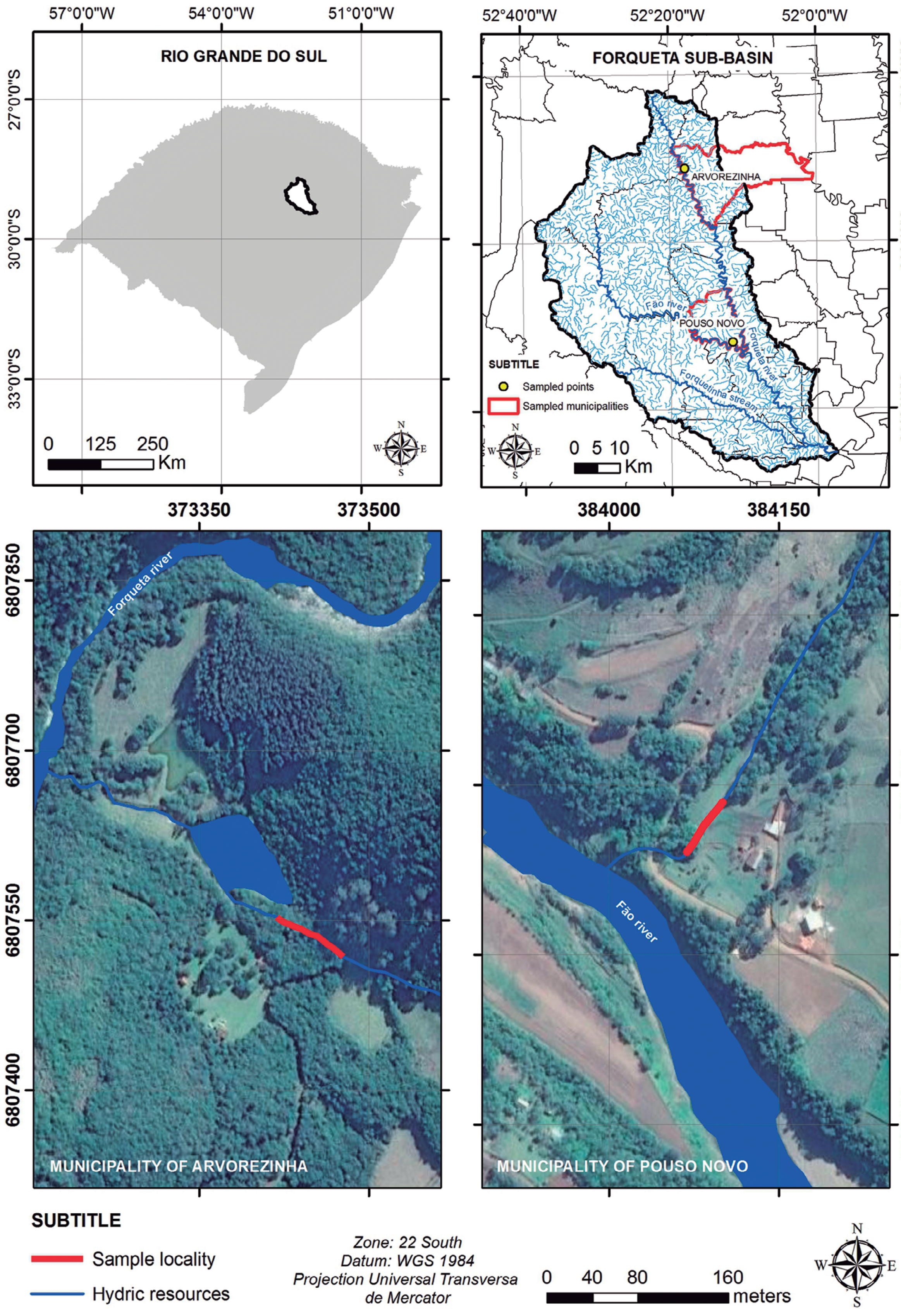

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Figure 1. Map of Rio Grande do Sul showing the Forqueta River Sub-Basin and collection points in the municipalities of Arvorezinha and Pouso Novo.

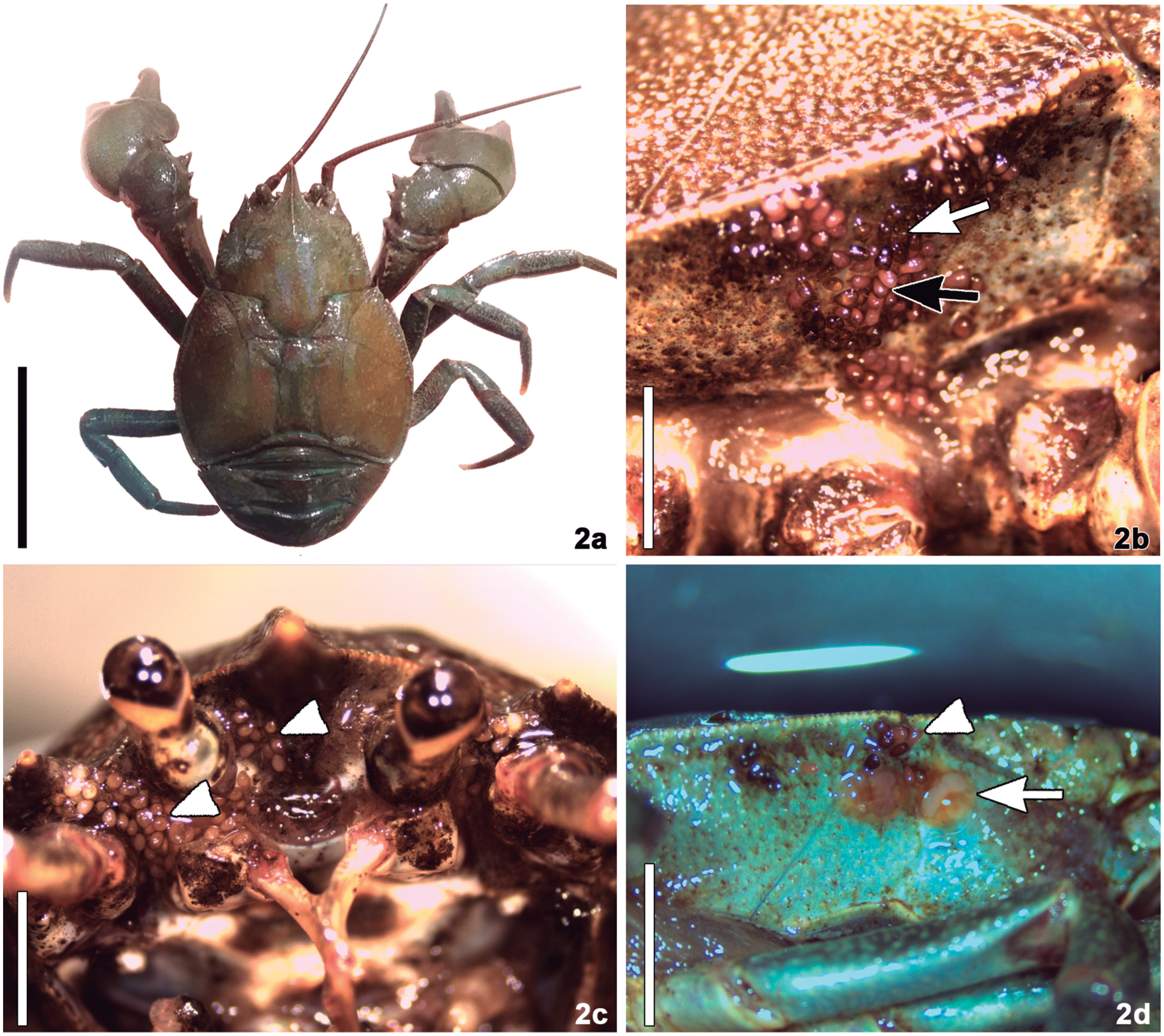

Figure 2. (2a) *Aegla grisella*. Scale bar = 20 mm. **(2b)** Pleural side of carapace showing unhatched eggs (black arrow) and hatched eggs (white arrow) of *Temnocephala grisella* **sp. nov.** Scale bar = 5 mm. **(2c)** Eggs of *Temnocephala grisella* **sp. nov.** deposited in the orbital cavity and mouthparts (head arrows). Scale bar = 2 mm. **(2d)** Pleural side of carapace showing unhatched eggs (head arrow) and live specimens of *Temnocephala grisella* **sp. nov.** (arrow). Scale bar = 5 mm.

specimens are kept in the laboratory for completion of the other studies. All material will be deposited at the 'Coleção Zoológica, Museu de Ciências Naturais da Univates (MCN/UNIVATES)' upon conclusion of these studies.

Results

Description. Based on 63 temnocephalans specimens collected from *A. grisella* and 34 specimens from *A. spinipalma*: 13 whole mounted adults from *A. grisella*, 12 whole mounted adults from *A. spinipalma*, 3 dissected cirri from *A. grisella,* and 2 dissected cirri from *A. spinipalma* measured.

External characteristics. Body (without tentacles) (Figs 3a and 8d) 1.42–4.05 mm (2.65 mm ± 700) long, 1.05–2.39 mm (1.69 mm ± 360) wide; adhesive disk ventral, subterminal, partially covered by the body (Fig. 3a) 276–790 (502 ± 116) long, 434–889 (597 ± 127) wide; disc peduncle 217–632 (424 ± 111) wide. Eyespots with red pigmentation (observations made on live specimens). Two EPs longer than wide (Figs 5 and 6) 347.5–447.5 (397.5 ± 58; 4) total length, 110–132.5 (121 ± 13; 4) total width; length of the anterior portion of the EP from the excretory pore 117–158 (138 ± 24; 4), length of the posterior portion of the EP from the excretory pore 230–289 (260 ± 34; 4); width of the external limit of the EP from the excretory pore 62–69 (65 ± 4; 4); width of the internal limit of the EP from the excretory pore 49–64 (56 ± 9; 4). The excretory pore is almost central horizontally, but displaced to the anterior portion of the plate. Seventeen percent of the total length of the EP is beyond the limit of the tentacles with the body. Ratio of total length of the EPs/total body length (without tentacles): 6.7: 1.

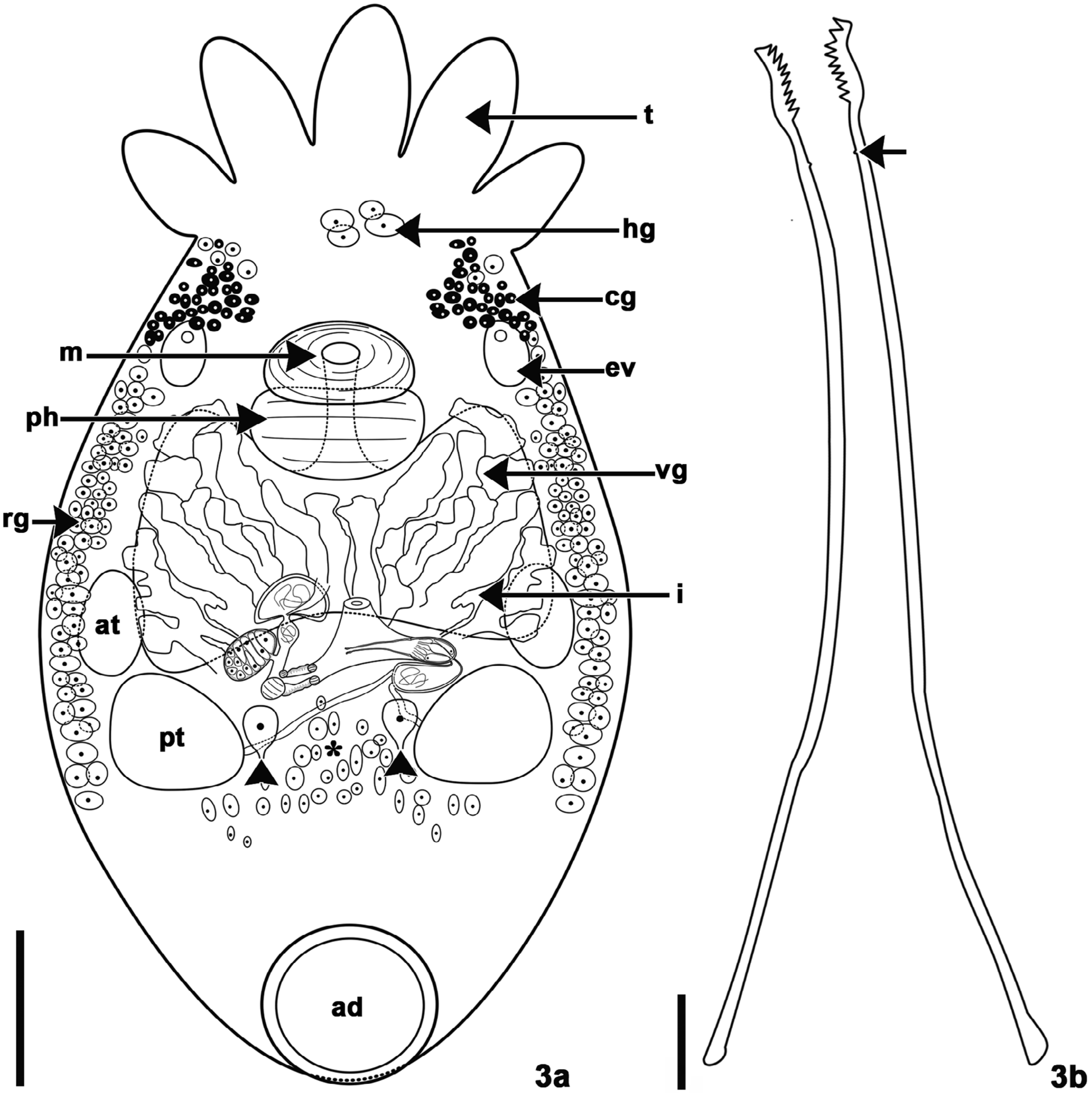

Figure 3. *Temnocephala grisella* **sp. nov. (3a)** Diagram of an adult specimen showing adhesive disk (ad), disc glands (asterisks), anterior testes (at), cyanophilous glands (cg), excretory vesicle (ev), paranephrocytes (head arrows), Haswell glands (hg), intestine (i), mouth (m), pharynx (ph), posterior testis (pt), rhabditogenic glands (rg), tentacles (t), and vitelline glands (vg). Scale bar = 500 µm. **(3b)** Cirrus, showing the proximal limit of the introvert (arrow). Scale bar = 20 µm.

Glands. Rhabditogenic glands (Figs 3a and 8d) forming bunches (average 92 cells) extending from the level of the Haswell glands to the end of the posterior testes, in lateral fields of the body, 30-90 (67 ± 15) in diameter, ducts inconspicuous. Grape-like bunches of cyanophilous glands (Figs 3a and 8d) (average 20 cells), located at the level of the excretory vesicles. Two groups of two Haswell glands (Fig. 3a), showing little affinity with hematoxylin/aceto-carmine/fast green, in front of the cerebral transverse band; diameter of largest cell 65–160 (107 ± 30). Disc glands between adhesive disc and genital complex, 30–80 (52 ± 16; 24) in diameter, including two pairs of large, round, more central paranephrocytes, 52.5–160 (107 ± 32; 24) long (Fig. 3a).

Reproductive system. Female. Vitellarium arborescent and thin (Fig. 3a); vagina elongated 65–137 (99 ± 23; 10) total length (Figs 4a, 8b - 8c); divided into two portions, before (BPS) and after (APS) the proximal sphincter. BPS portion 40–87 (60 ± 15; 9) long, 37–75 (48 ± 11; 9) wide, with thin wall (Figs 4a and 8b); APS portion 25–52 (38 ± 9; 13) long, 40–75 (57 ± 10; 13) wide, with strong muscular wall (Figs 4a, 8a - 8b). Ovary 97–242 (145 ± 29; 23) long, 82–172 (113 ± 25; 23) wide, located in the middle of the BPS portion of the vagina (Figs 4a, 8a - 8c). Proximal vaginal sphincter asymmetrical 62–105 (83 ± 13; 16) total diameter (Figs 4a, 8a and 8c), diameter of anterior portion 15–27 (24 ± 4; 14) (Figs 4a and 8b - apvs), diameter of posterior

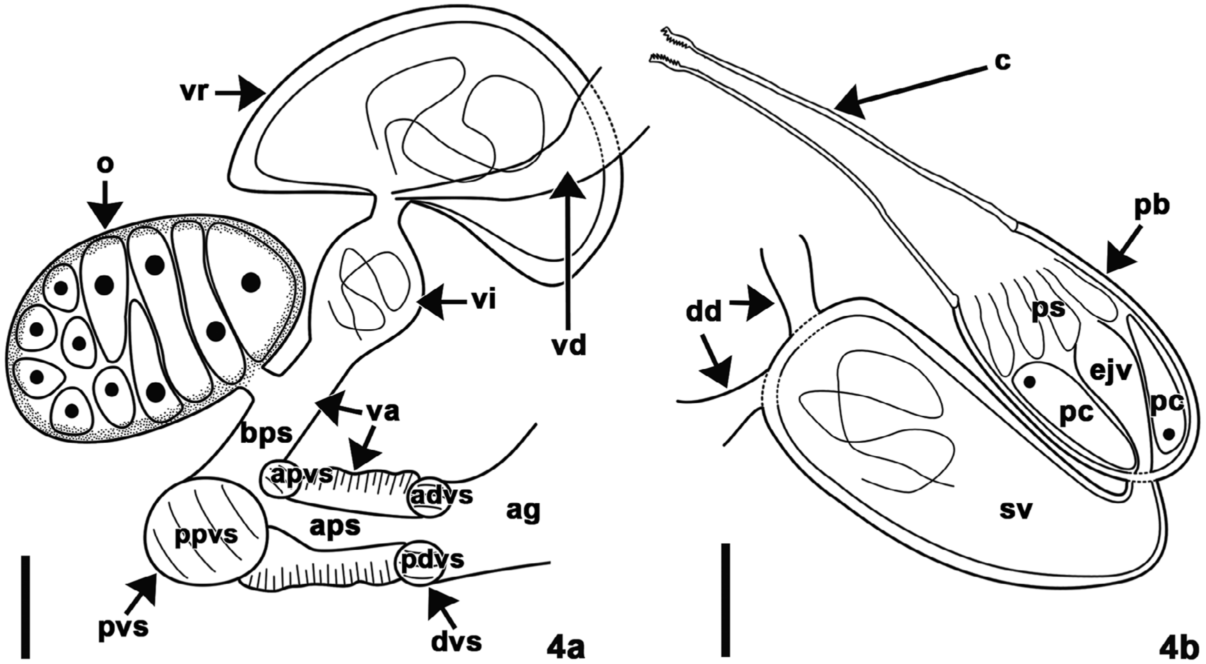

Figure 4. *Temnocephala grisella* **sp. nov. (4a)** Female reproductive system, showing: Anterior portion of the distal vaginal sphincter (advs), after proximal sphincter of the vagina (aps), anterior portion of the proximal vaginal sphincter (apvs), before proximal sphincter of the vagina (bps), distal vaginal sphincter (dvs), genital atrium (ga), ovary (o), posterior portion of the distal vaginal sphincter (pdvs), posterior portion of the proximal vaginal sphincter (ppvs), proximal vaginal sphincter (pvs), vagina (va), vitelline duct (vd), vesicula intermedia (vi), and vesicula resorbens (vr). Scale bars = 100 µm. **(4b)** Male reproductive system, showing: Cirrus (c), deferent vessels (dd), ejaculatory vesicle (ejv), prostatic bulb (pb), prostatic cells (pc), prostatic secretions (ps), and seminal vesicle (sv). Scale bars = 100 µm.

portion 27–65 (45 ± 14; 14) (Figs 4a and 8b - ppvs); distal vaginal sphincter symmetrical 40–85 (56 ± 13; 16) total diameter (Figs 4a, 8a - 8b), diameter of anterior portion 15–37 (24 ± 6; 16) (Figs 4a and 8c - advs), diameter of posterior portion 15–37 (25 ± 7; 16) (Figs 4a and 8c - pdvs). Vesicula intermedia 35–100 (66 ± 21; 11) long (Figs 4a and 8a); vesicula resorbens usually full of sperm, 60–287 (151 ± 60; 15) long, 100–257 (188 ± 44; 15) wide, wall thickness 2.5–22 (10 ± 8; 8) (Figs 4a and 8a).

Male. Four testes rounded to oblique (Figs 3a and 8d); deferent vessels unite in large, pyriform seminal vesicle 70–245 (146 ± 42) long, 52–125 (85 ± 23) wide, wall thickness 2.5–7.5 (4 ± 2; 20) (Figs 4b and 8a); prostatic bulb short, 145–400 (255 ± 64) long, 57–192 (120 ± 33) wide, wall thickness 2.5–20 (11 ± 5; 19) (Figs 4b and 8a); cirrus long and slightly curve 195–212 (202 ± 9; 3) long (Figs 3b, 4b, 7 and 8a); shaft 165–185 (174 ± 10; 3) long, with maximum width at base 65–72 (68 ± 4; 3); introvert 25–30 (27 ± 2; 3) long, with width at base 15 (n=3), with maximum width 15–17 (17 ± 1, 3) at level of swelling. Introvert´s swelling with approximately 27 rows of spines, and 9 short and thick spines in each row (Figs 3b and 7). Ratio of total body length (without tentacles):/total length of cirrus 14.2: 1; ratio of total length of cirrus/maximum width of shaft at its base 3: 1; ratio of total length of cirrus/total length of introvert 7.5: 1.

Taxonomic summary.

Type host. *Aegla grisella* Bond-Buckup & Buckup, 1994 (Crustacea, Anomura).

Other host. *Aegla spinipalma* Bond-Buckup & Buckup, 1994 (Crustacea, Anomura).

Type locality. Tributary creek of the Forqueta river, Perau de Janeiro, Arvorezinha, Rio Grande do Sul, Brazil (28º51’9.85”S; 52º17’55.02”W).

Other locality. Tributary creek of the Fão river (29°12’2.81”S; 52°11’31.80”W), Pouso Novo, Rio Grande do Sul, Brazil (29°12’2.81”S; 52°11’31.80”W).

Site. Branchial chambers and body surface; eggs cemented on external surfaces of exoskeleton (Figs 2b-2d).

Prevalence. 92.4%.

Average intensity of infestation. 14.8

Helminth specimens deposited. ‘Coleção Helmintológica do Instituto Oswaldo Cruz’: CHIOC 38212 (HOLOTYPE); CHIOC 38213 (cirrus). ‘Coleção de Invertebrados do Instituto Nacional de Pesquisas da Amazônia’: INPA 663 (paratype); INPA 664 (cirrus). ‘Colección de Invertebrados, División Zoología Invertebrados, Museo de La Plata’: MLP-He 7100 (paratype); MLP-He 7101 (cirrus).

Host specimens deposited: ‘Coleção de Crustáceos, Departamento de Zoologia, UFRGS’: 6119 - 6142 (*A. grisella*); ‘Coleção Zoológica, Museu de Ciências Naturais da Univates (MCN/UNIVATES)’: ZAUMCN 1072-1076 (*A. grisella*).

Etymology. The specific epithet *grisella* refers to the type host and act as a reminder of the importance of its preservation.

Remarks. The EPs of the species hosted by crustaceans usually present a great variation in shape, however *T. grisella* **sp. nov.** presents an EPs’ shape similar to *Temnocephala pignalberiae* Dioni, 1967 (Seixas et al. 2015b). In the male reproductive system, the seminal vesicle has a thin muscular wall (4 µm thick on average), in contrast with a strong

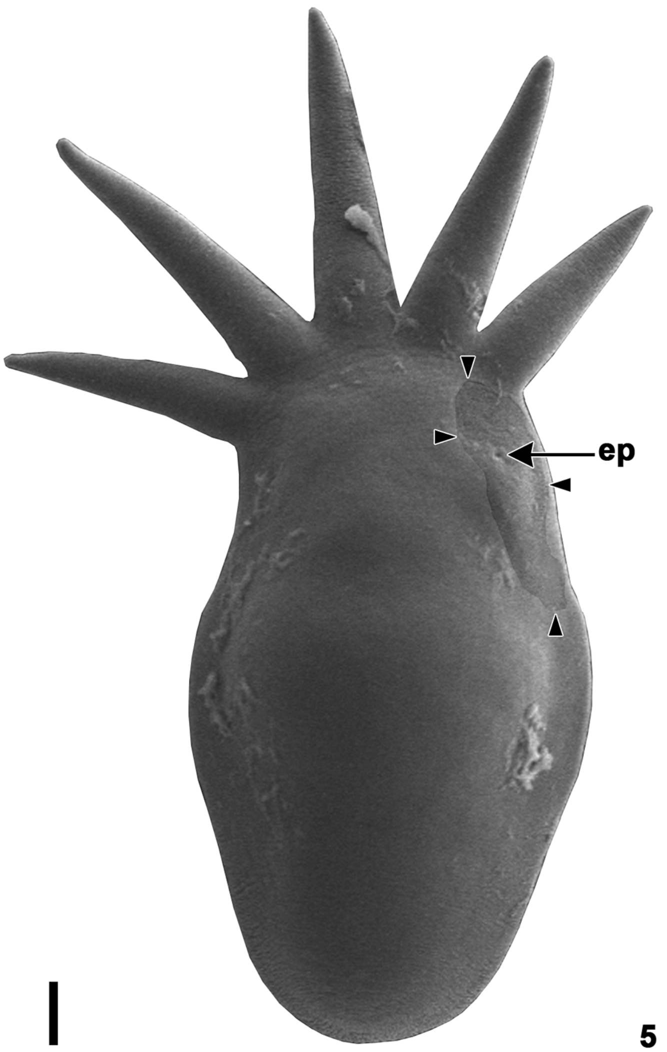

Figure 5. Scanning Electron Microscopy of *Temnocephala grisella* **sp. nov.** showing the highlighted epidermal 'excretory' syncytial plate; excretory pore (ep) and limits of the plate (head arrows). Scale bar = 100µm.

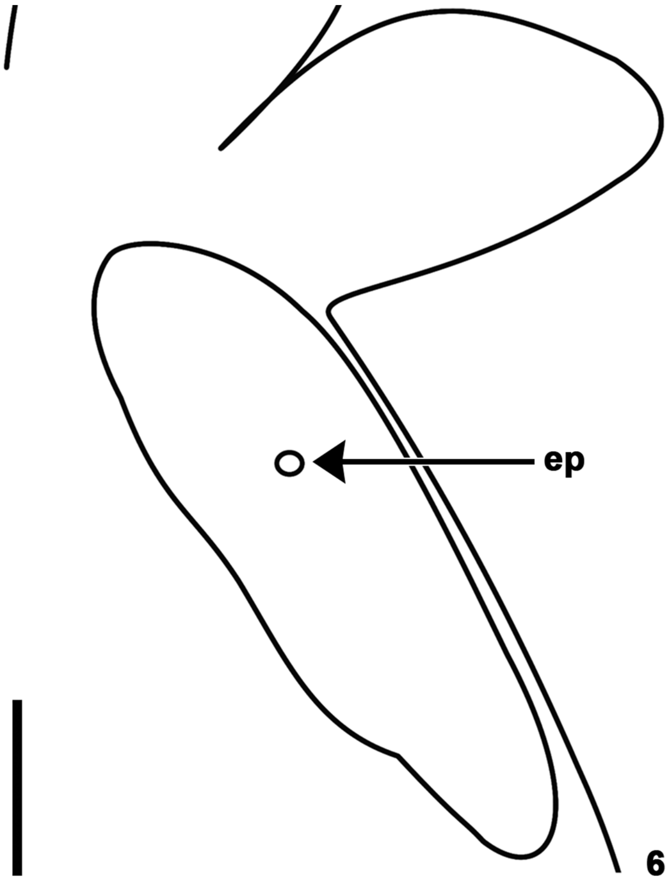

Figure 6. Diagram of the epidermal 'excretory' syncytial plate of *Temnocephala grisella* **sp. nov.**, showing the limit of the plate and the excretory pore (ep). Scale bar = 100µm.

muscular wall of the prostatic bulb (11 µm thick on average). The cirrus showed intraspecific variation of the curvature from straight to slightly curve. The introvert portion of the cirrus has a small variation in length (25-30 µm) but the same measure in width at base (15 µm) in all specimens measured. The total length of the cirrus is three times bigger than the maximum width of the shaft at its base. The posterior pair of the testes is two times bigger than the anterior pair.

Discussion

Temnocephala chilensis, *T. axenos*, *T. talicei* and *T. mertoni* present cirrus measuring, on average, between 123-149 µm long (Damborenea & Cannon 2001, Volonterio 2007), while *T. cyanoglandula* has the largest cirrus among anomuran crabs temnocephalans, having an average length of 256 µm (Amato et al. 2003). Among these species, *T. grisella* **sp. nov.** has a cirrus of intermediate size, measuring 179 µm on average (Table 1). Dioni (1967c) studied specimens of *T. axenos* ectosymbiont on species of *Aegla* and *Parastacus* Huxley, 1879 from Uruguay and Brazil, finding a great cirrus' size variability (125-150 µm), although, due to the lack of data on female reproductive system, is impossible to compare with the new species described in the present work. Nonetheless, both cirri measurements presented by Dioni (1967c) and Volonterio (2007), on their description of Uruguayan specimens of *T. axenos*, differ from that of *T. grisella* **sp. nov.** (Table 1).

Lamothe-Argumedo (1968) also found a great cirrus' size variability (144-206 µm) while re-describing *T. mexicana*. Although the range was similar to *T. grisella* **sp. nov.**, the shape of the cirrus differed. In fact, the cirrus' shape of *T. mexicana* presented by the author differs greatly from the original description of the species. Vayssière (1898) describe the cirrus as a little curved, with an "exsertile" portion at its outer end, meaning that it have a portion projected beyond the organ, which is visible in the diagram provided by the author. The cirrus described by Lamothe-Argumedo (1968) doesn't have this characteristic introvert and was similar to *T. mertoni* by having a slightly sinuous portion in the shaft. The single vaginal sphincter of *T. mexicana,* evidenced by the diagram presented, is also similar to *T. mertoni*. These cirrus and vaginal sphincter characteristics differs *T. mexicana* and *T. mertoni* from *T. grisella* **sp nov.**

Figure 7. Cirrus of *Temnocephala grisella* **sp. nov.**, showing the limit of introvert-shaft (arrow). Scale bar = 25µm.

Damborenea & Cannon (2001), on a neotropical temnocephalans revision, pointed out the absence of any muscular structure (sphincter) in *T. chilensis* vagina. They also recorded a conic cirrus with a swollen introvert measuring an average of 149 µm in length. All these characteristics differs from *T. grisella* **sp nov.** The authors also assert the lack of sphincter on *T. talicei*. Volonterio (2009), while re-describing *T. talicei*, has shown the existence of a single conspicuous and asymmetric distal sphincter, pointing out similarities of this species with *T. mertoni.* Likewise *T. talicei* and *T. mertoni*, *T. cyanoglandula* also have only one vaginal sphincter, which is also distal and asymmetric (Seixas et al. 2015a).

Temnocephala dionii have a unique cirrus with a “groove between introvert and the shaft” and a single vaginal sphincter (Ponce de León et al. 2015), which differentiates *T. dionii* from the new species. This cirrus’ shape characteristics have some similarities with the original description of *T. mexicana*’ cirrus, pointing out the importance of a future revision of *T. mexicana*.

Temnocephala axenos, alike *T. grisella* **sp. nov.**, has two vaginal sphincters, one proximal asymmetric and a symmetric distal, but they greatly differ on size. In the re-description made by Volonterio (2007), *T. axenos* presented both vaginal sphincters with similar average sizes (43 µm proximal e 45.5 µm distal), whereas *T. grisella* **sp. nov.** presents one proximal large vaginal sphincter (83 µm on average) and a smaller distal one (56.5 µm on average). The author also pointed out that both sphincters of *T. axenos* are located at the final portion (distal) of the vagina, very close to one another. In contrast, in *T. grisella* **sp. nov.**, the vaginal portion between the proximal and distal sphincters is quite long with a strong muscular wall (Fig. 4a - aps), measuring 38 µm in length on average. Therefore, in *T. grisella* **sp. nov.**, the proximal sphincter is located in the middle of the vagina, while the distal one is at the tip end of the organ. The total vaginal length of *T. grisella* **sp. nov.** is larger than *T. axenos*, and both species have a vesicula intermedia, rather than seminal receptacles, that it is also slightly larger in specimens of *T. grisella* **sp. nov.** (Table 1).

Volonterio (2007) described *T. axenos*’ EPs like “elliptical excretory syncytia, small, extends from base of external tentacles to level of anterior portion of intestine”, however she did not provide character measurements. *Temnocephala grisella* **sp. nov.** has elongate EPs, wider in the area surrounding the excretor pore. The excretory pore is central and in the anterior portion of the plates. *Temnocephala grisella* **sp. nov.**’ EP is the larger in total length (397.5 µm on average) among epibionts temnocephalans on crustaceans already registered, *T. cyanoglandula*’s being the second largest, with a total length of 284.4 µm on average (Seixas et al. 2015b). The larger than wide EPs of *T. grisella* **sp. nov.** are evidenced with the ratio of total length of the EPs/total body length. Six EPs, approximately, could occupy the total length of the body while in the wider than long EPs’ species, such as *Temnocephala trapeziformis* Amato, Amato & Seixas, 2006 (Seixas et al. 2015b), 17 EPs are necessary to occupy the total length of the body.

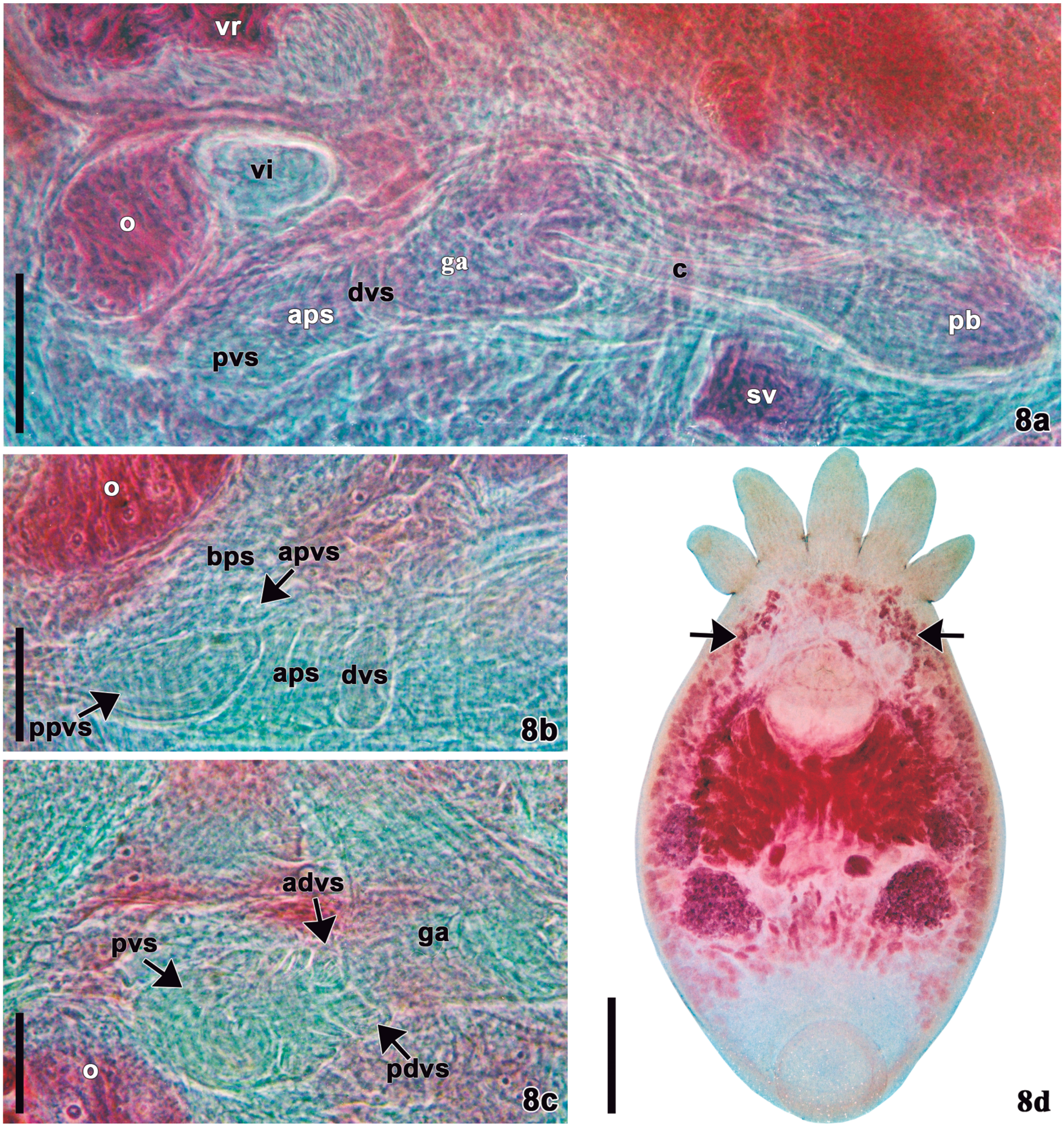

Figure 8. *Temnocephala grisella* **sp. nov. (8a)** Reproductive system, showing: after proximal sphincter - vagina (aps), cirrus (c), distal vaginal sphincter (dvs), genital atrium (ga), ovary (o), prostatic bulb (pb), proximal vaginal sphincter (pvs), seminal vesicle (sv), vesicula intermedia (vi), and vesicula resorbens (vr). Scale bar = 100 µm. **(8b-8c)** Partial female reproductive system. **(8b)** after proximal sphincter of the vagina (aps), anterior portion of the proximal vaginal sphincter (apvs), before proximal sphincter of the vagina (bps), distal vaginal sphincter (dvs), ovary (o), and posterior portion of the proximal vaginal sphincter (ppvs). Scale bars = 50 µm. **(8c)** anterior portion of the distal vaginal sphincter (advs), genital atrium (ga), ovary (o), posterior portion of the distal vaginal sphincter (pdvs), and proximal vaginal sphincter (pvs). Scale bars = 50 µm. **(8d)** Adult specimen, showing the cyanophilous glands (arrows). Scale bar = 500 µm.

Table 1. Morphometric data from *Temnocephala grisella* (present work) comparing with *Temnocephala mertoni* and *Temnocephala axenos* (Volonterio 2007). Measurements are in micrometers (µm), unless otherwise indicated.

	***Temnocephala mertoni* Volonterio (2007)** **Range (mean ± SD; N)**	***Temnocephala axenos* Volonterio (2007)** **Range (mean ± SD; N)**	***Temnocephala grisella* (present work)** **Range (mean ± SD; N)**
Total body length (without tentacles)	1,04 - 1,48 mm (1,26 ± 0,14; 11)	1,16 - 2,66 mm (1,75 ± 0,44; 13)	1.42 - 4.05 mm (2.65 ± 0.70; 25)
Total body width	0,50 - 0,73 mm (0,64 ± 0,08; 11)	0,82 - 1,83 mm (1,44 ± 0,35; 13)	1.05 - 2.39 mm (1.69 ± 0.36; 25)
Vagina (total length)	58 - 92 (75 ± 12; 15)	38 - 107 (70 ± 22; 15)	65 - 137 (99 ± 23; 10)
Proximal vaginal sphincter (total diameter)	_	31 - 60 (43 ± 9; 15) A	62 - 105 (83 ± 13; 16) A
Distal vaginal sphincter (total diameter)	36 - 56 (44 ± 6; 15) A	27 - 62 (45.5 ± 12; 15) S	40 - 85 (56 ± 13; 16) S
Vesicula intermedia (length)	29 - 127 (56.5 ± 24; 15)	33 - 91 (50 ± 17; 14)	35 - 100 (66 ± 21; 11)
Seminal vesicle (length)	83 - 176 (130 ± 26; 15)	81 - 212 (146 ± 43; 15)	70 - 245 (146 ± 42; 25)
Seminal vesicle (width)	43.5 - 107 (79 ± 21; 15)	58 - 154 (90 ± 27. 14)	52 - 125 (85 ± 23; 25)
Prostatic bulb (length)	83 - 136 (108 ± 15; 14)	69 - 161 (108 ± 33; 15)	145 - 400 (255 ± 64; 25)
Prostatic bulb (width)	54 -100 (82 ± 13; 15)	47 -107 (68 ± 19; 14)	57 - 192 (120 ± 33; 25)
Cirrus (length)	123 -158 (138 ± 10; 15)	129 -163 (141 ± 11; 15)	195 - 212 (202 ± 9; 3)
Shaft (width at base)	38 -56 (46 ± 6; 15)	31 -54 (42 ± 6; 15)	65 - 72 (68 ± 4; 3)
Introvert (length)	24-31 (27 ± 3; 15)	18 -29 (24 ± 3; 14)	25 -30 (27 ± 2; 3)
Introvert (maximum width)	13-16 (14 ± 1; 15)	11 -13 (12 ± 0.9; 14)	15 - 17 (17 ± 1;3)

SD = Standard deviation; N = number of specimens measured for a given character; A = asymmetric; S = symmetric.

Temnocephala mertoni' EPs have a similar shape to the new species described in the present work, however their measurements, as well as *T. axenos*, have not been provided. Observing the illustrations of the species description, *T. mertoni*' EPs extend from the limit of the tentacles up to the intestine (Volonterio 2007), while in *T. grisella* **sp. nov.**, the EPs start before the limit of the tentacles and extend until almost the half of the intestine, suggesting a larger overall size.

Amato et al. (2003) distinguished *T. cyanoglandula* by the unique appearance of its cyanophilous glands, which form "two irregular-shaped, grape-like bunches of cells, located in the anterior portion of the body, at the level of mouth and pharynx". The same structures are visible in *T. grisella* **sp. nov.** (Figs 3a and 8d), but, even utilizing the same staining methods, they do not appear to be as conspicuous nor its ducts visible, as they are in *T. cyanoglandula*. According Volonterio (2007), *T. axenos* has four paranephrocytes and *T. mertoni*, one pair. Similar to *T. mertoni*, *T. grisella* **sp. nov.** also have one pair of large, round, more central paranephrocytes (Fig. 3a - head arrows).

The host *A. grisella* was included in the list of endangered species of the State of Rio Grande do Sul in the category of vulnerable, according to IUCN (The World Conservation Union) criteria (Marques et al., 2002). In addition, the sampling point at the municipality of Arvorezinha, although well preserved, has recently been threatened by the construction of a hydroelectric power plant. The 'Perau de Janeiro' is home to some records of highly restricted and endemic species, such as the amphibian *Melanophryniscus admirabilis* Di Bernardo, Maneyro & Grillo, 2006, a critically endangered species (Fonte et al. 2014). Due to these threats, the records of associated fauna of these crustaceans at this locality becomes even more necessary and can aid in the conservation policy of aeglids and its epibionts, as well as the environment that has undergone a series of actions that threaten the original biodiversity of the region. The accelerated process of degradation becomes even more worrying in a region where a great part of the fauna is still unknown, thus, it is essential to carry out taxonomic, ecological and environmental studies whose results make the elaboration of conservation actions possible.

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Author contributions

Samantha Alves Seixas - 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.

Norton Dametto - Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation;

Eduardo Périco - 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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Non-invasive hair sampling of Neotropical otters

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Abstract: Sampling wild animal populations using non-invasive techniques is advised when dealing with threatened species. Hair samples provide ecological information like species and individual identification. However, hair trapping is scarcely used in otters, due to their aquatic habits. Most studies are with captive individuals, so there is the need to test non-invasive hair trapping methods in otters in the wild. The aim of this study was to develop a simple and cost-effective method to collect hair from otter species in a non-invasive way. The study was carried out in the Paranapanema River, São Paulo State, Brazil, with the Neotropical otter (*Lontra longicaudis* Olfers, 1818), a protected species. Hair traps (wooden sticks and tree roots with adhesive tape or wax bands) were set during six nights on river banks, otter trails and scent-marking sites. Traps were baited with otter fresh spraints from other river locations. From the 23 traps, 10 (43.7%) were successful in collecting otter hairs, mostly guard-hair. The sticks were much more efficient than the roots at capturing otter hair (70.6.% vs. 0%) as well as adhesive tape when compared to wax (71.4% vs. 0%). Method simplicity and efficiency suggest that it can be a cost-effective way for collecting otter hairs without the need for capturing individuals. This method can be used for: assessment of local otter distribution; collecting otter hair samples for sex and individual identification (by molecular analysis), trophic ecology (by isotopic analyses), ecotoxicology (by contamination analysis) or behaviour ecology (by hormonal and stress levels analysis). More trapping campaigns should be implemented to further test the method's efficiency.

Keywords: *hair trap, Lontra longicaudis, non-invasive methods, Brazil.*

Coleta não invasiva de pelos de lontra Neotropical

Resumo: O uso de técnicas de amostragem não-invasivas é aconselhado quando se trabalha com espécies ameaçadas de animais selvagens. Amostras de pelo fornecem informações ecológicas, como a identificação ao nível da espécie e do indivíduo. No entanto, a coleta de pelo é pouco usada em lontras, devido aos seus hábitos aquáticos. A maioria dos estudos é feita com indivíduos em cativeiro, existindo por isso a necessidade de testar métodos não invasivos de coleta de pelos de lontras na natureza. O objetivo deste estudo foi desenvolver um método simples e com uma boa relação custo-benefício para coletar pelos de espécies de lontra de maneira não invasiva. O estudo foi realizado no rio Paranapanema, Estado de São Paulo, Brasil, com a lontra Neotropical (*Lontra longicaudis* Olfers, 1818), uma espécie protegida. Armadilhas de pelo (estacas de madeira e raízes de árvores com fita adesiva ou bandas de cera depilatória) foram colocadas durante seis noites nas margens do rio, em trilhas e locais de marcação de lontra. As armadilhas foram iscadas com dejetos frescos de lontra de outros locais do rio. Das 23 armadilhas, 10 (43.7%) foram eficazes na coleta de pelos de lontra, maioritariamente pelos-guarda. As estacas foram muito mais eficientes que as raízes na captura de pelos de lontra (70.6.% vs. 0%) tal como a fita adesiva quando comparada com a cera (71,4% vs. 0%). A simplicidade e a eficiência do método sugerem que esta pode ser uma maneira econômica de coletar pelo de lontra sem a necessidade de capturar indivíduos. Este método pode ser usado para: levantamento da distribuição local da lontra; coleta de amostras de pelo de lontra para identificação sexual e individual (por meio de análise molecular); ecologia trófica (por meio de análise isotópica); ecotoxicologia (por meio de análise de contaminantes); e ecologia comportamental (por meio da determinação de níveis hormonais reprodutivos e ligados ao estresse). Mais campanhas de armadilhagem devem ser implementadas para melhor avaliar a eficiência do método.

Palavras-chave: *armadilha de pelo, Lontra longicaudis, métodos não-invasivos, Brasil.*

Introduction

Monitoring or sampling wild animal populations is important to increase our scientific knowledge on the structure and functioning of ecosystems and to inform managers and decision-makers, particularly when dealing with elusive and low density species, still lacking relevant ecological information. At the same time, there is an increasing pressure from the public, environmental and scientific bodies to use non-invasive techniques, in particular when surveying mammals (Sikes & Gannon 2011).

Among the non-invasive sampling methods that have been developed to survey mammals, hair samples have the advantage of providing ecological information, like species and individual identification (e.g. Foran et al. 1997), hormonal and stress levels (Koren et al. 2002, Macbeth et al. 2010), diet reconstruction based on carbon and nitrogen isotope values (e.g. Hobson et al. 2000, Mitani et al. 2009, Walter et al. 2014) and exposure to environmental contamination (e.g. Pereira et al. 2006). Hair traps are commonly used to sample hairs from wild free-moving animals (e.g. Baker 1980, Mowat & Strobeck 2000, Vine et al. 2009, Monterroso et al. 2014) as they do not require checking on a daily basis such as snares and leg-hold, Hancock or box traps. However, hair trapping in semi-aquatic mammals is scarcely used due to their aquatic habits while active that difficult its implementation.

Otters are semi-aquatic mammals, most of them elusive and shy species, that rest on land but use water in most of its daily activities and most of the times their dens are on the water edges opening directly to water (Kruuk 2006). Traditional capture methods, such as box/cage traps, tend therefore to have low capture success (Kranz 1995). Although there are some studies addressing the practicability of various field techniques for capturing and radio-tracking otters (e.g. Ó Néill et al. 2008), modified body-snares or foot-hold traps (Ben-David et al. 1998, Newsome et al. 2009) or Hancock traps (Mitchell-Jones et al. 1984) are not advised due to safety concerns (e.g. sensitivity to stress) especially since all but one (North American river otter - *Lontra canadensis* Schreber, 1777) out of the thirteen existing otter species in the world have a conservation status of Endangered (EN), Vulnerable (VU) or Near Threatened (NT) and decreasing population trends in most of their range (IUCN 2017). Nevertheless, most of otter species still lack information in some critical parameters, such as population size and structure, occupancy patterns, or genetic diversity. Therefore, non-invasive methods that supply biological samples such as hair trapping should be pursued.

There are still very few published studies on otter hair trapping methods and most of them with captive individuals. To obtain DNA-based population estimates of river otter, Depue & Ben-David (2007) developed and tested, first in captivity and then in the wild, three traps to capture otter hairs: 1. modified body-snare, 2. modified foot-hold trap, and 3. bucket trap. The first two provided an effective technique for obtaining hair from individual river otters, with a success rate of 94% and 3–20 guard hairs per capture. Another study, directed to the test methods for estimating the number and recording the distribution of river otter (Johnson et al. 2013), used wire body snares and knaplock hair snag to collect otter hairs for genotyping with the first being more effective than the second. Their data suggested that hair collection was an efficient and cost-effective technique for monitoring the abundance of river otter with known latrine sites. In regard to both studies, and although they did not restrained animals in any trap type, one might argue that breakaway body-snares still causes some type of stressful otter interaction with the trap.

Two other studies were conducted with captive Eurasian otters (*Lutra lutra* Linnaeus, 1758). Anderson et al. (2006) developed a successful hair trapping method with two clay pipes with Velcro straps with cylindrical geometry placed following initial trails. The success rate of the trap was 0.71 hair samples collected per trap night (five nights out of seven) and hair was successfully used for DNA extraction; however, otters only approached the traps after these were baited with chicks. The other study was conducted by Kuhn (2010) who tested two designs of hair traps (a wooden box and sticks stuck into the soil), two distinct materials (a double-sided adhesive tape and the hook side of Velcro brand fastener) and three types of lures in the box (food - chicks, foreign spraints and cod liver oil). The 6-day test resulted in successful capturing otter hairs, and up to 52 hairs/24 hours were found glued on the adhesive tape that was fastened to the lid of the box. The set of sticks with Velcro strips was the less effective device with only five hairs collected in four days and spraints were the most effective lure.

The approaches above described revealed high potential for successful hair trapping but there is still the need to test non-invasive hair trapping methods in otters in the wild. They also addressed just two other species, the North American river otter and Eurasian otter, the two most know otter species.

The Neotropical otter (*Lontra longicaudis* Olfers, 1818) is considered “Near Threatened”, both on a global scale (Rheingantz & Trinca 2015) as well as in Brazil (Rodrigues et al. 2013). Robust ecological information is still lacking on population size, area of occupancy, genetic diversity, population connectivity, as well as on interactions with man (Barbieri et al. 2012, Rheingantz & Trinca 2015), and exploring the application of non-invasive techniques for collecting hair samples is important.

The aim of this study was to develop a simple, cost-effective and rapid method to collect otter hair samples in a non-invasive way for future use in distribution studies, and in isotopic and molecular analysis. To our knowledge this is the first study of Neotropical otter hair capture method.

Material and Methods

The study was carried out in a 10 km stretch of the Paranapanema River, in Angatuba and Paranapanema municipalities, in São Paulo State, Brazil (Figure 1). This river stretch is located downstream of the Jurimirim hydroelectric station, with bordering landscape composed of riverine forests (with some degree of human disturbance including areas used by fishermen, small fishing and living houses, stretches of riparian deforestation) and agro-silvo-pastoral fields (e.g. *Eucalyptus*, corn and cotton plantations, cattle breeding pastures) (IBGE 2017). The area is characterized by a sub-tropical climate, with rainy summers and dry winters, and average temperatures of 22°C in the warmest month (March) and 17°C in the coldest (June) (CEPAGRI 2017).

This study site was chosen because regular presence of Neotropical otters was previously detected, based on fresh spraint (term used specifically for otter scats) daily observation and the identification of

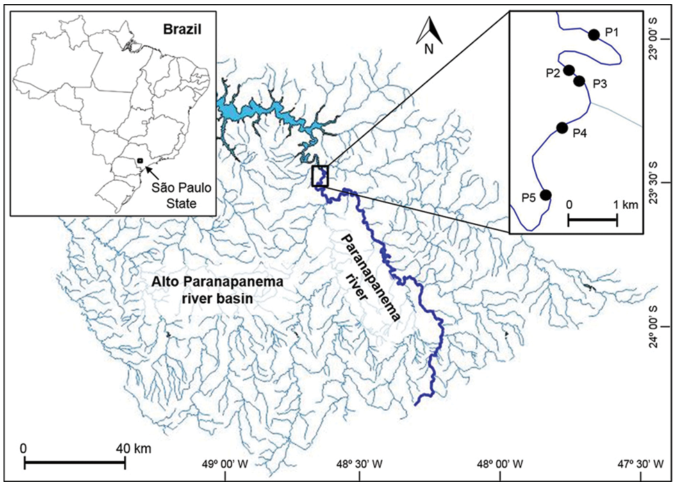

Figure 1. Study area location within Alto Paranapanema river basin, São Paulo State, Brazil. Black dots indicate sampling points (from P1 – 23º28´50´´S, 48º37´12´´W to P5 - 23º30´57´´S, 48º37´55´´W)

several holts. Otters use holts and shelters even near disturbed areas (Santos & Reis 2012). Nevertheless we selected sampling points outside the main human fishing areas and with adequate riparian cover. Hair traps consisted of rectangular 60 cm wooden sticks with double-sided adhesive tape (Axton 50mm light brown) wrapped around the part of the stick that was not buried. A variation was also tested using wax bands instead. When superficial tree roots were available these were preferred to sticks, as the first are more natural elements to otter habitat and involved less costs (Figure 2). Attaching the adhesive tape to roots was more difficult than the wax bands so the latter were more used in the roots. A total of 23 traps (17 sticks and six tree roots) were set on river banks, on otter trails and scent-marking sites. The number (ranging between two and seven) and type of trap that were placed at each sampling point depended on the area, structure, and substrate at the site. The low number of used roots reflects their low availability near the sampling points. The distance between the sticks varied between 40 to 60 cm depending on the site characteristics. Trap deployment was conducted on the 30th May 2017.

To attract otter to the traps, we decided not to use traditional baits as fish, as otters do not readily take fish that they have not caught themselves (Kruuk 2006). Based on the knowledge that otters are territorial and mark intensively their territories and that spraint deposition is associated with territoriality and resource defence, and it is a powerful mean of intraspecific communication (Kruuk 1992), we baited each trap with fresh spraints from the same river but from other locations, the further possible from the sampled point to maximise the probability of being from a different individual. Sprants also have the advantage of decomposing slowly and not being eaten by other species nor stolen (Kuhn 2010).

Hair traps were checked after two and six nights. In the first checking, collected hairs were removed with sterile forceps and placed in individual plastic bags for future processing in the laboratory. Hair traps were removed at the end of day 6 since on the last night started to rain intensively and river water level started to increase, and would soon submerge some of the traps. During the sampling period there were no rain and water level remained stable. To avoid species misidentification, all sampled hairs were processed for observation of cuticular impressions and medullar pattern (Quadros & Monteiro-Filho 2006a, Martin et al. 2009) and identified to species level using mammal hair keys and guides (Quadros & Monteiro-Filho 2006b, Kuhn & Meyer 2010, Quadros & Monteiro-Filho 2010). To check for differences between visits (two and six nights), we run a statistical test for "difference between two proportions" using STATISTICA software (version 13).

Results

Four of the five sampling points gave positive results. From the 23 traps, 10 (43.7%) were successful in collecting otter hairs, mostly guard-hair (Figure 3, Table 1). Other otter hairs were also collected but as only guard-hairs are used for processing, we only considered these.

The sticks were much more efficient than the roots at capturing otter hair (70.6.% vs 0%) as well as adhesive tape when compared with wax (71.4% vs 0%).

In the second visit to the traps (after six nights) some sticks were muddy and four sampling points were marked with spraints and the remaining two had claw marks, confirming that otters visited the sampling points during the study period. In the first visit (after two nights) just one trap had collected otter hairs (five in total, two of which

Figure 2. Sampling with sticks (left) and using roots (right)

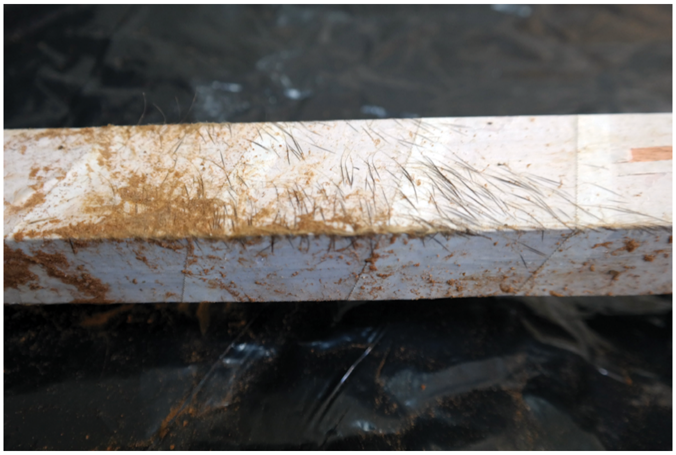

Figure 3. Otter hair trap (stick with adhesive tape) with dozens of otter hairs

Table 1. Number of Neotropical otter guard-hairs collected with adhesive tape and wax fastened to wooden sticks and tree roots in Paranapanema river (São Paulo State, Brazil)

Trap	Sampling Point	Stick (S)/Root (R)	Adhesive tape/Wax	Number of collected otter hair	
				After 2 nights	After 6 nights
1	1	S1	Adhesive tape	2	74
2	1	S2	Adhesive tape	0	0
3	1	S3	Adhesive tape	0	3
4	1	S4	Adhesive tape	0	7
5	1	S5	Adhesive tape	0	0
6	2	S1	Wax	0	0
7	2	S2	Adhesive tape	0	0
8	2	S3	Adhesive tape	0	32
9	2	S4	Adhesive tape	0	30
10	2	S5	Adhesive tape	0	6
11	2	S6	Adhesive tape	0	3
12	2	R1	Wax	0	0
13	3	S1	Adhesive tape	0	236
14	3	S2	Adhesive tape	0	331
15	4	S1	Adhesive tape	0	22
16	4	S2	Adhesive tape	0	0
17	5	S1	Wax	0	0a
18	5	S2	Wax	0	0b
19	5	R1	Wax	0	0
20	5	R2	Wax	0	0
21	5	R3	Wax	0	0
22	5	R4	Wax	0	0
23	5	R5	Wax	0	0

[a]23 and [b]19 hairs of *E. barbara* were collected in this trap

guard-hairs). After six nights, many more hairs were collected in all sites (p = 0.0005 - significant difference of proportions of sticks with adhesive tape between visits; p = 0.009 - significant difference of sticks between visits). Some hairs were removed in the field with forceps and that proved to be more difficult with wax traps than with adhesive tape. Therefore, hair was removed from several sticks afterwards in the laboratory to prevent further manipulation in the field. Although field observation already pointed that, with the exception of two traps, no hair from other species was collected, this was additionally confirmed by cuticular impressions and medullar pattern observation. The non-otter hairs were of tayra (*Eira barbara*) and here the only hair trapped in wax.

We found that tayra hair captured in wax is far more difficult to clean than otter hair from adhesive tape (both had 70% - alcohol applied but wax needed a longer cleaning process). In addition, the former presented a more difficult cuticular identification probably due to both the more extensive clean-up process and to the initial damaging by the wax.

Discussion

The simplicity and efficiency of the method used suggest that it can be a cost-effective way for collecting otter hairs without the need for capturing individuals. The use of fresh spraints may induce the investigation by other otters and may be a key element for the method efficiency. Males may respond more intensively to fresh spraints than females and this may have influence on the type of study and objectives, so further tests including molecular analysis are needed.

Except for tayra, no hair from other non-otter species otter was collected. This is probably due to the specificity of the sampling points (steep margins near water in trails mostly possible to be accessed by semi-aquatic species rather than terrestrial species) and due to the use of fresh otter spraints as bait. Such specificity helps diminishing cross-contamination of DNA based on hair samples. The tayra is a typical forest species but also can be found in the vicinity of aquatic habitats (Nowak 1999) and can also use altered environments that provide resources (e.g. agriculture and plantations) (Presley 2000, Hunter & Barrett 2011) as was the case of the surrounding environment of the sampling point were the hairs were collected.

The higher capture success in sticks when compared with roots was rather surprising as we expected that the latter, being more natural elements to the otter habitat, would capture more hair. A possible reason is the novelty in the otter environment investigated by the resident otters. Nevertheless, we must remind that the low availability of adequate roots near the sampling points led to an unbalanced number of the two types of traps used. Although both glue types captured hairs, only adhesive tape captured otter hairs. Additionally wax proved to be more inadequate as it damages the hair and decreases effectiveness of cuticular impressions and future molecular analysis. For this we recommend adhesive tapes.

This was a short-term study (six nights) and capture efficiency may vary if a longer capture campaign is conducted and with a higher number of traps. However, the minimum capture efficiency of our hair traps (one otter sample/2–6 trap-nights) was similar to the results of Depue & Ben-David (2007) for river otter (one otter capture/2–228 trap-nights) and higher than live-capture rates for the same species (one otter capture/21–315 trap- nights; Melquist & Hornocker 1983, Shirley et al. 1983, Serfass et al. 1996, Blundell et al. 1999, Belfiore 2008). The significant difference between the success of otter hair capture after two (low) and after six (high) days may be result of otters not using that specific part of the territory every night, so larger periods of sampling (several days/weeks) will improve capture efficiency.

The capture success in different study sites is presumably related to otter abundance (Depue & Ben-David 2007). In this study, although otter numbers are not known, the daily presence of new otter spraints in most of the sampling points may suggest a high local otter density. Nevertheless it can also be a response of the same individual(s) to novel elements in its territory due to the short duration of the study (no time for habituation) and this needs further investigation. The high capture efficiency and success of hair traps was also likely a result of the high availability of otter holts and/or presence of otter signs of presence where the traps were deployed.

Our method can be used for the following tasks: a) assessment of local otter distribution; b) collecting otter hair samples for sex and individual identification (by molecular analysis); trophic ecology (by isotopic analyses); ecotoxicology (by contamination analysis) or behaviour ecology (by hormonal and stress levels analysis). Additionally, hair samples have proved to be a much more reliable (Anderson et al. 2006) and cost-effective (Johnson et al. 2013) source of DNA than spraints. Molecular and isotopic analysis success from the capture hairs in this study will be explored in future works but at the moment we can report that 11 otter hairs were selected for isotopic analyses and successfully used for establishing an isotopic signature and trophic niche for the Neotropical otter.

This method can be implemented in other regions for otter species with identified holts and/or tracks, in areas of usual otter marking. The degree of success is expected to be lower in areas where the habitat use is low, or where it is more difficult to identify otter holts (e.g. Eurasian otter in Mediterranean habitats where holts are mainly under vegetation), or in areas of low otter population density.

There are some possible method limitations that should be considered. One is loss of adhesive capabilities. These type of materials can lose their stickiness because of humidity but using stronger adhesive types may be more intrusive to animals. Consequently we advise not the use of stronger adhesive tape or wax but instead to check traps for loss of adherence and change tapes if and when necessary. In our case, during the six days of trapping, we had no need to change the adhesive tape or the wax but we advise checking for stickiness loss every three days especially in tropical (humid) environments. Also, the use of glue material in animal tissue may decrease effectiveness for ecotoxicological or molecular analysis. An alternative could be the use of velcro stripes but they seem to be less effective in capturing hairs, especially hairs with roots that are needed for molecular analysis. We must also take into consideration the possibility of water level variation in the river or stream were this method will be applied. Tropical environments (not only but especially) can be subjected to sudden and intense water level variations due to intense rain. If traps are subjected both to rain and especially to submersion, trap efficiency and hair sample analysis will decrease considerably. Consequently trapping campaigns should be conducted outside high rain and humidity periods if possible. Additionally, the use of these traps can affect or harm other animal such as insects. We did not found many insects captured in the traps, nevertheless, this is always a risk. Trap deployment should try to minimize non-targeted accidental catches.

More trapping campaigns should be implemented to further test the method's efficiency, with a higher number of trapping sites and a longer duration. Also, molecular analysis of the hairs collected should be performed to test individual identification, and test the DNA extraction efficiency in relation with hair cleaning processes. Although wooden sticks and tree roots can be used, sticks appear to be considerably more attractive for otter scent marking. Additionally, the use of other otter spraints as bait should be tested in relation with the distance from the sampling points as the response individuals might, by hypothesis, be stronger to neighbours than to distant animals.

Author Contributions

Nuno M. Pedroso: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Sofia V. Dias: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Thais Rovere Diniz-Reis: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Margarida Santos-Reis: Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Luciano Martins Verdade: Contribution to data collection; Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision, adding intellectual content.

Conflicts of interest

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

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Inventory

Ichthyofauna of the Una river in the Paraíba do Sul Paulista River Valley, Southeastern of Brazil

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Abstract: This study focus on an inventory of the ichthyofauna of the Una river, a tributary of the Paraíba do Sul river, located in the region of Paraíba do Sul River Valley, in the State of São Paulo. Sampling was carried out in three sampling areas along the channel of the Una river between April 2016 and March 2017. For the collection of fish specimens, was used angling, fyke nets, cast nets, dragnets and hand nets. A total of 1.534 specimens were collected, which corresponds to five different orders, 14 families, 26 genera and thirty species. The study revealed that the most significant number of reported species are from the orders Siluriformes and Characiformes. The Characidae family was the most representative concerning the wealth of species and *Astyanax* aff. *bimaculatus* (two spot *Astyanax* | lambari-do-rabo-amarelo) was the species with the highest number of individuals captured. The freshwater ichthyofauna of the Una river is composed of eight species considered allochthonous and one exotic. Of the thirty species listed in this study, five are new records for the Paraíba do Sul river basin.

Keywords: *introduced species, new occurrence records, diversity, endemic species.*

Ictiofauna do rio Una, Vale do Paraíba do Sul Paulista, sudeste do Brasil

Resumo: Este trabalho teve como objetivo realizar o inventário da ictiofauna do rio Una, afluente do rio Paraíba do Sul na região do Vale do Paraíba do Sul Paulista. As coletas foram realizadas em três áreas de amostragem ao longo do canal do rio Una entre os meses de abril de 2016 e março de 2017. Para a coleta dos espécimes de peixes foram utilizadas varas de pesca, covos, tarrafas, redes do tipo tela de arrasto manual e puçás. Foi coletado um total de 1.534 exemplares pertencentes a cinco ordens, 14 famílias, 26 gêneros e trinta espécies. Siluriformes e Characiformes foram as ordens com o maior número de espécies registradas. A família Characidae foi a mais representativa em relação a riqueza de espécies e *Astyanax* aff. *bimaculatus* (lambari-do-rabo-amarelo), a espécie com maior número de indivíduos capturados. A ictiofauna do rio Una apresentou em sua composição oito espécies de origem alóctone e uma exótica. Das trinta espécies listadas neste trabalho, cinco são novos registros de ocorrência para a bacia do rio Paraíba do Sul.

Palavras-chave: *espécies introduzidas, novos registros de ocorrência, diversidade, espécies endêmicas.*

Introduction

Estimates show that among more than 33.900 species of fish described on the planet, 13.000 exclusively inhabit freshwater environments (Nelson et al. 2016, Eschmeyer & Fong 2017). Much of this freshwater fish species richness is found in tropical waters of the Neotropical Region, especially in the South American continent, where recent surveys indicate the presence of 5.160 valid species (Reis et al. 2016). Brazil, the largest country of Neotropics, also presents the most diverse ichthyofauna in the world, where more than 3.300 species are currently registered (Froese & Pauly 2017). This high number of species occurs mainly because of its geographical position and size of its hydrographic network (Agostinho et al. 2007).

Among the large Brazilian hydrographic systems is the set of basins that drains the southeastern region of the country, generally called the Brazilian East (Menezes 1972). These basins are characterized by being independent, small in size and draining directly into the Atlantic Ocean (Agostinho et al. 2007). As a result of their respective geological and evolutionary histories, these basins present ichthyofaunas with high differentiation (Ribeiro 2006). One of the main basins of the Eastern Brazilian System, the Paraíba do Sul river basin, stands out for its highly endemic ichthyofauna, to the point of being considered a distinct ecoregion among the other regions forming this specific region of ichthyofauna (Abell et al. 2008).

Despite its intense environmental degradation, the Paraíba do Sul river basin is home to a highly diversified ichthyofauna (Teixeira et al. 2004). Currently, this basin counts for the registration of 130 species of freshwater fish, 71 of which occurring in the State of São Paulo. However, ichthyofauna surveys are not common in the basin, especially in the areas belonging to the State of São Paulo, which have not been systematically inventoried yet. Many of the hydrographic basin's smaller tributaries remain poorly studied regarding its fish species composition (Bizerril 1999, Melo et al. 2006, Oyakawa & Menezes 2011).

The Una river, one of the most important tributaries of the Paraíba do Sul river in the State of São Paulo (Marengo & Alves 2005), is an example of a river virtually unknown from the ichthyological point of view. In this context, the objective of the present study is to carry out the first inventory of Una river fish species, which may serve as a tool in the development of conservation strategies for this area.

Material and methods

1. *Study Area*

The Paraíba do Sul river is formed by the union of the Paraitinga river and Paraibuna river in the State of São Paulo. The Paraíba do Sul river runs a distance of just over 1.000 km until it drains into the Atlantic Ocean in Atafona, a city in the State of Rio de Janeiro (Hilsdorf & Petrere Jr 2002). In addition to the territory of São Paulo, the Paraíba do Sul river basin also drains the States of Minas Gerais and Rio de Janeiro, in a total area of approximately 57.000 km^2 (Bizerril 1999).

The hydrographic basin of the Una river occupies a total area of 476 km^2 distributed among the territory of the cities of Taubaté, Tremembé, Pindamonhangaba and Redenção da Serra, in the Paraíba do Sul River Valley region in the State of São Paulo (Batista et al. 2005). The Una river is formed by the union of the Santa Luzia river and the Almas stream in Taubaté, crossing a distance of 36 km and flowing from the south to the north until its confluence with the Paraíba do Sul river in the city of Tremembé, SP. As it crosses rural districts, the river receives several tributaries, having its course adapted to the relief (Prado & Abreu 1995) (Figure 1). If the length of its longest tributary is added, the Una river has a total length of approximately 70 km (Targa 2009).

The Una river runs at altitudes varying between 500 and 900 meters of elevation. The basin is limited by the mountain range of the Serra do Quebra-Cangalha, to the Northeast, and by the mountain range of the Serra do Jambeiro, to the Southwest. For this reason, the topography of the basin in its upper portion is rugged, smoothing towards its lower course (Prado & Abreu 1995). The natural vegetation, typical of the Atlantic Forest, is currently restricted to mountainous areas, such as on the slopes of the Serra da Mantiqueira, or inside small landowners properties in the form of small natural reserves (Freitas Junior & Marson 2007). The prevailing climate in the Una river basin is subtropical, with a dry winter and a hot and humid summer (Devide et al. 2014).

The study area of this work consists of three collecting locations, here called area I, area II and area III located along the main channel of the Una river. Area I (23º 05 '03.7 "S - 45º 29' 00.5" W) is the upper region, located near the headwaters of the Una river, in the rural area of Taubaté. This region is characterized by the presence of riparian vegetation formed by medium to large trees and Gramineae, which are eventually submerged during the rainy season. The substratum is predominantly composed of sand, gravel, stones and plant material of allochthonous origin (Figure 2a, Figure 2b). Area II (23º 01 '39.8 "S - 45º 30' 24.2" W) is the intermediate portion of the Una river, located in a floodplain region. There is riparian vegetation composed of small to large trees, herbaceous vegetation and grasses (Gramineae) in constant contact with water. The substratum is predominantly composed of sand (Figure 2c, Figure 2d). Area III (22º 55 '53.5 "S - 45º 31' 24.3" W) corresponds to the lower portion of the Una river, located less than six hundred meters from the confluence with the Paraíba do Sul river in Tremembé. The riparian vegetation is formed by grasses and trees from medium to large size. The substratum is formed by sand, gravel, rocks and shale (Figure 2e, Figure 2f).

2. *Fish Collecting*

Monthly sample collections were carried out between April 2016 and March 2017 in the three sampling areas within a delimited area of one hundred fifty meters each. Samples were performed by three people who spent up to four hours in each sampling area each time. Fishes were collected using angling, fyke nets (in the backwater area), and cast nets with fishnet mesh measurement varying from 0.5 to 3.0 cm between opposite knots. Dragging fish along the riparian vegetation with the aid of dragnets and seine nets were also used. Collection among rocky area in the riverbanks with the help of hand nets was also carried out.

The collected fish was immediately fixed in 10% formalin and then transferred to 70% ethanol solution. The specimens were identified with the use of identification keys and their status confirmed by specialist (Prof. Dr. Francisco Langeani Neto-DZSJRP). All the specimens sampled are deposited in the Coleção Científica do Laboratório de Zoologia da Universidade de Taubaté-CCLZU.

Fishes were classified in native, indigenous or autochthonous species (species which occur naturally in the basin); allochthonous species (species from different hydrographic basins within the Neotropical Region which were introduced into the Paraíba do Sul river); and exotic species (species from other continents which artificially entered the Paraíba do Sul river) (Oyakawa & Menezes 2011).

Results

A total of 1.534 specimens of freshwater fish was collected, belonging to five orders, 14 families, 26 genera and thirty species (Table 1, Figure 3, Figure 4). The order Siluriformes (43.3%, n = 13 species) was predominant regarding the number of species captured, followed by Characiformes (36.6%, n = 11 species), Perciformes (13.3%, n = four species), Gymnotiformes (3.4%, n = one species) and Synbranchiformes (3.4%, n = one species).

The most representative families were the Characidae (seven species), Loricariidae (six species) and Cichlidae (four species). Characidae was also the most representative family concerning the number of specimens collected (44.2%), followed by the families Loricariidae (23.4%) and Cichlidae (9%). *Astyanax* aff. *bimaculatus* was the species with the highest number of specimens collected (37.4%), followed by *Hypostomus* cf. *luetkeni* (12.6%) and *Corydoras nattereri* (8.6%).

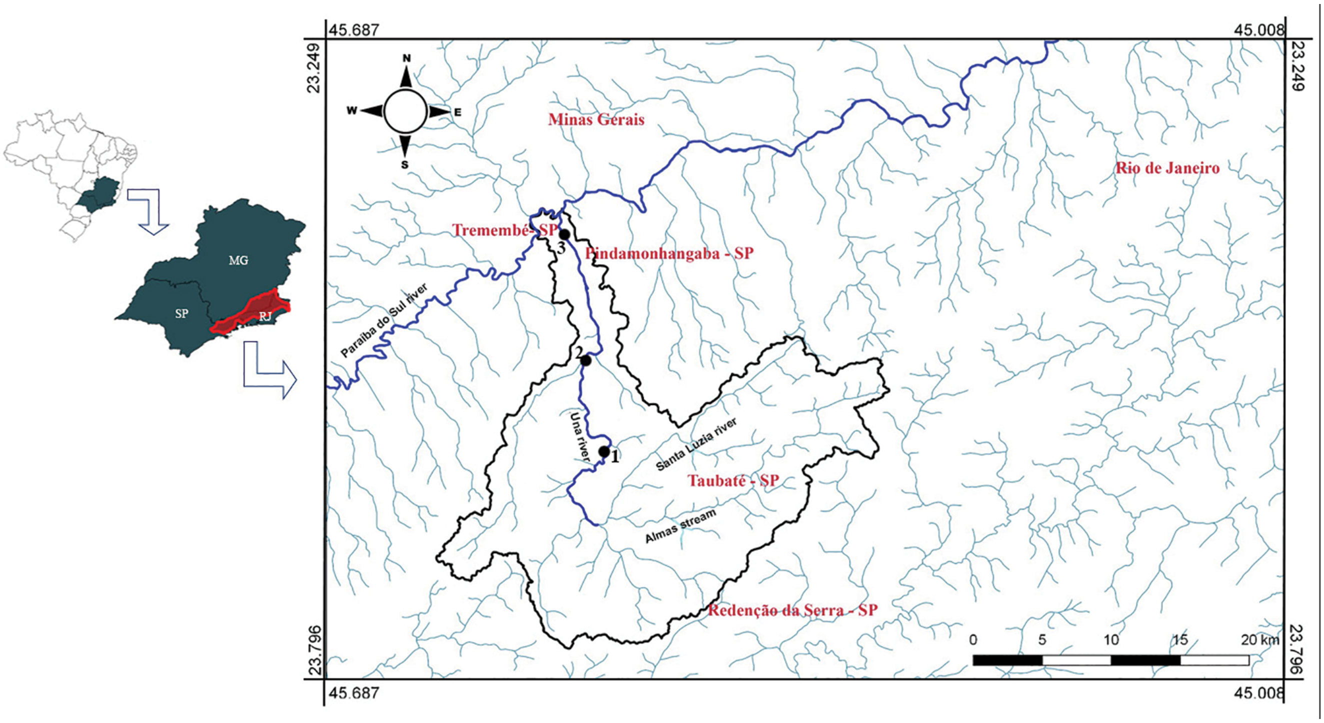

Figure 1. Hydrographic map of the Paraíba do Sul river basin encompassing the States of São Paulo (SP), Minas Gerais (MG) and Rio de Janeiro (RJ), highlighting the limits of the Una river basin, Paraíba do Sul River Valley, State of São Paulo, Brazil. Sampling areas: 1) area I: 23º 05 '03.7 "S - 45º 29' 00.5" W; 2) area II: 23º 01 '39.8 "S - 45º 30' 24.2" W; 3) area III: 22º 55 '53.5 "S - 45º 31' 24.3" W.

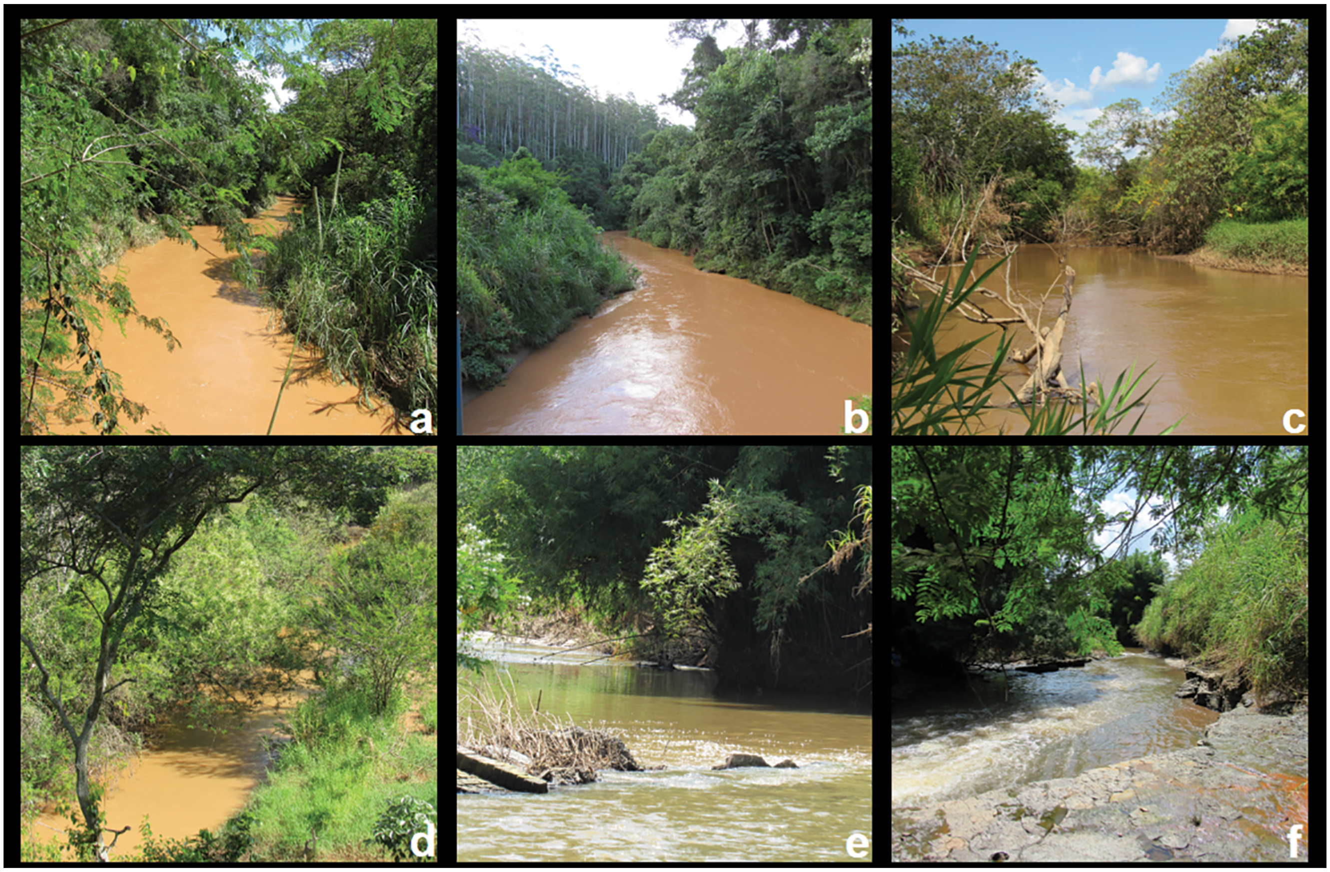

Figure 2. Overview of the three sampling areas along the Una river, in the Paraíba do Sul River Valley, in the State of São Paulo, Brazil. a-b) area I - 23º 05' 03.7" S - 45º 29' 00.5" W; c-d) area II - 23º 01' 39.8" S - 45º 30' 24.2" W; e-f) area III - 22º 55' 53.5" S - 45º 31' 24.3" W.

Table 1. Taxonomic list of collected species in the tree sampling areas along the Una river, Paraíba do Sul River Valley, São Paulo, in the period from April 2016 to March 2017. (*) Allochthonous species, (**) exotic species.

Order	Family	Species
Characiformes	Parodontidae	*Apareiodon affinis* (Steindachner, 1879)*
	Prochilodontidae	*Prochilodus lineatus* (Valenciennes, 1836)
	Crenuchidae	*Characidium pterostictum* Gomes, 1947*
	Characidae	*Astyanax* aff. *bimaculatus* (Linnaeus, 1758)
		Astyanax giton Eigenmann, 1908
		Astyanax parahybae Eigenmann, 1908
		Bryconamericus iheringii (Boulenger, 1887)*
		Hyphessobrycon eques (Steindachner, 1882)*
		Metynnis sp.*
		Oligosarcus hepsetus (Cuvier, 1829)
	Erythrinidae	*Hoplias malabaricus* (Bloch, 1794)
Siluriformes	Trichomycteridae	*Trichomycterus albinotatus* Costa, 1992
	Callichthyidae	*Corydoras nattereri* Steindachner, 1877
		Hoplosternum littorale (Hancock, 1828)
	Loricariidae	*Ancistrus multispinis* (Regan, 1912)*
		Hypostomus affinis (Steindachner, 1877)
		Hypostomus cf. *luetkeni* (Steindachner, 1877)
		Parotocinclus maculicauda (Steindachner, 1877)
		Rineloricaria cf. *steindachneri* (Regan, 1904)
		Rineloricaria sp.
	Heptapteridae	*Pimelodella lateristriga* (Müller & Troschel, 1849)
		Rhamdia quelen (Quoy & Gaimard, 1824)
	Pimelodidae	*Pimelodus maculatus* La Cepède, 1803*
	Auchenipteridae	*Glanidium melanopterum* Miranda Ribeiro, 1918
Gymnotiformes	Gymnotidae	*Gymnotus carapo* Linnaeus, 1758
Synbranchiformes	Synbranchidae	*Synbranchus marmoratus* Bloch, 1795
Perciformes	Cichlidae	*Cichlasoma portalegrense* (Hensel, 1870)*
		Crenicichla lacustris (Castelnau, 1855)
		Geophagus brasiliensis (Quoy & Gaimard, 1824)
		Oreochromis niloticus (Linnaeus, 1758)**

Of the thirty species recorded here, eight are considered as allochthonous: *Apareiodon affinis Characidium pterostictum*, *Bryconamericus iheringii*, *Hyphessobrycon eques*, *Metynnis* sp., *Ancistrus multispinis*, *Pimelodus maculatus* and *Cichlasoma portalegrense*. *Apareiodon affinis, C. pterostictum, B. iheringii, A. multispinis* and *C. portalegrense* also new records for the Paraíba do Sul river basin. One species was considered exotic: *Oreochromis niloticus*.

Discussion

The predominance of Siluriformes (catfish) and Characiformes (characins) in the composition of the ichthyofauna of the Una river corroborates the pattern of fish biodiversity and abundance of the Paraíba do Sul river basin (Bizerril 1999), the rivers of the Brazilian East (Bizerril 1994), the freshwater environments of Brazil (Buckup et al. 2007) and the Neotropical Region (Reis et al. 2016). In general, the ichthyofauna recorded in the Una river is very similar to that reported for the Paraíba do Sul river basin in the works of Bizerril (1999) and Teixeira et al. (2004). Twenty-two species listed for the Una river had previously been cited in Element Occurrence (EO) Reports produced by those authors to Paraíba do Sul river basin. Considering only the portion of this basin in the São Paulo State, Oyakawa & Menezes (2011) registered 71 species, of which 19 species were listed in the present study.

Astyanax aff. *bimaculatus* was the most abundant species in the three sampling areas along the Una river. It belongs to a group of species sharing similar color pattern, composed of at least 22 valid species distributed in practically all the hydrographic basins of South America, representing, certainly, one of the most abundant morphotypes in the continent (Lucena & Soares 2016). This freshwater fish species shows reduced size, high reproduction rate, and rapid growth. It is an opportunistic feeder, relatively unselective for zooplankton, detritus, higher plants and sometimes the scales of fish. This behavior certainly

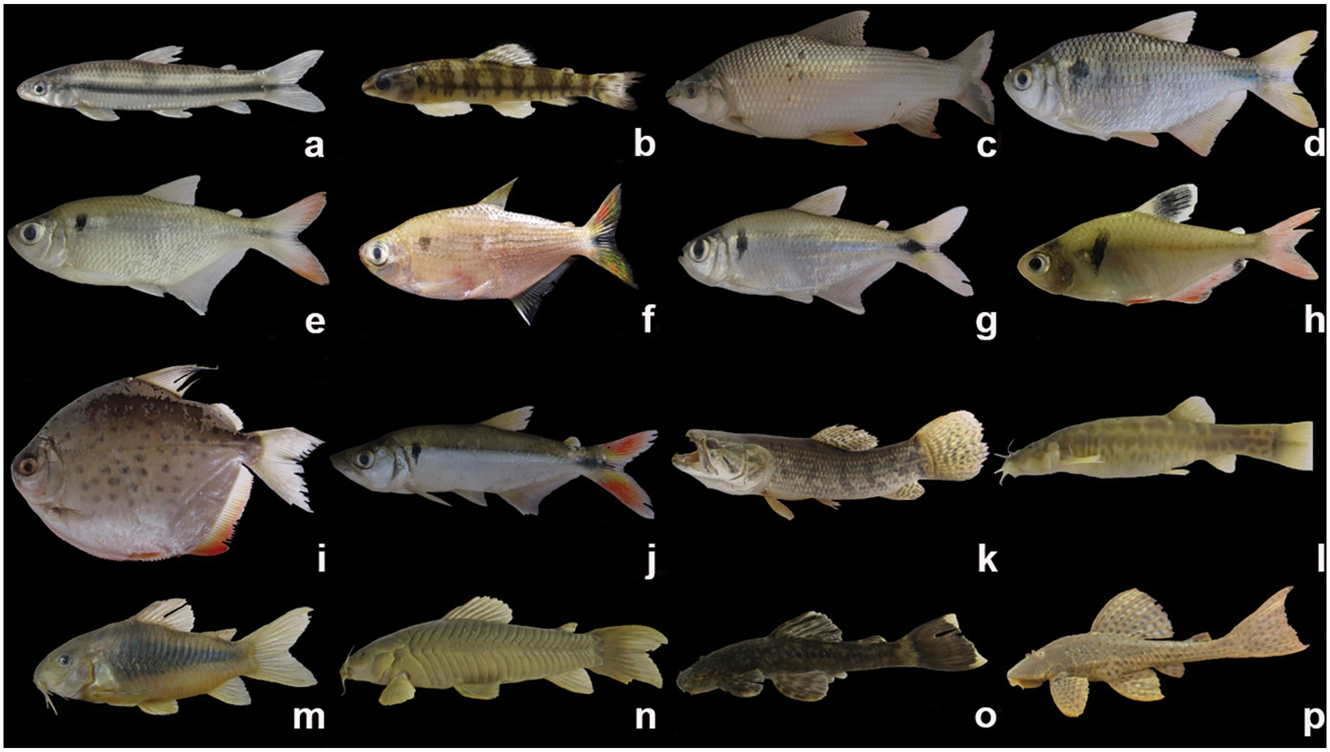

Figure 3. Registered species in the Una river in the period from April 2016 to March 2017. a) *Apareiodon affinis*, 39,9 mm; b) *Characidium pterostictum*, 46,6 mm; c) *Prochilodus lineatus*, 250,0 mm; d) *Astyanax* aff. *bimaculatus*, 73,4 mm; e) *Astyanax giton*, 72,2 mm; f) *Astyanax parahybae*, 82,0 mm; g) *Bryconamericus iheringii*, 49,1 mm; h) *Hyphessobrycon eques*, 28,0 mm; i) *Metynnis* sp., 84,9 mm; j) *Oligosarcus hepsetus*, 63,8 mm; k) *Hoplias malabaricus*, 244,1 mm; l) *Trichomycterus albinotatus*, 141,3 mm; m) *Corydoras nattereri*, 44,4 mm; n) *Hoplosternum littorale*, 165,1 mm; o) *Ancistrus multispinis*, 73,2 mm; p) *Hypostomus affinis*, 151,9 mm.

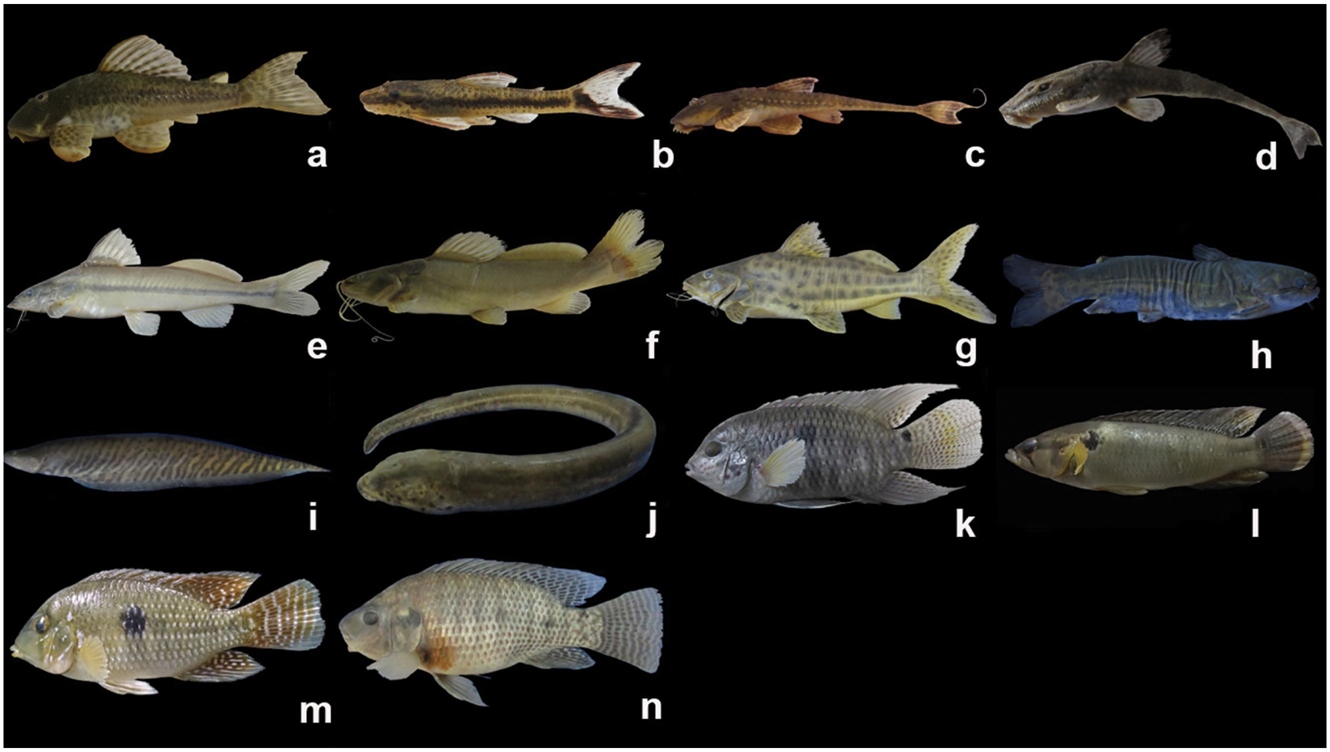

Figure 4. Registered species in the Una river in the period from April 2016 to March 2017. a) *Hypostomus* cf. *luetkeni*, 113,1 mm; b) *Parotocinclus maculicauda*, 31,5 mm; c) *Rineloricaria* cf. *steindachneri*, 112,1 mm; d) *Rineloricaria* sp., 149,5 mm; e) *Pimelodella lateristriga*, 107,2 mm; f) *Rhamdia quelen*, 155,3 mm; g) *Pimelodus maculatus*, 210,5 mm; h) *Glanidium melanopterum*, 180,4 mm; i) *Gymnotus carapo*, 195,4 mm; j) *Synbranchus marmoratus*, 480,0 mm; k) *Cichlasoma portalegrense*, 100,0 mm; l) *Crenicichla lacustris*, 97,1 mm; m) *Geophagus brasiliensis*, 121,7 mm; n) *Oreochromis niloticus*, 190,0 mm.

favors its distribution in most different environments that occur (Arcifa et al. 1991, Esteves & Galleti Jr 1995, Melo 2001, Andrian et al. 2011).

Other two abundant species recorded in this work were Heptapteridae, *Pimelodella lateristriga*, and the Callichthyidae, *Corydoras nattereri*. *Astyanax* aff. *bimaculatus* and *P. lateristriga* are generalist species, showing great *plasticity in their foraging behavior, which favor* their high incidence in the environments they inhabit (Mazzoni et al. 2010). *Corydoras nattereri* belongs to a group composed of freshwater fish species which withstand the stress of low oxygen levels in water. They have been known to inhabit stretches of slow water in rivers, areas where there is a higher sediment deposition rate (Reis 2003). This species showed greater abundance in the intermediate portion of Una river, located in a floodplain region, where the river presents lower current and high sediment accumulation.

Among the 19 native species of the Paraíba do Sul river basin sampled in the Una river, *Corydoras nattereri* and *Parotocinclus maculicauda* have not been formally recorded along the São Paulo portion of the basin. Their known distribution lies within tributaries located in the States of Rio de Janeiro and Minas Gerais. In this work, Bizerril (1999) mentioned the preference of *C. nattereri* for stretches of sediment-laden water along the final portions of the Paraíba do Sul river located in the State of Rio de Janeiro. Vieira & Rodrigues (2010) listed the presence of *P. maculicauda* in the Muriaé river, a tributary of Paraíba do Sul river located in the State of Minas Gerais.

Among the eight allocthonous species aforementioned for the Una river, *Hyphessobrycon eques*, *Metynnis* sp. and *Oreochromis niloticus* are economically important. The reduced size and reddish coloration of *H. eques* make it an appreciated species in the aquarium trade. This species occurs naturally in the Amazon basin and the Paraguay river and has been introduced in the Upper Paraná river basin and the Brazilian East coastal rivers (Lima et al. 2007). The introduction of ornamental fish species into lotic ecosystems usually occurs through intentional release of specimens into freshwater bodies (Fuller et al. 1999, Semmens et al. 2004, Moraes et al. 2017). In the Paraíba do Sul river basin, this situation is aggravated by the presence of the most extensive ornamental freshwater fish aquaculture park in Brazil, located in the Minas Gerais portion of the basin (Magalhães et al. 2002).

Oreochromis niloticus is the most exploited species in freshwater fish farm cage systems in the world. This fact is mainly due to its high productivity and tolerance diverse environmental conditions (Carvalho et al. 2010). *Metynnis* spp. are commonly used in aquarium ornamentation. This genus is frequently cited in articles and inventories along the Paraíba do Sul river basin as allochthonous into the system (Moraes et al. 2017). In fact, according Ota (2015), there are no occurrence records of *Metynnis* in Southeast coastal rivers until the 1990s.

The presence of *O. niloticus* and *Metynnis* in the Paraíba do Sul river basin is attributed to irregular escapes from cage systems of freshwater fish farms. These cages are prone to allow fish to escape during floods caused by heavy rains (Orsi & Agostinho 1999, Moraes et al. 2017). The presence of this kind of enterprise is quite common along the Una river, mainly in their medium and lower courses, located in the municipalities of Taubaté and Tremembé.

Other allochthonous species listed to the Una river, *Apareiodon affinis*, *Characidium pterostictum*, *Bryconamericus iheringii*, *Ancistrus multispinis* and *Cichlasoma portalegrense* are new reports records for the Paraíba do Sul river basin. Among these species, *A. affinis* presents the largest geographical distribution in Brazilian systems, being frequently found in the Paraná and Uruguay basins, in addition to other hydrographic systems located in well sampled regions (Godoy 1975, Pavanelli 1999, 2007). In this study, *A. affinis* and *C. pterostictum* were restricted to the final portion of the Una river, near the it confluence with Paraíba do Sul river in Tremembé. It is still not possible to point out the causes of the presence of these five species in the Una river. However, it is probable that future biogeographic analysis of the region will elucidate this issue.

The presence of a relatively high number of species from other freshwater systems is not a particular feature of the Una river but also of the Paraíba do Sul river basin, where currently there are records of more fifty non-native species (Vieira & Rodrigues 2010). In a recent analysis, the introduction of species has been pointed out as one of the leading causes of extinction of freshwater fish species in this basin, as in lotic ecosystems in general (Bizerril 1999, Simberloff 2003).

The species list of the Una river contributes to fill part of knowledge gap regarding the fish fauna distribution in the Paraíba do Sul river basin in the State of São Paulo, by the inventory of an area unstudied until this moment, being able to serve as subsidies for the elaboration of future management actions in the Paraíba do Sul Valley region.

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Author Contributions

Juliano Rodrigues Honorio: Substantial contribution in the concept and design of the study; contribution to manuscript preparation, data collection and in the data analysis and interpretation.

Itamar Alves Martins: Contribution to manuscript preparation, data analysis and interpretation, critical revision and in the adding to intellectual content.

Conflicts of interest

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

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Article

Continental ichthyofauna from the Paraíba do Norte River basin pre-transposition of the São Francisco River, Northeastern Brazil

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Abstract: Freshwater ichthyofauna from Brazil Northeast region was considered as being poorly known until recent years, with a considerable number of publications becoming available in the last decade. The present study provides an inventory of freshwater fish species from the Paraíba do Norte River basin located in Paraíba State, Brazil. This inventory is intended to contribute to the of knowledge to the regional fish diversity, pre-transposition of the São Francisco River. Collecting data was obtained from ichthyological databases of both national and foreign institutions. A total of 47 freshwater fish species are registered within the Paraíba do Norte River basin, represented by 38 genera, 20 families and six orders. Characiformes, comprising 47% (22 species), Cichliformes, and Siluriformes are among the most representative orders, 19% (9 species) each, of total recorded species. Seven species of Cichliformes are reported as introduced species in this basin. Cyprinodontiformes and Gobiiformes also registered in this region and correspond to 5% (two species) and Gymnotiformes, Perciformes and Synbranchiformes, 2% (one species each) of total recorded species. Paraíba do Norte River basin stands out in the current national scenario as it comprises the first region from the Mid-Northeastern Caatinga freshwater ecoregion to receive water from the transposition of the São Francisco River. The current inventory is important as it provides scientific data related to the ichthyofauna of Paraíba do Norte River basin prior to the commencement of the river transposition process. An identification key is also given for the freshwater fish species of the region.
Keywords: *Freshwater fishes, Neotropical Region, diversity, identification key.*

Ictiofauna continental da bacia do Rio Paraíba do Norte pré-transposição do rio São Francisco, Nordeste do Brasil

Resumo: A ictiofauna de água doce do Nordeste brasileiro durante muito tempo foi considerada pouco conhecida. No entanto, este paradigma vem sendo mudado nas últimas décadas com aumento significativo no número de publicações desta ictiofauna. Para contribuir com mais informações sobre a diversidade de peixes do Nordeste brasileiro, o presente estudo realizou um inventário das espécies de peixes de água doce da bacia do rio Paraíba do Norte, localizada no Estado da Paraíba, pré-transposição do Rio São Francisco. Os dados foram provenientes de acervos ictiológicos de instituições nacionais e estrangeiras. Foram registradas 47 espécies, distribuídas em 38 gêneros, 20 famílias e seis ordens de peixes na bacia do rio Paraíba do Norte. As ordens mais representativas foram Characiformes (22 espécies/47% do total), Cichliformes e Siluriformes (9/19%). Sete espécies de Cichliformes são reportadas como introduzidas na bacia. Além das ordens citadas, foram registrados dois Cyprinodontiformes and Gobiiformes (2/5%), e Gymnotiformes, Perciformes e Synbranchiformes (1/2% cada). A bacia do Paraíba do Norte tem se destacado no cenário atual por ser a primeira da região Nordeste Médio-Oriental a receber águas da transposição do rio São Francisco. Com isso, este inventário é de fundamental importância, por apresentar dados da ictiofauna da bacia do rio Paraíba do Norte antes da transposição. Esse trabalho também apresenta uma chave para identificação das espécies de peixes da bacia.
Palavras-chave: *Peixes de água doce, Região Neotropical, Inventário, Chave de identificação.*

Introduction

Knowledge regarding freshwater fish fauna in Brazil used to be heterogeneous, with major studies focusing on the South and Southeast regions of the country with little emphasis being placed on the Northeast region (Rosa et al. 2003, Langeani et al. 2009, Ramos et al. 2014). Lack of research has limited the classification of the local ichthyofaunistic diversity and delayed biogeographic evaluation of groups of fishes from Brazilian Northeastern region (Rosa et al. 2003, Ramos et al. 2014). This paradigm has recently changed with the publication of a book chapter entitled "Diversity, patterns of distribution and conservation of fishes from Caatinga" in Rosa et al. (2003) listed 240 species of freshwater fishes for the Caatinga biome, comprising the broadest evaluation about the freshwater ichthyofauna from this region to date. Various studies have since been undertaken which the understanding of the regional ichthyofauna through publications, including: Ramos et al. (2005), Alves et al. (2008), Nascimento et al. (2011), Cardoso (2012), Gurgel-Lourenço et al. (2013), Novaes et al. (2013), Sánchez-Botero et al. (2013), Camelier & Zanata (2014), Nascimento et al. (2014), Paiva et al. (2014), Silva et al. (2014), Ramos et al. (2014), Silva et al. (2015), Gurgel-Lourenço et al. (2015), Rodrigues-Filho et al. (2016), Costa et al. (2017), Teixeira et al. (2017) and Oliveira-Silva et al. (2018).

Paraíba do Norte River basin is located entirely within the Paraíba State and it comprises one of the main basins from the Mid-Northeastern Caatinga freshwater ecoregion (MNCE - *sensu* Rosa et al. 2003, Albert et al. 2011). This ecoregion bears hydrographic basins situated between São Francisco and Parnaíba River, and supplies water to Alagoas, Pernambuco, Paraíba, Rio Grande do Norte, Ceará States, as well as a small portion of Piauí State (Rosa et al. 2003). The hydrographic network Paraíba State is comprised basins of medium to short length such as those from Paraíba do Norte and Piranhas-Açu Rivers, as well as those of the Curimataú, Camaratuba, Mamanguape, Miriri, Gramame, Guaju and Abiaí Rivers, respectively (AESA 2017). Knowledge about the ichthyofauna from these river basins is limited, with most of the data available in grey literature.

Taxonomical studies related to freshwater fishes from Paraíba do Norte River basin are restricted to list of species from reservoirs such as those of Marinho et al. (2006), Montenegro et al. (2012) and Costa et al. (2017). Other studies are focused on ecological populational dynamics and communities, including those of Medeiros & Maltchik (2001), Montenegro et al. (2010), Montenegro et al. (2011), and Costa (2015). Studies on the taxonomy and systematics of freshwater fishes from Paraíba do Norte River basin is scarce. This is problematic and contributes to misleading future researches focusing on the biological aspects of the local species due to misidentifications.

Recently, the Paraíba do Norte River has been artificially connected to São Francisco River basin through a water transposition project (Integration Project of São Francisco River with hydrographic basins from the North Northeast – known as PISF). This project aims to eradicate hydrological shortage in the Northeast Semi-arid region (Pittock et al. 2009, Brasil 2004). The transposition began in March 2017, along the east axis, which takes the waters of the São Franscisco River to the Paraíba do Norte River. Considering the difference in species composition (São Francisco, with 241 species and NMCE with 94, Barbosa et al. 2017; Oliveira-Silva et al. 2018, respectively) and its natural history, it is expected the occurrence of biological invasions between basins, a factor already observed in researches involving the transposition of basins (e.g., Ellender & Weyl, 2014; Shelton et al. 2016), which could lead to the extinction of species in these ecosystems to the detriment of competition for space and resources (Pysek & Richardson, 2006). The current study aimed to provide an inventory of the ichthyofauna from Paraíba do Norte River basin prior to the transposition project. Additionally, this study provides the first identification key to freshwater species from this basin.

Material and Methods

1. *Study area*

The hydrographical basin of Paraíba do Norte River is located between latitudes 06º51'31" and 08º26'21" South, and longitudes 34º48'35" and 37º02'15" North (Lima & Melo 1985, Xavier et al. 2013) (Figure 1). Its area ranges a total of 20,071.83 km^2 (comprising 32% of the total extension of Paraíba State), and it is considered the second largest hydrographical basin of this State.

Paraíba do Norte River is the most extensive river that drains the Western of Borborema plateau in Northeastern Brazil. Its origin is located in Alto da Serra de Jabitacá (Monteiro municipality), and runs in a Southeast-Northeast direction for about 360 km until it reaches the Atlantic Ocean (Cabedelo municipality). This river crosses over one of the most drought stricken regions of the State (Cairiri Paraibano) and disembogues in the Littoral flatland. This area is characterized by a humid climate and prevalence of typical Atlantic forest vegetation (Silva 2003).

This river basin is divided into three portions, the higher, middle and lower portions. The main river course of the higher portion is named Meio River, and extends from its origin to Boqueirão municipality. Waters from Taperoá River, its main affluent, supply this region. The largest reservoir of this basin, Epitácio Pessoa, is currently located in the junction between these rivers. The middle portion extends from Taperoá River mouth to Paraibinha River confluency, among Itatuba and Natuba municipalities. The lower portion of this river basin begins downstream of this reservoir and extends until the river mouth which runs into the Atlantic Ocean (Lima & Melo 1985, Silva 2013, AESA 2017).

The region surrounding Paraíba do Norte River is characterized by irregular regimes of droughts and floods, semi-arid climate, and impermeable riverbeds in the high and middle portions. These characteristics induce an intermittent superficial water-flow (Silva 2013). Typical Caatinga vegetation prevails in these portions and it is characterized by the presence of deciduous shrubs and xerophytic plants (Simões et al. 2008). Semi-arid climate, type BS'h (warm and dry), has temperature of 26ºC and a mean annual precipitation of 600 mm, respectively (Köppen, 1936; Sudene 1990). The lower portion of the basin is situated in the Atlantic forest vegetation with a perennial regime of superficial water-flow. The climate in this portion is humid tropical (*Am*) with temperatures between 24º and 27ºC and a mean annual precipitation between 900 and 1800 mm, respectively (Lima & Melo 1985).

2. *Data collection*

Databases from ichthyological collections of Universidade Federal da Paraíba (UFPB) and Universidade Federal Rio Grande do Norte (UFRN) were accessed for collecting data. Other national and foreign

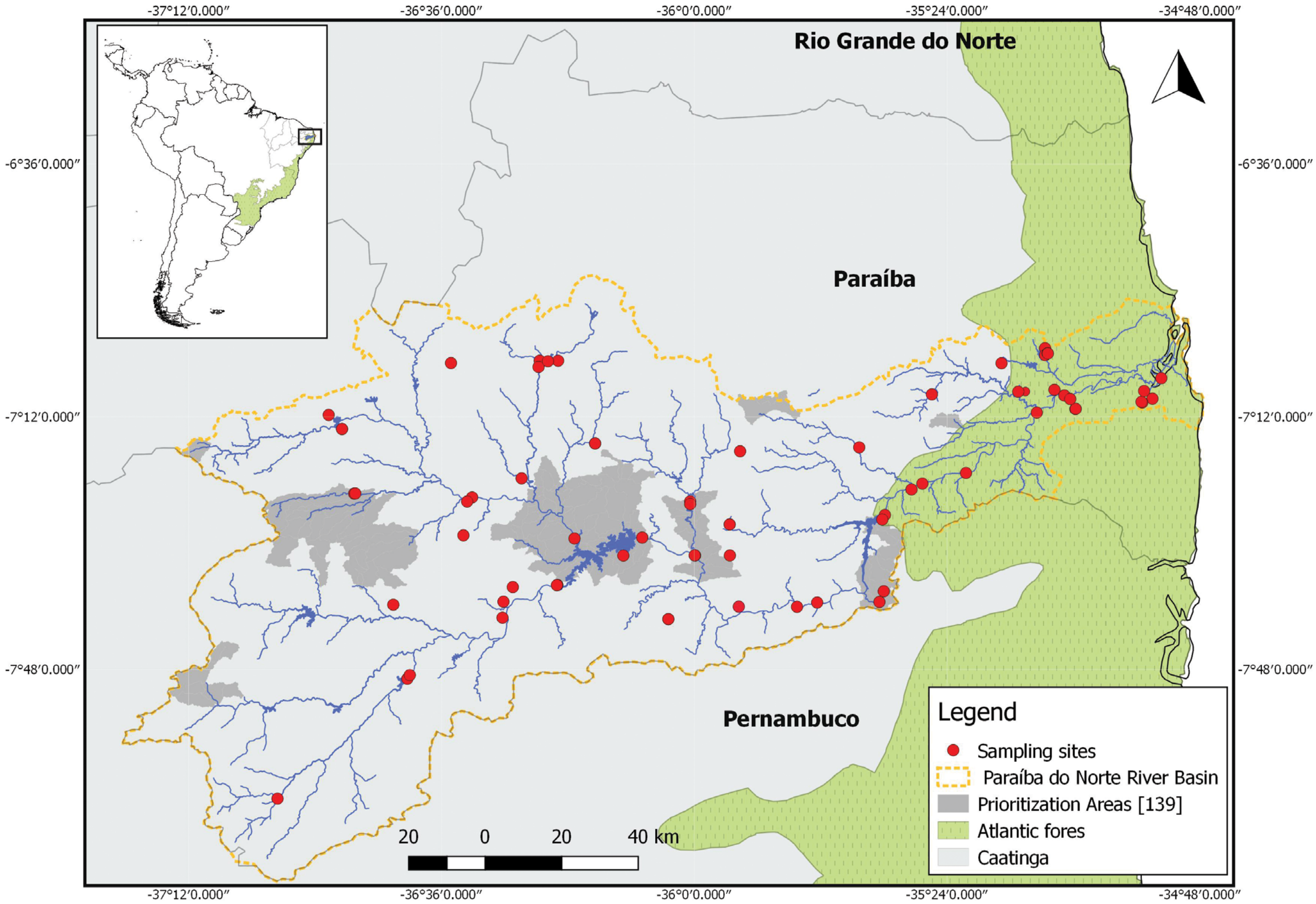

Figure 1. Map of Paraíba do Norte River basin, Paraíba State, Brazil, showing sampling sites.

institutions that bear a variety of representatives from the Neotropical region were also consulted, including: Museu Nacional, Rio de Janeiro (MNRJ), Museu de Zoologia da Universidade de São Paulo (MZUSP), Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP), Universidade Federal da Bahia (UFBA), all located in Brazil; Museum of Comparative Zoology, Harvard University (MCZ) and Smithsonian National Museum of Natural History (NMNH) in the U.S.A; Museum fur Naturkunde, Berlin, Germany (ZMB); Muséum National D' Histoire Naturelle, Paris, France (MNHN); Natural History Museum (NHM), London, U.K. Digital database platforms were also accessed for obtaining data: PRONEX/Neodat (http://www.mnrj.ufrj.br), Specieslink (www.specieslink.org), GBIF (www.gibf.org), and Portal Biodiversidade/ICMBIO (https://portaldabiodiversidade.icmbio.gov.br). However, all the material included in the species list (Table 1) was analyzed and deposited in the UFPB and UFRN collections. Other institutions, whose curators claimed to have material from the studied basin, as well as data obtained from online platforms, did not register any different species when compared to the species found in the collections previously accessed (UFPB and UFRN). Therefore the data were used only to increase the distribution of the samples in the drainage. In total data from 71 sampling points were recorded (Figure 1). Some sampled areas were lacking geographic coordinates, displaying only the name of the municipalities they belonged to. In such cases, we used an approximated coordinate based on the municipality location. For that reason, we did not present any list with the geographic information of the sampling areas.

Data available in the scientific collections and online databases are results from independent initiatives of a variety of research projects. Thus, these results do not represent equivalent samplings and/or standardized collecting methodology, indicating its unfeasibility on providing comparisons about the abundance of specimens between the collection sites.

A dichotomous identification key was prepared using material held in the fish collections from UFPB and UFRN. Meristic and morphometric data were taken from Hubbs & Lagler (2004). Classification follows Eschmeyer et al. (2018). The conservation status was classified according to the Brazilian lists of endangered species, Portaria nº 445, December 17, 2014 (Brasil 2014), and 'data deficiency' (DD) species lists (ICMBio 2016).

Results

Material from Paraíba do Norte River basin are available in the fish collections at MCT and MCZ only. A total of 47 species of freshwater fishes are recorded and classified into 38 genera, 20 families and six orders (Table 1). There are 22 species of Characiformes that represents 47% of total recorded species, which comprises the largest order in the region (Figure 2). Species of Characiformes are

Table 1. List of Fish species of Paraíba do Norte River basin, Paraíba State, Brazil. Endemic: of MNCE; Allochthonous: introduced from other regions; Autochthonous: native to the region; Exotic: introduced from other countries. DD = deficient data, LC = Last concern, NE = not evaluated, EN = endangered, UFPB = Universidade Federal da Paraíba, UFRN = Universidade Federal do Rio Grande do Norte.

ORDER/Family/Species	Origin	Status	Caatinga	Forest Atlantic	Voucher
CHARACIFORMES					
Prochilodontidae					
Prochilodus brevis Steindachnner, 1875	Autochthonous	LC	X		UFPB 04069
Triportheidae					
Triportheus signatus (Garman, 1890)	Autochthonous	LC	X	X	UFPB 10600
Anostomidae					
Leporinus piau Fowler, 1941	Autochthonous	LC	X	X	UFPB 03665
Leporinus taeniatus Lütken 1875	Autochthonous	LC	X		UFPB 04167
Characidae					
Astyanax bimaculatus (Linnaeus, 1758)	Autochthonous	LC	X	X	UFPB 03668
Astyanax fasciatus (Cuvier, 1819)	Autochthonous	LC	X	X	UFPB 02888
Cheirodon jaguaribensis Fowler, 1941	Endemic	DD	X	X	UFPB 11194
Compsura heterura Eigenmann, 1915	Autochthonous	LC	X	X	UFPB 11200
Hemigrammus marginatus Ellis, 1911	Autochthonous	LC	X	X	UFPB 11202
Hemigrammus rodwayi Durbin, 1909	Autochthonous	NE	X	X	UFPB 11203
Hemigrammus unilineatus (Gill, 1858)	Autochthonous	NE		X	UFPB 11245
Hyphessobrycon parvellus Ellis, 1911	Autochthonous	LC		X	UFPB 11284
Serrapinnus heterodon (Eigenmann, 1915)	Autochthonous	LC	X	X	UFPB 11283
Serrapinnus piaba (Lütken, 1875)	Autochthonous	LC		X	UFPB 11207
Crenuchidae					
Characidium bimaculatum Fowler, 1941	Endemic		X	X	UFPB 11199
Curimatidae					
Psectrogaster rhomboides Eigenmann & Eigenmann, 1889	Autochthonous	LC	X		UFPB 10607
Steindachnerina notonota (Miranda Ribeiro, 1937)	Autochthonous	LC	X	X	UFPB 11206
Erythrinidae					
Erythrinus erythrinus (Bloch & Schneider, 1801)	Autochthonous	LC		X	UFPB 11247
Hoplerythrinus unitaeniatus (Spix & Agassiz, 1829)	Autochthonous	LC		X	UFPB 11603
Hoplias malabaricus (Bloch, 1794)	Autochthonous	LC	X	X	UFPB 01075
Parodontidae					
Apareiodon davisi Fowler, 1941	Endemic	EN	X	X	UFPB 00741
Serrasalmidae					
Metynnis lippincottianus (Cope 1870)	Autochthonous	LC		X	UFPB 11286
SILURIFORMES					
Callichthyidae					
Callichthys callichthys (Linnaeus, 1758)	Autochthonous	LC		X	UFPB 04077
Megalechis thoracata (Valenciennes, 1840)	Autochthonous	NE		X	UFPB 11246
Auchenipteridae					
Trachelyopterus galeatus (Linnaeus, 1766)	Autochthonous	LC	X		UFPB 03667
Heptapteridae					
Pimelodella enochi Fowler, 1941	Endemic	LC	X		UFRN 00443
Rhamdia quelen (Quoy & Gaimard, 1824)	Autochthonous	LC		X	UFPB 11240
Loricariidae					
Hypostomus pusarum (Starks, 1983)	Endemic	LC	X	X	UFPB 06183

Continuation Table 1.

ORDER/Family/Species	Origin	Status	Caatinga	Forest Atlantic	Voucher
Parotocinclus cf. *cearensis* Garavello, 1977	Autochthonous	DD	X	X	UFRN 01583
Parotocinclus jumbo Britski & Garavello, 2002	Autochthonous	LC	X		UFPB 04189
Parotocinclus spilosoma (Fowler, 1941)	Endemic	LC	X	X	UFPB 04107
CYPRINODONTIFORMES					
Poecilidae					
Poecilia reticulata Peters, 1859	Allochthonous	NE	X	X	UFPB 10209
Poecilia vivipara Bloch & Shneider, 1801	Autochthonous	LC	X	X	UFPB 01076
GYMNOTIFORMES					
Gymnotidae					
Gymnotus carapo Linnaeus, 1758	Autochthonous	LC		X	UFPB 11273
GOBIIFORMES					
Gobiidae					
Awaous tajasica (Lichtenstein, 1822)	Autochthonous	LC	X	X	UFPB 04104
Eleotridae					
Eleotris pisonis (Gmelin, 1789)	Autochthonous	LC		X	UFPB 11191
CICHLIFORMES					
Cichlidae					
Astronotus ocellatus (Agassiz, 1831)	Allochthonous	NE	X		UFPB 10605
Cichla monoculus Spix & Agassiz, 1831	Allochthonous	NE	X	X	UFPB 10609
Cichla ocellaris Bloch & Shneider, 1801	Allochthonous	NE	X	X	UFPB 06693
Cichlasoma orientale Kullander, 1983	Endemic	LC	X	X	UFPB 11234
Coptodon rendalli (Boulenger, 1896)	Exotic	NE	X	X	UFPB 02883
Crenicichla menezesi Ploeg, 1991	Autochthonous	LC	X	X	UFPB 11225
Geophagus brasiliensis (Quoy & Gaimard, 1824)	Autochthonous	LC	X	X	UFPB 10619
Oreochromis niloticus (Linnaeus, 1758)	Exotic	NE	X	X	UFPB 09985
Parachromis managuensis (Günther, 1867)	Exotic	NE	X		UFRN 01971
PERCIFORMES					
Sciaenidae					
Plagioscion squamosissimus (Heckel, 1840)	Allochthonous	NE	X		UFPB 09983
SYNBRANCHIFORMES					
Synbranchidae					
Synbranchus sp.	Autochthonous	NE	X	X	UFPB 06245

spread among 17 genera and nine families. No introduced species of Characiformes were verified in this basin. The second largest order is Cichliformes and Siluriformes with 9 species each (representing 19% of total recorded species, each). Cichiformes is divided into eight genera and one family. Six introduced species of Cichliformes were registered (*Astronotus ocellatus*, *Cichla ocellaris*, *Cichla monoculus*, *Coptodon rendalli*, *Parachromis managuensis* and *Oreochromis niloticus*). The nine species of Siluriformes are distributed in seven genera and four families. Cyprinodontiformes and Gobiiformes with two species each, representing 5% of total recorded species. Within of Cyprinodontiformes, *Poecilia reticulata,* is considered an exotic species. Gymnotiformes, Perciformes and Synbranchiformes comprise one species each, representing 2% of total recorded species.

Characidae (Characiformes) is the most diverse family with 10 species (or 21.3% of total recorded species) followed by Cichildae (Perciformes) and Loricariidae (Siluriformes) with nine and four species corresponding to 19.2% and 8.5% of total recorded species, respectively (Figure 3). Three species of Cichlidae are native to this region. Thus, Loricariidae comprises the second most representative group in the basin when considering only native species. Cichlidae is the third most representative group together with Erythrinidae, both with three species each (6.4% of total recorded species). Anostomidae, Callichthyidae, Curimatidae, Heptapteridae and Poeciliidae two species each, representing 4.3% of total recorded species for each family. Poeciliidae has one introduced species, *Poecilia reticulata.* A single species is verified for each of the other ten families (Auchenipteridae,

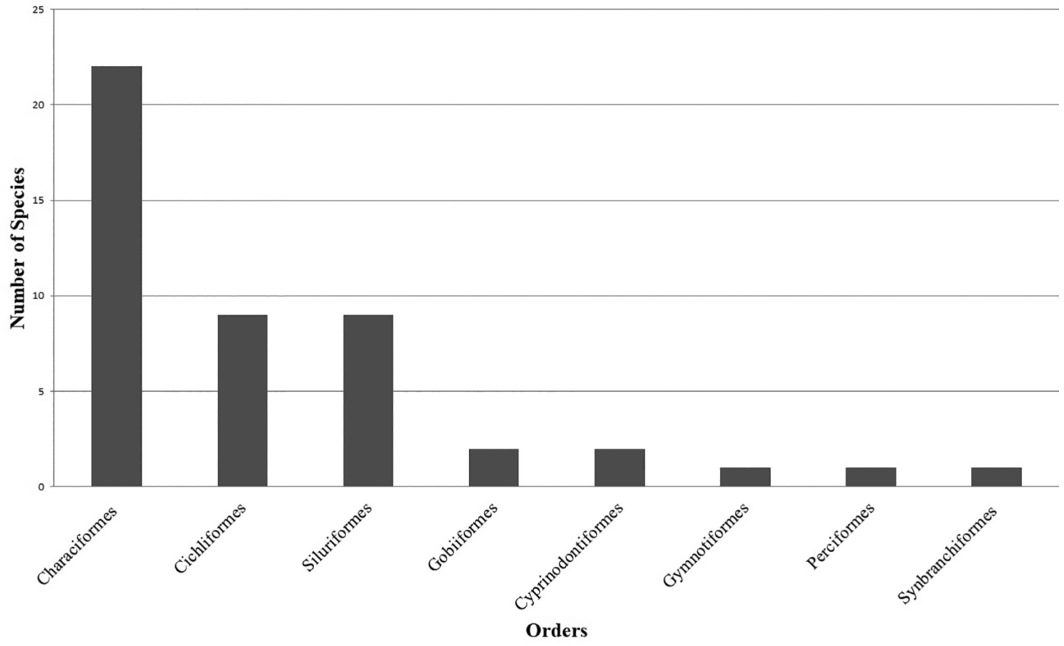

Figure 2. Number of species by order caught in Paraíba do Norte River basin, Paraíba State, Brazil.

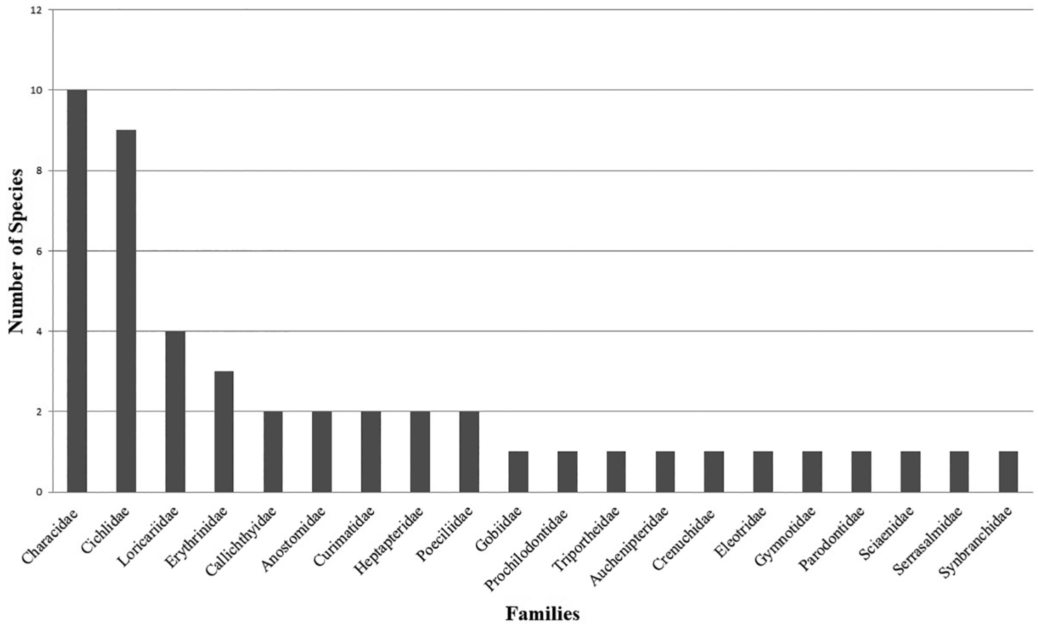

Figure 3. Number of species by family caught in Paraíba do Norte River basin, Paraíba State, Brazil.

Crenuchidae, Eleotridae, Gobiidae, Gymnotidae, Parodontidae, Prochilodontidae, Serrasalmidae, Sciaenidae, Synbranchidae and Triportheidae). *Plagioscion squamosissimus* is also an introduced species that belongs to the family Sciaenidae.

Eight introduced species and 39 native species are observed out of 47 species registered in Paraíba do Norte River basin. A total of 37 native species are exclusively from freshwater environment and two species are marine-estuarine, including *Awaous tajasica* and *Eleotris pisonis*. Seven freshwater species are endemic to the MNCE (*stricto* Rosa et al. 2003) (Table 1), in which *Parotocinclus spilosoma* is endemic to Paraíba do Norte River basin. *Apareiodon davisi* is the only threatened species among those endemic species from the MNCE. This species is currently classified as "in danger" according to the Official National List of Threatened Faunistic Species – Fishes and Aquatic Invertebrates (Brasil 2014).

Species herein evaluated are recorded from 74 sampling sites distributed throughout the three portions (higher, middle, and lower) of Paraíba do Norte River basin. A total of 5,498 specimens were counted within the 597 sampling sites. Twelve collecting sites are located within the limits of prioritization areas for conservation of the Caatinga biome (Figura 1) and 14 inside the Conservation Units.

The ichthyological collection at UFPB holds the largest number of representatives from Paraíba do Norte River basin with 4,001 specimens available and comprising 46 fish species, and distributed within 396 lots. Thus, it contains 97.9% of total ichthyofauna from the studied basin. UFRN has another important fish collection representing the diversity of the studied basin. It bears 905 specimens available, comprising 28 species (59.6% of total ichthyofauna), and distributed within 142 lots. MCZ has 219 specimens distributed within 30 lots for 30 species (63.8% of total ichthyofauna). MCT holds 366 specimens with 24 lots for 17 species (36.2% of total ichthyofauna). MZUSP only has three lots belonging to the type series of *Parotocinclus jumbo* (MZUSP 69513 – Holotype; MZUSP 69514 and MZUSP 69519 – Paratypes), a species described in Britski & Garavello (2002). The type locality of this species is Paraíba do Norte River, under bridge from PB-408 road, near Umbuzeiro municipality, Paraíba State, Brazil. MNRJ has a single lot from this basin (MNRJ 21924) that is regarded to be the paratype of *P. jumbo*, with same collecting data as the holotype of this species.

Twenty-two native species out of 39 species recorded from the studied basin occur in regions under dominion of both Caatinga and Atlantic forest biomes. Six and 11 species occur exclusively in these biomes, respectively (Table 1). Five exotic species out of eight species recorded in the area occur in both biomes. Three exotic species occur exclusively in the Caatinga biome while no exotic species are found exclusively in the Atlantic forest biome.

Discussion

Paraíba do Norte River basin has 47 fish species that correspond to 54.6% of total ichthyofauna from MNCE and 19.5% of total ichthyofauna from Caatinga biome when compared to the number of species registered in these areas (86 and 240 species, respectively, according to Rosa et al., 2003).

Siluriformes becomes the most representative group when considering only autochthone ichthyofauna. Cichliformes comprises the second most representative order in the region. This result is in agreement with Bizerril (1994), Mazzoni (1998), Castro (1999), Reis et al. (2003), Rosa et al. (2003), Buckup et al. (2007), Serra et al. (2007), Albert & Reis (2011), and Ramos et al. (2014) that supported Characiformes and Siluriformes as the group of greatest representation in the neotropical riverine systems. Lowe-McConnel (1999) reported that Siluriformes exceeds Characiformes in number of species in the South American ichthyofauna. However, the taxonomical composition varies in different basins (Bizerril 1994). Characiformes order stands out from those groups in the, Parnaíba, São Francisco and Paraná basins. At family level, Characidae and Loricariidae are the most representative groups in the neotropical region when considering native species (Reis et al. 2003, Buckup et al. 2007), which is in accordance to the current results for Paraíba do Norte River basin.

One collecting site from this basin is available at the list of species from MCZ. The collectors are Maj. J.M.S. Coutinho and Dr. Justa from the expedition Thayer that occurred between 1865 and 1866 in Brazil under leadership of Louis Agassiz. The collecting site is Paraíba do Norte River near João Pessoa municipality and collecting date is 2 August 1865 (Dick 1977, Higuchi 1996). This material is very valuable because it contributes to the ichthyofauna database from this region prior to environmental impacts that have since taken place in the Northeast Brazilian region.

Environmental degradation is common in this region, directly affecting the local freshwater ichthyofauna. Anthropic changes such as dam constructions, deforestation, implementation of powerplants and distilleries, and distribution of ichthyo-toxics in the natural habitat for eradication of weed species, introduction of allochthonous species, and alterations on river courses are examples. Reduction of fish populations or extinction of local species may have occurred due to these anthropic interferences (Rosa et al. 2003). Introduction of exotic species is also very common in Northeast Brazil (Leão et al. 2011, Levis et al. 2013) as observed in other areas of the country (Gomes et al. 2008, Biagioni et al. 2013, Frota et al. 2016). Many allochthonous species were intentionally introduced in this region through national campaigns from the Departamento Nacional de Obras Contra a Seca (DNOCS) in the 1930 decade, especially in the MNCE basin. Tucunaré (*Cichla ocellaris and C. temensis*) and pescada-do-Piauí (*Plagioscion squamosissimus*), originally from Amazonas and Parnaíba basins respectively, are examples of introduced species through DNOCS campaigns. African tilapias (*Oreochromis niloticus* and *Coptodon rendalli*) (Gurgel & Fernando 1994, Reaser et al. 2005, Paiva & Mesquita 2013) are also examples of species introduced through DNOCS. *Parachromis managuensis* is registered for the first time in Paraíba do Norte River basin at Soledade dam. This Cichlidae species is originally from the east side of Central America (Conkel 1993), and has been introduced in many countries, including Brazil (Agasen et al. 2006, Barros et al. 2012). Barbosa et al. (2006) first recorded *P. managuensis* in Northeast Brazil at the middle portion of São Francisco River. It piscivorous and aggressive species that reaches up to 65 cm total length (Conkel 1993, Barbosa et al. 2006, Barros et al. 2012).

Metynnis lippincottianus, known locally as "peixe CD", is reported to occur at the lower portion of Paraíba do Norte River basin according to the fishing community. This species was previously recorded in other regional basins such as Gramame in Paraíba State (Beltrão et al. 2009), Pratagi (Paiva et al. 2014), Maxaranguape, Ceará-Mirim and Pirangí River basins (Nascimento et al. 2014), all located in Rio Grande do Norte State. Beltrão et al. (2009) considers *M. lippincottianus* as an introduced species in Gramame River basin. However, other authors recognize it as a native species from Northeast river basins Canan & Gurgel (1997), Rosa et al. (2003), Morais et al. (2012), Nascimento et al. (2014), and Paiva et al. (2014). *Metynnis roosevelti* Eigenmann, 1915 is usually cited in many studies although this species has been considered a junior synonym of *M. lippincottianus* (Cope 1870) in Zarske & Géry (1999). The oldest record of *Metynnis* in the MNCE region is provided in Starks (1913) as *M. maculatus* (Kner 1858), collected in Papary lake, Trairi River basin, Rio Grande do Norte State. The species was collected 20 years prior to the introduction of fish species in the region as described earlier in this study. There is no species from this genus listed as introduced species from the Northeast region (Leão et al. 2011, Levis et al. 2013, Gurgel & Oliveira, 1987). Thus, *M. lippincottianus* is supported herein as a non-introduced species in the MNCE region.

Two marine-estuarine species were observed at the studied basin, *Awaous tajasica* and *Eleotris pisonis*. These species have broad distributions along the Brazilian coast, and occasionally occur in the freshwater environment (Kullander 2003). It is important to notice that the first species also occurs in the Caatinga biome, which is an area outside the Atlantic forest region. Another important record is the presence of the, *Cheirodon jaguaribensis*, described from Jaguaribe River, Ceará State in Fowler (1941) and considered restricted to this basin (Reis et al. 2003, Buckup et al. 2007). However, the current results also recognize this species to occur in Paraíba do Norte River basin. Occurrences of this species in other basins within the MNCE ecoregion in Texeira et al. (2017) and Gouveia et al. (2017) indicate that *C. jaguaribensis* is not restricted to the Jaguaribe River.

There are seven official Conservation Units (UCs) in Paraíba do Norte hydrographic network: Parque Estadual do Poeta e Repentista Juvenal de Oliveira (419,51 ha), Parque Estadual Mata do Xém-Xém (182,00 ha), Área de Proteção Ambiental (APA) do Cariri (18.560,00 ha), Área de Proteção Ambiental (APA) das Onças (36.000,00 ha) all under State supervision; and three private reserves, including Reservas Particular do Patrimônio Natural (RPPN), RPPN Fazenda Almas (3.505,00 ha), RPPN Fazenda Santa Clara (750,50 ha) and RPPN Fazenda Pacatuba (266,53 ha) with a total of 59.685,54 ha of protected land. There are fish records in RPPN Fazenda Almas and Fazenda Pacatuba, and at the Parque Estadual Mata do Xém-Xém. Twenty-seven species were recorded from the UCs, comprising 71% of the native ichthyofauna of Paraíba do Norte River basin.

The source of the Paraíba do Norte River is located at Serra de Jabitacá, a region known to also separate water to the Pajeú River sub-basin which is an affluent from the São Francisco River. Paraíba do Norte River basin shares 20 native species with São Francisco River basin out of 38 native species recognized in the present study. Ten species originally from this basin were introduced at MNCE basins: *Brycon hilarii* (Valenciennes, 1850), *Conorhynchos conirostris* (Valenciennes, 1840), *Franciscodoras marmoratus* (Lütken, 1874), *Megaleporinus elongatus* (Valenciennes, 1850), *Myleus micans* (Lütken 1875), *Pachyurus francisci* (Cuvier, 1830), *Pachyurus squamipennis* Agassiz, 1831 and *Pimelodus maculatus* Lacepède, 1803 (Gurgel & Oliveira 1987). Neither of these species was recorded in the Paraíba do Norte river basin in this work.

Water transposition between different river basins has contributed to impacts in the diversity and abundance of local fish fauna (Izique 2005). Transposition of waters from São Francisco River to MNCE basins aimed to supply water for local populations that suffer from regular drought crises due to intermittent river flows. Paraíba do Norte (Paraíba), Apodi-Mossoró (Rio Grande do Norte), Jaguaribe (Ceará) and Piranhas-Açu (Paraíba, Rio Grande do Norte) river basins were connected artificially to water channels from São Francisco River (Pittock et al. 2009). The first basin to receive waters from this process was Paraíba do Norte River basin, and thus it has its regime changed to perennial. According to Moreira-Filho & Buckup (2005), changes in species composition and abundance are imminent after water transposition took place. Consequences include competition among species, propagation of populations over others, and possibly extinction of native species.

The current inventory of freshwater fish species from Paraíba do Norte River basin is of importance as it provides an updated list of the local ichthyofauna, and contributes to the improvement of the knowledge about the diversity and evolutionary history of freshwater fish species from the region. The current study also provides an ichthyological overview of its composition prior to systemic anthropic interferences in the environment through the water transposition from São Francisco River, which will contribute to future comparative studies for understanding the environmental changes in the region.

Supplementary material

The following online material is available for this article:

Appendix: Identification key to fish species from Paraíba of North River basin

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Author Contributions

Telton Pedro Anselmo Ramos: participated in the data collection, wrote the text and confirmed the identifications.

Jéssica Alcoforado de Sena Lima: participated in the data collection and wrote the text.

Silvia Yasmin Lustosa Costa: participated in the data collection and wrote the text.

Márcio Joaquim da Silva: participated in the data collection and wrote the text.

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Leonardo Oliveira-Silva: participated in the data collection and wrote the text.

Conflicts of interest

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

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