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Erratum

Erratum: Biological survey of a cloud forest in southwestern Mexico: plants, amphibians, reptiles, birds, and mammals

In the article "*Biological survey of a cloud forest in southwestern Mexico: plants, amphibians, reptiles, birds, and mammals*" with DOI code number http://dx.doi.org/10.1590/1676-0611-bn-2017-0444 published at Biota Neotropica 18(2): e20170444,

Where you read:	Should be read:
(...) and four threatened species (e.g., *Boa imperator*, *Thamnophis godmani*, and *Trimorphodon quadruplex*; Figure 2, Appendix 2).	(...) and four threatened species (e.g., *Boa sigma*, *Thamnophis godmani*, and *Trimorphodon biscutatus*; Figure 2, Appendix 2).
In particular, *Carpinus caroliniana*, *Eupherusa poliocerca*, *Herpailurus yagouaroundi*, and *Trimorphodon quadruplex* are threatened (...).	In particular, *Carpinus caroliniana*, *Eupherusa poliocerca*, *Herpailurus yagouaroundi*, and *Trimorphodon biscutatus* are threatened (...).

And in the "*Appendix 2 - List of amphibian and reptile species recorded in a cloud forest of southern Mexico*",

Where you read:	Should be read:
Dryophytes eximius	*Dryophytes arboricola*
Plectrohyla bistincta	*Sarcohyla bistincta*
Plectrohyla pentheter	*Sarcohyla pentheter*
Marisora unimarginata	*Marisora brachypoda*
Boa imperator	*Boa sigma*
Trimorphodon quadruplex	*Trimorphodon biscutatus*
Geophis sieboldi	*Geophis occabus*

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Inventory

Abundance and composition of the medium to large-sized mammals in a private area of a REDD+ project in Acre, Brazil

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Abstract: The implementation of private areas focused on conservation of species and habitats, combined with REDD+ policies, has become an important ally for biodiversity conservation, expanding the conservation areas of the most varied habitats, covering key groups such as large mammals, which are extremely important for the maintenance of ecosystem services. In the upper region of the Purus River in Acre, Brazil, an inventory was carried out using camera-traps, of medium and large mammals community in a private REDD+ area, known as the Purus Project. A total of 19 species of mammals were recorded with an effort of 1859 trap-nigths, including rare, endangered and key species. It is estimated that the richness for the area is of 22 species. Endangered and rare species such as the tapir (*Tapirus terrestris*) and the short-eared dog (*Atelocynus microtis*) presented high rates of relative abundance compared to other Conservation Units (UCs). The richness of medium to large-sized mammals recorded in the Purus Project underscores the importance of REDD+ in private areas for the conservation of this group, given the challenges for inclusion and creation of new protected areas. REDD+ projects in privates' areas become an important component for conservation of species and the connection between public conservation units favoring the spread of species and populations between areas, and consequently the gene flow.

Keywords: *Private Areas, Conservation, Camera-trap, Maintenance of Biodiversity.*

Abundância e composição de mamíferos de médio e grande porte em uma área privada de projeto REDD+ no Acre, Brasil

Resumo: A implementação de áreas privadas voltadas para conservação de espécies e habitats, aliadas a políticas de REDD+, tem se tornado importante aliada para conservação da biodiversidade, ampliando as regiões de conservação dos mais variados habitats, abrangendo grupos chaves como grandes mamíferos, extremamente importantes para manutenção dos serviços ecossistêmicos. Na região do alto rio Purus, Acre, Brasil, foi realizado um inventário utilizando armadilhas fotográficas, da comunidade de mamíferos de médio e grande porte de uma área privada de REDD+, o Projeto Purus. Foram registradas 19 espécies de mamíferos, com um esforço de 1.859 armadilhas-noite, incluindo chaves, raras e ameaçadas de extinção. Estima-se que a riqueza esperada para a área seja de 22 espécies. Espécies ameaçadas e raras como a Anta (*Tapirus terrestris*) e o Cachorro-do-mato (*Atelocynus microtis*) apresentaram taxas de abundância relativa elevadas, comparadas com outras UCs. A riqueza de médios e grandes mamíferos registrados na região do Projeto Purus ressalta a importância de REDD+ em áreas privadas para conservação deste grupo, dado os desafios para inclusão e criação de novas áreas protegidas públicas. Assim projetos de REDD+ em áreas privadas se tornam um importante componente para conservação de espécies e para a conexão entre Unidades de Conservação públicas favorecendo a dispersão de espécies e populações entre áreas, e consequentemente o fluxo gênico.

Palavras-chave: *Áreas privadas, Conservação, Armadilhas Fotográficas, Manutenção da Biodiversidade.*

Introduction

Although the surface of the Earth is covered by approximately 16,200 areas of legal protection with about 28 million km² (Watson et al. 2014), most of these areas cover very restricted geographic regions and habitats (Gallo et al. 2009). In Brazil, for example, about 1,500,436 km² of area are included in protected areas (MMA 2017). Most of these areas cover regions with little productive soils and low implantation costs (Norton 2000, Scott et al. 2001, Rouget et al. 2003). According to Norton (2000), the network of existing legal protected areas in the world is not adequate for biodiversity conservation, due to the costs to create and maintain these areas, as well as factors such as decrease and changes in the regulation of these areas (Gallo et al. 2009, Marques & Peres 2015). In a review, Rodrigues et al. (2004) showed that 11.4% of approximately 11,600 vertebrates are covered in public Conservation Units (UCs), and demonstrate that the percentage of area already protected in a given country or biome is a very poor indicator of additional conservation needs.

A possible solution to conservation biodiversity, would be to include private areas conservation (Gallo et al. 2009). According to Pence et al. (2003), associating private and public areas for conservation could save up to 80% of the costs directed to UCs. Although Private Conservation Areas are ignored from conservation statistics, academia and even national conservation plans, studies show the ability to conserve millions of hectares of land (Norton et al. 2000, Scott et al. 2001, Rouget et al. 2003, Gallo et al. 2009). For example, private areas preserve twice more types of vegetation than public conservation units in Australia and South Africa (Gallo et al. 2009), improving the connection between UCs, favoring maintenance of local fauna and flora. This association is important because it increases the conservation area in the region, protecting distinct and endangered habitats (Gallo et al. 2009, Polack et al. 2016).

In this scenario, areas that have REDD+ projects meet the need to combine conservation between private and non-private areas. REDD+ Policies (Reducing Emissions from Deforestation and Forest Degradation) has sought to enhance actions of initiatives that focus on the conservation of biodiversity (Harvey et al. 2010). REDD+ is of particular importance in this scenario, since it considers the need to monitor biodiversity and not only the carbon stock of the areas (Harvey et al. 2010). This broad vision is needed for the sustainability of REDD+, since the fauna and flora are closely related, and the conservation of mammalian dispersers and seed predators is of extreme importance for the conservation of trees of high commercial value (Terborgh et al. 2008, Estes et al. 2011, Culot et al. 2017). However, most REDD+ projects have failed in goals and plans, including conservation and monitoring of biodiversity (Panfil & Harvey 2016). In a review, Panfil & Harvey (2016) found that in 80 REDD+ projects listed around the world, none provided detailed information on the sampling design, methodology for measuring indicators, or how data would be analyzed, although some indicated monitoring of biodiversity. In addition, none reported data on impacts of population sizes of threatened species nor provided other direct measures of status of fauna communities. This makes it difficult to assess how REDD+ projects have fulfilled their roles in biodiversity conservation.

Among the most varied vertebrate taxa, mammals of medium and large sizes stand out because they play fundamental roles in the regulation and structuring of forest ecosystems, acting in different ecological services, such as seed dispersal and predation, fruit and seedling removal, agent pollinators and top-chain predators. Much of the world's mammal diversity is found in the neotropical region (Brown 2014). 701 species of the 5,400 known mammals (Schipper et al. 2008), occur in Brazil, a total of 399 species were identified in the Amazon region, and the most diverse, with approximately 58% of the endemic species of the region (Paglia et al. 2012).

Despite their great importance for tropical forest ecosystems, large mammals are severely threatened by the advance of the agricultural frontier and recent projects to create hydropower plants (Peres 2001, Soares-Filho et al. 2006, Benchimol & Peres 2015). In addition, hunting activity in the Amazon has shown to be an important cause in the loss of species during the "Anthropocene age" (Peres 2000a, Dirzo et al. 2014, Peres et al. 2016), capable of altering different ecosystem services, such as seed dispersal, and also causing changes in the structure of the plant community (Peres 2000b, Stoner et al. 2007, Terborgh et al. 2008, Dirzo et al. 2014, Culot et al. 2017). The disappearance of mammals in forest ecosystems can have a direct effect on the structure of the plant community and the food chain (Stoner et al. 2007, Dirzo et al. 2014). The main change is the increase in the abundance of plant species that have no dispersion associated with vertebrates, to the detriment of other species with large seeds that are dispersed by large vertebrates (Terborgh et al. 2008, Culot et al. 2017). Such a change in plant community caused by defaunation is detrimental to the carbon market, since large biomass trees can have their recruitment reduced by the absence of their dispersers, reducing the carbon stock of the areas (Bello et al. 2015, Peres et al. 2016)

Acre is considered of extreme importance for the conservation of mammals. The State has approximately 86% of its territory covered by forests and is situated in one of the areas with the most endemic plants of the Amazon (Maury 2002, INPE 2016). Approximately 47% of the State is covered by protected areas, including public UCs and Sustainable Use Units totaling 5,198,273 ha (SEMA 2010). In addition, in Acre, mammal richness is estimated at 203 species, corresponding to 29% of Brazilian mammals (SEMA 2010) and more than half of its territory is considered as "extreme" and "very high" protection areas for the conservation of biodiversity, identified by the MMA (2002).

The knowledge on wild mammals in the Amazon region is still very scarce and has several gaps. Studies on medium and large mammals in the state of Acre have been carried out mainly in public protected areas, and use a variety of sampling methods to record the existing fauna, from census surveys, linear transects (Calouro 2005), track counting (Borges et al. 2014), camera-traps (Botelho et al. 2012) to other methods (Borges et al. 2015).

Given the lack of information related to the occurrence, distribution and the need to understand the importance of private areas for mammal conservation, here we present the first study of medium to large-sized mammals, with a consistence sampling design and methodology in a REDD+ project area. The main objective was to identify the community of medium to large mammals in an area of REDD+, evaluating mainly the richness of species composition, their abundances, and their activity patterns. We also held brief comparisons with studies conducted in public conservation units.

Material and Methods

1. *Study area*

The study was conducted in an area of REDD+ known as the Purus Project, located in a forest area of 34,702 ha in the municipality of Manoel Urbano, state of Acre, Brazil. The project area is divided into two plots (Seringal Itatinga and Seringal Porto Central) located along the Purus River (Figure 1), with only 1.4% of the forest area being converted into an open area or pasture with more than 98% forest (Eaton & Andrade 2012). The Purus Project is bordered by the Cazumbá-Iracema Extractive Reserve, and is located near the Indigenous Area of the upper Purus River and the Chandless State Park, functioning as an extension of protected areas in the upper Purus region. The area is inhabited by traditional communities, with occur in low human density (0.29 habitants /km²), which are concentrated on the banks of the river (Eaton & Andrade 2012). Two main vegetable typologies were identified in the area: Open Forest with Palm of Alluvial Influence, which is smaller and concentrated in areas near the river and Open Forest with Bamboo and Palms covers the largest area of the Purus Project area, located further away from the Purus River (Eaton & Andrade 2012).

2. *Sampling of mammals*

The community of medium and large mammals was inventoried with the use of 10 BUSHNELL TROPHY CAM HD traps. Sampling stations were installed, consisting of a tree-tied trap approximately 30 to 40 cm above the ground. The sampling stations were installed at regular distances of 1 km from each other, in two lines of 4.5 to 5 km (Tobler et al. 2008), covering an area of 10 km² approximately. The lines were positioned perpendicularly to the Purus River (Figure 1), as well as by Borges et al. (2015) in Chandless State Park, near to Purus Project. No baits were used at the sampling stations. The traps operated continuously (24 hours a day) for 120 days in the first sampling campaign (June 2013 to September 2013) and 150 days during the second campaign (December 2013 to May 2014). The traps were programmed to shoot at intervals of 30 seconds between the photos, always making double shots to help identify the species. During the field work for the installation of traps, occasional mammalian recordings were carried out by visualization, vocalization and traces.

The conservation status of species was classified according to categories used by the International Union for the Conservation of Nature (IUCN) and the Red Book of Brazilian Fauna Threatened with Extinction (MMA 2014). We used the relative abundance index (RAI) as a measure of the abundance of species in each trap station, since relative abundance indices have strong relation with the abundance of a species (Carbone et al. 2002, O'Brien et al. 2003).

The RAI consists of the number of independent records of a given species at a station, divided by the number of days of operation of this collection station multiplied by 100 (ind/100 night-traps). Each record was considered independent if: (1) the interval between consecutive photos of the same species is greater than 1 h; (2) photographs of

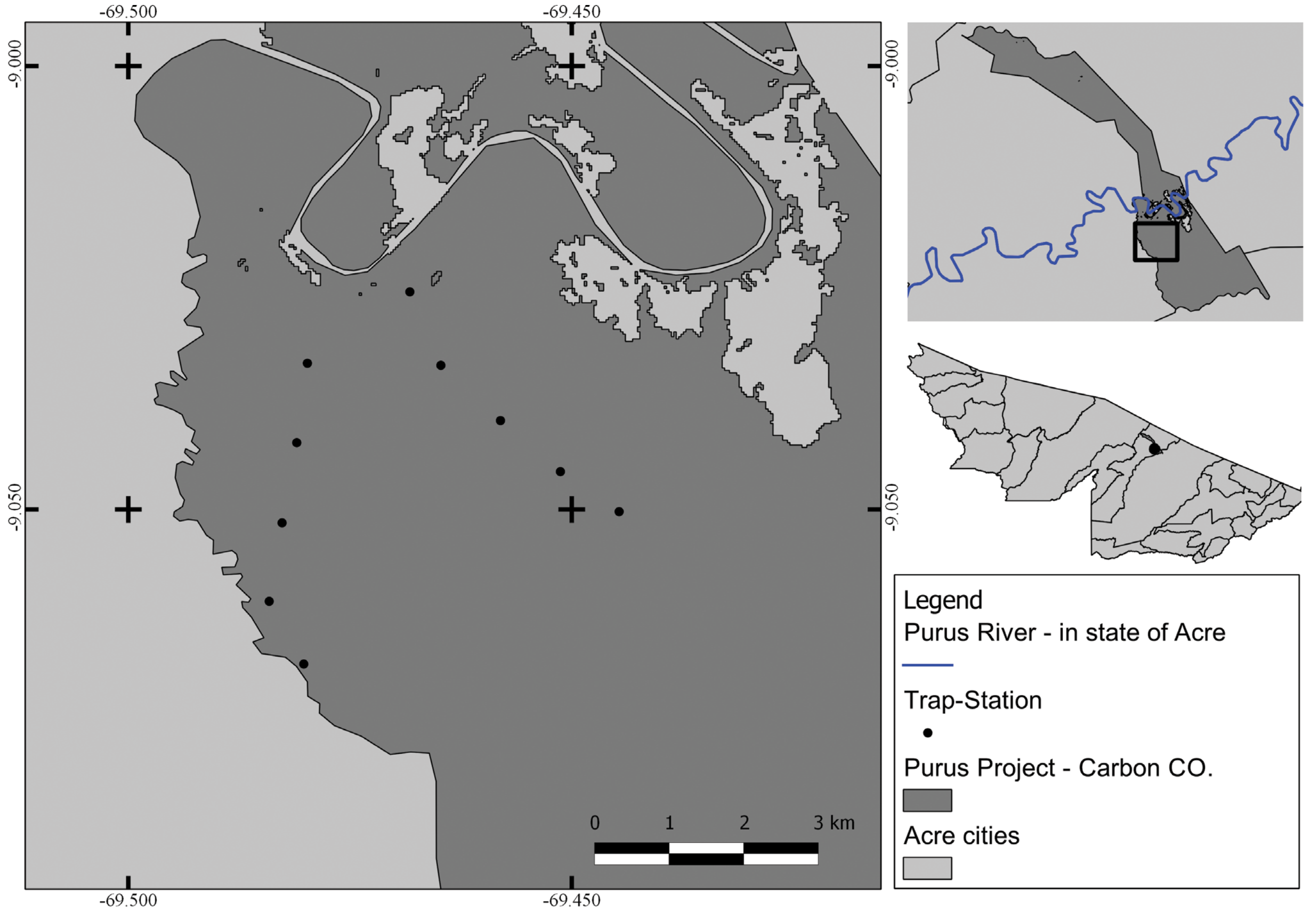

Figure 1. Location of the Purus Project and camera-traps stations in Manoel Urbano, Acre, Brazil.

different individuals of the same species when differentiation was possible (O'Brien et al. 2003). Social species in which more than one individual is recorded in the same photo are considered a single record, so the relative abundance of *Pecari tajacu*, for example, is given as the no. of groups/100 night-traps. The activity pattern was estimated for species that had more than nine independent records (Harmsen et al. 2010). The number of records was counted at 1-hour intervals.

To evaluate if the sampling effort was enough to record most of the species in the area, accumulation curves were constructed by plotting the number of species observed as a function of the sampling effort of night-traps using 1000 randomizations (observed richness - Mao Tao). Species richness expected for the area was estimated using the richness estimator Jack-knife 1. As the method using camera-traps is not a suitable method for primate studies, records of such individuals were excluded from these analyses.

Results

With an effort of 1,859 night-traps, a total of 22 species of mammals (including three species of primates) were recorded, distributed in nine orders and 15 families. Occasional visualizations and traces were added and the number of mammal taxa recorded in the Purus Project reaches 27 (Table 1). The richest order, including occasional visualization and trace data, was Primates with seven species, followed by the order Carnivora with six.

With a total of 19 species, the cumulative curve of species showed no tendency to reach asymptote, which suggests the registration of new species with increased sampling effort. According to the richness estimator Jack-knife 1, the species richness estimated for the area is of 22 species (Figure 2).

The most abundant species were *Pecari tajacu* (1.56), followed by *Dasyprocta* sp. (1.51) and *Mazama americana* (1.18) (Table 1). Among the recorded species, two are considered endangered (*Tapirus terrestris* and *Myrmecophaga tridactyla*) (Figure 3) and four others are data deficient species (Table 1), based on the lists of endangered species of IUCN or MMA. Most species had a predominantly diurnal activity pattern, *A. microtys* and *Dasyprocta* sp. showed activity pattern throughout the day, starting at sunrise reducing to sunset, while *M. pratti* has your activity peak at sunrise and sunset only, and *E. barbara* started from sunrise ending at 14:00. Only four species presented higher nocturnal activity, *Dasypus* sp. with activity peak between 20:00 and 23:00, *C. paca* and *L. pardalis* at midnight and the dawn, *T. terrestris* showed your activity peak at 22:00 reducing your activity at the dawn, at 04:00, with some records during the day (Figure 3).

Discussion

The Purus Project area presented a considerable richness of medium and large terrestrial mammals with 22 species are registred, results close to those found in conservation units such as Alto Tarauacá Extractive Reserve with 23 (Botelho 2013), 25 in Los Amigos, Peru (Tobler et al. 2008) and 27 in Chandless State Park, Acre (Borges et al. 2015). Although there is a difference in the sample effort employed, this finding is of high importance, since private areas may be of great value for the conservation of mammals and ecosystems as a whole (Gallo et al. 2009, Negrões et al. 2011), especially if allied with REDD+ goals and plans.

The number of species recorded in the Purus Project may be related to the fact that the area is poorly deforested and has a low human density, which is characterized by habitat quality and low hunting pressure. Several studies have demonstrated the deleterious effects of habitat fragmentation and high hunting pressure. These two factors are essential for maintaining biodiversity in Amazonian ecosystems, making it possible to achieve one of the REDD+, as demonstrated in Peres (2001) and Michalski & Peres (2007). Robinson and Bennett (2000) argue that forest ecosystems have a capacity to support hunting pressure of up to one inhabitant per km². As in the Purus Project the human density is approximately 0.29 hab/km² (approximately 100 hab/347.02 km^2) there is a greater probability of registering species susceptible to hunting pressure such as the tapir.

Among the species of mammals registered in the Purus Project area, two are categorized on the lists of endangered species of IUCN and MMA. Despite the large living area, such species are susceptible to hunting pressure and habitat loss. The tapir (*Tapirus terrestris*), for example, is a targeted species by hunters and is vulnerable, with slow reproduction, and can quickly suffer a reduction of its abundance in areas with high hunting pressure (Bodmer et al. 1997, Peres 2000a, Calouro 2005). Considered the largest terrestrial mammal in South America, the tapir is categorized as vulnerable to extinction by the International Union for the Conservation of Nature (IUCN). A possible extinction of this species entails the loss of a large number of ecosystem services, as a function of which it participates in processes such as dispersal, predation and grazing (of small seedlings). Therefore, acting in the control of plant populations and considered a key species for seed dispersal in the forest, especially large ones (Peres et al. 2016).

The record of naturally rare species, like *Cabassous unicinctus* and *Atleocynus microtis* (Leite-Pitman & Williams 2011, Anacleto et al. 2013), in the Purus Project area, demonstrating the additional role of REDD+ in fill gaps in the distribution of these species and to contribute with information about their ecology. *C. unicinctus*, for example, is a rare armadillo, with only a few records confirmed in the Amazon (Tobler et al. 2008, Anacleto et al. 2013). The also rare short-eared dog (*A. microtis*), also known as the short-eared fox, draws attention to the high number of records in the Purus Project. This species of fox was recorded 12 times in six different collecting seasons. The relative abundance of 0.65 ind/100 night-trap is among the highest recorded so far elsewhere in the Amazon, (Tobler et al. 2008) with about 2.9 ind/1000 night-traps in the Peruvian Amazon, (Koester et al. 2008) in the Jamari National Forest in Rondônia (Pimenta 2012) and even in the lower Purus have a relative abundance of 0.45. Information on *Atelocynus microtis* is extremely scarce. It is known that the species is the most solitary of the South American canids, has an omnivorous diet and its main habitat is near water bodies (Leite-Pitman & Williams 2011). In the Purus Project, all canine records were made of solitary individuals, it is noteworthy that none of the capture stations were mounted along water bodies.

Although not considered endangered, or even considered rare, the record of the puma (*Puma concolor*) is important in the study area. Because it is a large feline, the puma is a key species for the functioning of ecosystems (Terborgh et al. 2001). Through ingestion of their prey, large predators perform top-down control in ecosystems contributing to their balance and diversity (Estes et al. 2011). Another feline that plays a similar role in the control of species is the ocelot

Table 1. Mammals recorded through camera-trap in the Purus Project. Record type: P (camera-trap), T (trace), V (visualization), Voc (vocalization). RAI: No. of records in camera-traps/effort x 100. Conservation status: LC (Least Concern), VU (Vulnerable), DD (Data Deficient) and NT (Near Threatened), according to the Ministry of Environment (MMA) (2014) and International Union for the Conservation of Nature (IUCN) (2017).

Order FAMILY	**Common name**	**Record Type**	**RAI**	**Conservation Status**	
				MMA 2014	**IUCN 2017**
Artiodactyla					
CERVIDAE					
Mazama americana (Erxleben, 1777)	Red brocket deer	**P**	1.18		**DD**
TAYASSUIDAE					
Pecari tajacu (Linnaeus, 1758)	Collared peccary	**P; T**	1.56		**LC**
Carnivora					
CANIDAE					
Atelocynus microtis (Sclater, 1883)	Short-eared dog	**P**	0.65	**DD**	**NT**
FELIDAE					
Leopardus pardalis (Linnaeus, 1758)	Ocelot	**P**	0.54		**LC**
Puma concolor (Linnaeus, 1771)	Cougar	**P**	0.22		**LC**
MUSTELIDAE					
Eira barbara (Linnaeus, 1758)	Tayra	**P**	0.70		**LC**
Lontra longicaudis (Olfers, 1818)	Neotropical otter	**T**			**DD**
PROCYONIDAE					
Nasua nasua (Linnaeus, 1766)	Coati	**P**	0.27		**LC**
Cingulata					
DASYPODIDAE					
Cabassous unicinctus (Linnaeus, 1758)	Southern Naked-Tailed Armadillo	**P**	0.05		**LC**
Dasypus kappleri (Krauss, 1862)	Greater Long-nosed Armadillo	**P**	0.05		**LC**
Dasypus novemcinctus Linnaeus, 1758	Nine-banded armadillo	**P**	0.48		**LC**
Didelphimorphia					
DIDELPHIDAE					
Didelphis marsupialis Linnaeus, 1758	Black-eared opossum	**P**	0.11		**LC**
Lagomorpha					
LEPORIDAE					
Sylvilagus brasiliensis (Linnaeus, 1758)	Tapeti	**P**	0.05		**LC**
Perissodactyla					
TAPIRIDAE					
Tapirus terrestris (Linnaeus, 1758)	Lowland Tapir	**P; T**	0.59	**VU**	**VU**
Pilosa					
MYRMECOPHAGIDAE					
Myrmecophaga tridactyla Linnaeus, 1758	Giant anteater	**P**	0.22	**VU**	**VU**
Tamandua tetradactyla (Linnaeus, 1758)	Collared Anteater	**P**	0.05		
Primates					
ATELIDAE					
Alouatta puruensis Lönnberg, 1941	Purús Red Howler Monkey	**V**		**DD**	**LC**
CALLITRICHIDAE					
Saguinus weddelli (Deville, 1849)	Saddle-back tamarin	**P; V**	0.05		**LC**
Saguinus imperator (Goeldi, 1907)	Emperor tamarin	**V**		**DD**	**LC**

Continued Table 1.

Order FAMILY	**Common name**	**Record Type**	**RAI**	**Conservation Status**	
				MMA 2014	**IUCN 2017**
CEBIDAE					
Cebus unicolor Spix, 1823	Spix's White-fronted Capuchin	**P**	0.05		**LC**
Saimiri boliviensis (I. Geoffroy & de Blainville,1834)	Bolivian squirrel monkey	**P;V**	0.05		**LC**
PITHECIIDAE					
Plecturocebus cf. *toppini* (Spix,1823)	Red titi monkey	**V; Voc**			**LC**
Pithecia mittermeieri Gray, 1842	Gray's Bald-faced Saki	**V**			**LC**
Rodentia					
CUNICULIDAE					
Cuniculus paca (Linnaeus, 1766)	Spotted paca	**P**	0.43		**LC**
DASYPROCTIDAE					
Dasyprocta fuliginosa Wagler, 1832	Black agouti	**P**	1.51		**LC**
Myoprocta pratti (Pocock, 1913)	Green acouchi	**P**	0.59		**LC**
SCIURIDAE					
Hadrosciurus spadiceus Olfers, 1818	Southern Amazon red squirrel	**P**	0.38		**LC**

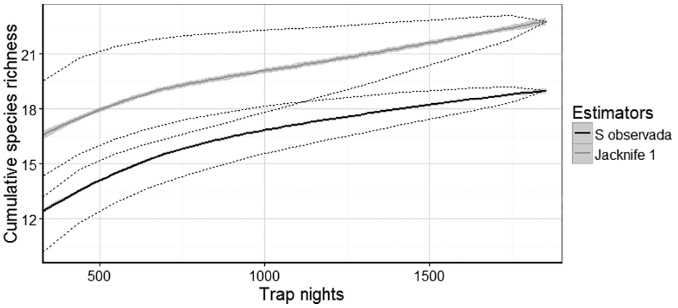

Figure 2. Cumulative curve of species (with standard deviation) of medium and large mammals registered at the Purus Project, Manoel Urbano - Acre.

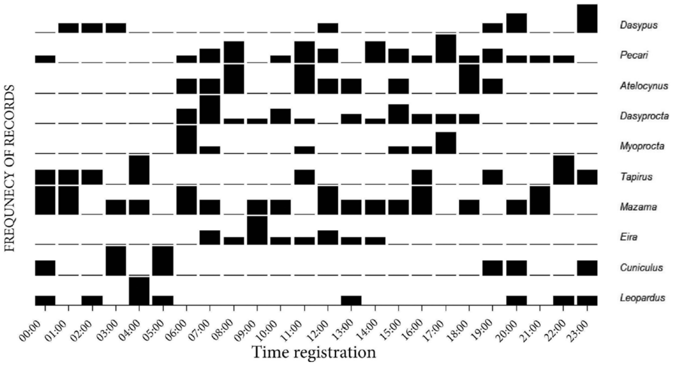

Figure 3. Summary of the activity pattern of the major taxa recorded in the Purus Project, Manoel Urbano, Acre.

(*Leopardus pardalis*), however, its relationship is with smaller prey, such as rats and marsupials of less than 1 kg, and even agouties. Through the predation of more competitive prey, ocelot contributes to a greater diversity of small mammals (Fonseca & Robinson 1990). Even with their great importance for the functioning of the ecosystem, felines are the target of conflicts with humans, especially in areas of high population density, because of the predation of domestic animals (Michalski et al. 2006, Marchini & Macdonald 2012). The relative abundance of these species in this region is considerable, especially due to the existence of residents.

The activity patterns of the species follow the description by Negrões (2011), and observerd by Botelho (2013) in RESEX Alto Tarauacá, Acre, Brazil. The high number of diurnal records of red deer, for example, is important. It is known that the species has a pattern of caternal activity (diurnal, crepuscular and nocturnal) (Azevedo 2008), but in areas with high hunting pressure, the species shows a decrease in the amount of diurnal records (Di Bitetti et al. 2008). Similarly, *P. tajacu* and *T. terrestris* presents activity pattern at day and night with different peaks. We observed that the three rodent's species presented differentiation in the activity pattern in response to a possible niche overlap, however more studies are needed to understand the patterns involved.

The richness of mammal species recorded through camera-traps in this study area underscores the importance of REDD+ projects in private areas for conservation, in view of the increasing challenges of inclusion and creation of new public protected areas and showed that most of the goals set by the project are met at this moment. Of the 21 conservation units in the state of Acre, only five have studies focusing on the mammal community. The Purus Project area surpasses the size of two protected areas (Area of Relevant Ecological Interest Japiim-Pentecoste and São Francisco National Forest) in the state and is near two other units, which highlights the importance of this area for conservation of biodiversity and demonstrates the potential of private areas for mammal conservation.

In the State of Acre, more specifically in the Purus River, information and even inventories of medium and large mammals are scarce. Thus, this initial study of mammals in the Purus Project area may serve as a basis for studies aimed at understanding the conservation status of mammals in the region, and in REDD+ project areas. REDD+ projects have, at times, difficulty on establishing plans and goals for the conservation of biodiversity (Panfil & Harvey 2016). Therefore, studies such as the one presented here, should increase in order to improve discussions and align methods of biodiversity monitoring in REDD+ projects, especially with cameras-traps since it allows comparisons at a global level (O'Brien et al. 2010, Rich et al. 2017).

Although, on average, have large home ranges and a wide distribution, medium and large mammals have been suffering increasingly with the rapid habitat reduction in recent years. The existence of private protected areas and the establishment of REDD+ project goals, can become an important component for the conservation of species and for the connection between UCs favoring the dispersion of species and populations between areas, and will consequently promote gene flow.

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Author Contributions

André Luis Moura Botelho: Substantial contribution to the design and design of the work; data acquisition, analysis and writing of the paper.

Luiz Henrique Medeiros Borges: Contribution in the analysis and interpretation of data, in the writing of the work and critical review adding intellectual content.

Brian McFarland: Contribution in the writing of the work and critical review adding intellectual content.

Conflicts of interest

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

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Inventory

Biodiversity of the metazoan parasites of *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae) in anthropized environments from the Batalha River, São Paulo State, Brazil

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Abstract: Biodiversity of fish parasites is a field of significant growth worldwide, whether due to the advancement of fish farms or the important role of these organisms as indicators of environment quality and ecosystem health, making them useful tools in the conservation and maintenance of the biodiversity as a whole. The objectives of this study were to evaluate the structure and composition of the parasitic fauna of *Prochilodus lineatus* collected from two structurally distinct stretches of the Batalha River. Fifty specimens of *P. lineatus* were collected between June 2015 and June 2016. Of these 50 hosts, 875 parasite specimens were collected, divided into 30 species, belonging to seven groups: Myxozoa, Monogenea, Digenea, Acanthocephala, Nematoda, Copepoda and Hirudinea. In addition to new records of known parasites of *P. lineatus* for this locality, 13 species were newly recorded parasitizing this fish, including a new monogenean species (*Tereancistrum* sp. n.), with Monogenea being the most representative class in the study. The findings of this study expands the known geographic distribution of these parasite species and helps to increase the knowledge of the biodiversity of these organisms in different hosts and environments. In general, the structure and composition of the *P. lineatus* parasitic fauna did not seem to be influenced by the structural characteristics of the two stretches studied, due to the migratory habits of the host.

Keywords: *Parasitic diversity, Curimbatá, Lentic ecosystems, Lotic ecosystems, Tietê-Batalha River basin.*

Biodiversidade dos metazoários parasitos de *Prochilodus lineatus* (Valenciennes, 1837) (Characiformes: Prochilodontidae) em ambientes antropizados no rio Batalha, Estado de São Paulo, Brasil

Resumo: A biodiversidade de parasitos de peixes é um campo de pesquisa com significativo crescimento mundial, seja pelo aumento das pisciculturas ou pelo importante papel desses organismos como indicadores da qualidade do ambiente e da saúde do ecossistema, tornando-os importantes ferramentas na conservação e manutenção da biodiversidade como um todo. O objetivo desse estudo foi de avaliar a estrutura e composição da fauna parasitária de *Prochilodus lineatus* coletados em dois trechos estruturalmente distintos do rio Batalha. Cinquenta espécimes de *P. lineatus* foram coletados entre junho de 2015 e junho de 2016. Desses 50 hospedeiros, foram coletados 875 espécimes de parasitos, divididos em 30 espécies, pertencentes a sete grupos: Myxozoa, Monogenea, Digenea, Acanthocephala, Nematoda, Copepoda e Hirudinea. Além dos novos registros para a localidade com parasitos já observados parasitando *P. lineatus* anteriormente, obteve-se o novo registro de 13 espécies parasitando este peixe, além do registro de uma nova espécie de monogenético (*Tereancistrum* sp. n.). Os achados desse estudo expandem a distribuição geográfica dessas espécies de parasitos e ajudam a aumentar o conhecimento da biodiversidade desses organismos em diferentes hospedeiros e ambientes. De modo geral, a estrutura e composição da fauna parasitária de *P. lineatus* não pareceu ser influenciada por características estruturais dos dois trechos estudados, devido aos hábitos migratórios do hospedeiro.

Palavras-chave: *Diversidade parasitária, Curimbatá, Ecossistemas lênticos, Ecossistemas lóticos, bacia hidrográfica do Tietê-batalha.*

Introduction

The tropics have an extensive diversity of plants and animals, with a large number of species and highly complex interactions when compared with temperate zones. The fish fauna follows this general ecological rule, both in aspects of taxonomy as well as in occupation and distribution in the environment (Lowe-McConnel 1999).

The fishes are the most diverse group of vertebrates, comprising at least 32,500 current known species, found in almost any type of aquatic environment and presenting enormous diversity in morphology, habitat occupation and biology (Nelson 2006, Rosa & Lima 2008). It is estimated that 13,000 species of fish live in strictly freshwater environments, and the tropics are the greatest maintainers of freshwater fish biodiversity on the planet, sheltering around 4,475 species, with the majority (2,587) in Brazilian territories alone (Langeani et al. 2009).

Even though vertebrate biodiversity in the neotropics has been studied extensively over the last four centuries (Rull 2011), there are still serious gaps in our knowledge of the biodiversity of invertebrates, particularly in parasitic faunas. Studies on parasitic biodiversity is extremely important, since parasitism plays a crucial role in an ecosystem's balance, regulating the abundance or density of host populations, stabilizing the trophic chains and structuring animal communities (Poulin & Morand 2004). In addition, parasites can also be used as biomonitoring tools for environmental impacts, especially in aquatic environments, as they manifest themselves in the face of environmental changes much faster than their hosts (Lafferty 1997, Lafferty & Kuris 2005, Silva-Souza et al. 2006, Sures 2008).

Biodiversity of fish parasites is a field of significant growth worldwide, whether due to the advancement of fish farms (Thatcher 2006), or the important role of these organisms as indicators of environment quality and ecosystem health, which are very useful tools in the conservation and maintenance of the biodiversity as a whole (Gómez & Nichols 2013). Several studies on the biodiversity of fish parasites in Brazilian basins have been carried out in recent decades (Eiras et al. 2011).

Prochilodus lineatus (Valenciennes, 1837), popularly known as Curimbatá or Curimatã, is a native species to the upper Paraná River basin, occurring throughout South America, along the Paraná-Paraguay and Paraíba do Sul River basins (Argentina, Brazil, Paraguay and Uruguay). This species belongs to the family Prochilodontidae, in the order Characiformes (Castro & Vari 2003). It is considered medium-sized and with migratory habits (Oyakawa et al. 2009). Its ecology and biology have been a consistent target of research as its populations have been constantly declining due to negative anthropic actions, such as dam construction and sport fishing (Rosa & Lima 2008, Oyakawa et al. 2009).

As a host, this species has also been the subject of several parasitological studies (Eiras et al. 1995, Martins et al. 2001, Adriano et al. 2002, Takemoto et al. 2002, Eiras et al. 2004, Lizama et al. 2004, Adriano et al. 2005, Lizama et al. 2005, Santos et al. 2005, Lizama et al. 2006, Cugliana et al. 2009, Takemoto et al. 2009, Eiras et al. 2011, Valladão et al. 2014). However, relatively little is known about the parasitic fauna of *P. lineatus* in the region of the Tietê-Baltalha basin, particularly in the Batalha River, where there is no published data. This highlights the need for studies that complement previously obtained data, and that contribute satisfactory knowledge of the biodiversity of parasites in this species in different aquatic ecosystems.

Therefore, this study aimed to carry out an ecological study of parasitic biodiversity of *P. lineatus* in two stretches of the Batalha River, State of São Paulo, Brazil, with distinct limnologic and structural characteristics to verify if there are differences between the parasitic infracommunities in the two stretches.

Material and Methods

1. *Study area*

The present study was conducted in two stretches of the Batalha River (Figure 1), each with quite different structural and limnological characteristics. The distance between the two stretches is approximately 135 Km.

2. *Stretch 1 – River channel – Reginópolis (SP)*

The stretch of the Batalha River, located in the municipality of Reginópolis, State of São Paulo (21º53'17"S and 49º13'31"W), is an anthropic area with predominantly lotic characteristics.

The area around the river in this stretch, though covered by native riparian forest for most of its extent, it used substantially for agricultural purposes, particularly cattle breeding and sugar cane, corn and eucalyptus plantations, causing the quantities of organic matter and leachate pollutants to be quite high. In addition, the municipality does not have sewage treatment, causing sewage to be released *in natura* in a tributary of the river a river (Santos & Heubel 2008, Estado de São Paulo 2010, Sistema Nacional de Informações sobre Saneamento 2016).

3. *Stretch 2 – DAE's water catchment lagoon – Piratininga (SP)*

The water catchment lagoon of the Departamento de Água e Esgoto (DAE) is located in the municipality of Piratininga, State of São Paulo (22º24'46"S and 49º05'05"W). It is a highly anthropized lentic ecosystem, with an area of approximately 170,000 m^2 and a water volume of 1,256,040 m^3/month. Agriculture, livestock and industrial activities in areas adjacent to the lagoon have unleashed irreversible environmental impacts in its structure, leading to silting and reduction of the few remnants of native vegetation, in addition to contributing to the significant increase of pollutant levels (heavy metals, organochlorine compounds, free radicals etc.) and endangering water quality. Despite this, the responsible regulatory department considers the water drawn from the lagoon as Class 2, that is, good quality for domestic use, human consumption, aquatic biodiversity protection and recreation (Brasil 2005).

4. *Fish sampling*

Fish collections were carried out between June 2015 and June 2016 with a total of nine collections. Of these, three were carried out on the Batalha River stretch of the municipality of Piratininga (June 2015, February and June 2016) and six were carried out in the stretch located in the municipality of Reginópolis (May, June and August 2015, February, March and May 2016).

For fish collection, standardized experimental fishing methodology was used, using nylon monofilament holding nets, with different mesh sizes (from 2 to 10 cm, alternate internodes) and variable heights. In each of the points the nets were installed perpendicularly and in half-moon,

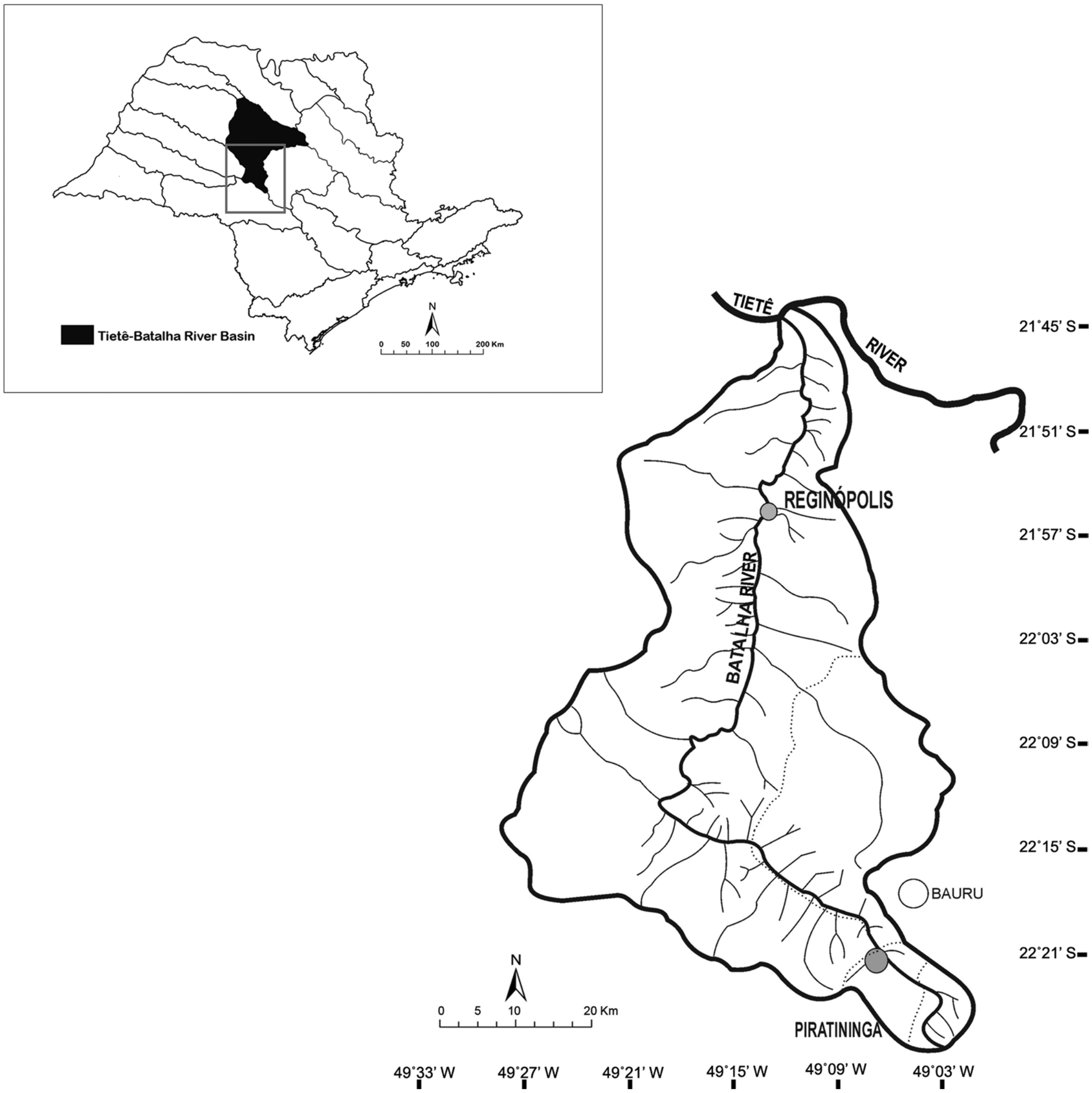

Figure 1. The Tietê-Batalha River Basin (BH-TB), located in the State of São Paulo, highlighting the course of the Batalha River between the two stretches studied, Reginópolis (SP) and Piratininga (SP).

at dusk (around 5pm) and the expense made at dawn (around 5am), resulting in approximately 12h of exposure.

After collection, fishes that were still alive were anesthetized with eugenol-based solution and euthanized through the physical method of medullary section. They were then packed in individual plastic bags and transported to the laboratory where they were frozen until necropsy, where measures of total length (cm), standard length (cm), weight (g) and sex were obtained. All collecting processes were made under the authorization of the Instituto Chico Mendes de Biodiversidade (ICMBio) through the Sistema de Autorização e Informação em Biodiversidade (SISBio) (authorization nº 40998-2). Additionally, the fish anesthesia and euthanasia methodologies were made following the guidelines of the Conselho Nacional de Controle de Experimentação Animal (CONCEA), and the research project was submitted to the Comitê de Ética no Uso de Animais (CEUA) da Universidade do Sagrado Coração (USC) (authorization nº 3295230615) before it could be performed.

5. *Parasite sampling, processing and identification*

For ectoparasite collection, the body, fins, nostrils, mouth, eyes and inner face of the operculums of each fish were separately washed with water and the contents of each external organ was filtered in a 53-µm mesh sieve. After this step, the gills were withdrawn and also submerged in aqueous solution and the vessel was shaken approximately 50 times and the contents passed through a 53-µm sieve. Afterwards, all the collected contents of the external organs were placed in Petridishes and analyzed individually under a stereomicroscope for parasite collection (modified from Eiras et al. 2006).

Then, through a longitudinal incision on the ventral surface of each individual, all organs were removed and separated. The visceral cavity and each organ were washed and filtered through a 75-µm mesh sieve, then placed in Petri dishes and examined separately under a stereomicroscope for the collection of endoparasites.

After collection, all parasites were fixed in 70% alcohol and kept in glass bottles until the time of staining/clarification and assembly with a slide and coverslip. Myxozoa were gel-mounted; Monogenea were mounted in Gray & Wess medium for the study of sclerotized structures (hooks, anchors, haptor bars, vagina and copulatory complex); Copepoda were clarified in lactic acid; Digenea and Acanthocephala were stained with carmine (Amato et al. 1991); and Nematoda were clarified with lactophenol (Eiras et al. 2006).

For parasite identification, specific bibliographies for each group with identification keys were used (Vicente et al. 1985, Lom & Arthur 1989, Boxshall & Montú 1997, Moravec 1998, Vicente & Pinto 1999, Gibson et al. 2002, Kabata 1992, Boeger & Vianna 2006, Thatcher, 2006; Cohen & Kohn 2007, Kohn et al. 2007).

6. *Statistical analysis*

Quantitative analyses for the characterization of infrapopulations and infracommunities were obtained through calculations of prevalence, mean abundance and mean intensity, as described Bush et al. (1997).

The community status of parasite species was classified according to Bush & Holmes (1986), classifying central species as presenting in 66% or more hosts, with secondary species present in between 33% and 65% of hosts and satellite species in fewer than 33% of hosts.

The structure of the parasitic community with prevalence equal to or greater than 10% was analyzed through its composition, numerical abundance, constancy of occurrence and species distribution, through the Pielou equitability index (J`), Brillouin diversity index (HB) and Margalef richness index (d) (Ludwig & Reynolds 1988).

The spatial distribution pattern of infrapopulations was calculated using the dispersion index (DI) and significance was tested using the statistical test *d*. The Green index (GI) was also applied to show how aggregated populations were (Ludwig & Reynolds 1988). These indexes were only calculated for species with a prevalence equal to or greater than 10%.

A species accumulation curve was run to verify sampling efficiency in conjunction with the first order Jackknife estimator (Magurran 1988) to determine the expected parasitic richness for the hosts studied. The levels of similarity between the communities of the two stretches were analyzed through the percentage similarity and Sorensen indexes (Wolda 1981).

Results

Fifty specimens of *P. lineatus* were collected and analyzed (37 from Stretch 1 and 13 from Stretch 2), with 12 females and 38 males. The hosts presented a mean standard length and weight of 28.45 ± 6.24 cm and 671.48 ± 542.74 g respectively. Of these 50 hosts, 875 specimens of parasites belonging to seven groups were collected: Myxozoa, Monogenea, Digenea, Nematoda, Acanthocephala, Copepoda and Hirudinea.

We found 30 parasite species (Table 1). The class Monogenea was the most representative (13 species, including a new species not yet described), followed by the Digenea subclass (six species) and by the Nematoda phylum (four species). The main site of infestation was the gills, parasitized by Myxozoa, Monogenea and Copepoda, followed by the body surface, parasitized by Monogenea, Digenea, Nematoda and Hirudinea and the intestine, parasitized by Digenea, Acanthocephala and Nematoda.

The most prevalent species were *Myxobolus* sp. (58%) and *Neoechinorhynchus curemai* Noronha, 1973 (56%), followed by Diplostomidae gen. sp. (28%) and *Tereancistrum* sp. n. (26%). Regarding mean abundance, the great majority of species presented low indexes, where the only exceptions were *N. curemai* (4.62) and *Tereancistrum* sp. n. with mean abundance of 16.04. Regarding the mean intensity, the highest indexes were for *Annelamphistoma* sp. (23.00) and *Tereancistrum* sp. n. (13.23). Regarding the community status, 28 species were classified as satellite, since they had low prevalence, and two species were classified as secondary (*Myxobolus* sp. and *N. curemai*). Dispersion and Green indexes of the parasites with prevalence above 10% indicated an aggregate distribution pattern for all species (DI > 1 and GI > 0) (Table 2).

Adult and larval forms were observed for nematodes, digeneans and acanthocephalans. Among the nematodes, the two species of *Contracaecum* were in the L4 larval stage, while *Procamallanus* (*Spirocamallanus*) *inopinatus* Travassos, Artigas & Pereira, 1928 and *Spinitectus asperus* Travassos, Artigas & Pereira, 1928 were adults. Among the digeneans, *Bellumcorpus* sp. and Diplostomidae gen. sp. were found in the metacercarial stage, and *Annelamphistoma* sp., *Colocladorchis* sp., *Unicoelium prochilodorum* Thatcher & Dossman, 2011, besides an unidentified species, were adults. Regarding the acanthocephalans, a species in the larval stage (cistacanth) was not identified, whereas the specimens of *N. curemai* were all found in the adult phase.

The accumulation curve of species showed a tendency to stabilize at 30 species, which indicates a good efficiency in parasite sampling. This can be confirmed by the first order Jackknife estimator that determined the expect richness as being 34 species, a value very close to that obtained (Figure 2).

Ecological indexes of diversity applied at the two sampling stretches showed the infracommunities of *P. lineatus* in the Batalha River as having high uniformity in the distribution of species (J’ = 0.92) and also high richness and diversity (d = 1.83 and HB = 0.81). Comparing the two stretches separately, Stretch 1 (Reginópolis-SP) was slightly more equitable (J’ = 0.93) and of greater richness (d = 1.96) (Table 3). Regarding the similarity between the parasitic infracommunities at the two sampling stretches, the results obtained were relatively high, with a percentage similarity of 72.5% and a Sorensen similarity of 0.73; more than 70% of the parasite species were common to the two stretches of the Batalha River studied.

Discussion

This work presents the first records of the parasite biodiversity of *P. lineatus* in the Batalha River, where the results show a metazoan community with high species richness and low abundance. The presence of two secondary species (*Myxobolus* sp. and *N. curemai*) indicates that there is also low species dominance. This low dominance can be explained by the eating habits of the host (Lizama et al. 2005), which is essentially characterized by ingestion of detritus and sediments, which includes a large variety of invertebrates (Fugi et al. 2001). Its general diet, with low specificity of food items, makes it extremely susceptible to

Table 1. Species, number of parasitized hosts (NPH), infestation/infection sites (IS), prevalence (P), range of variation (RV), mean abundance (MA), mean intensity (MI), community status (CS) and location of incidence (LI) of the parasites of *Prochilodus lineatus* from the Batalha River, Tietê-Batalha Basin, State of São Paulo, Brazil.

Taxon	NPH	IS	P (%)	RV	MA	MI	CS	LI
Myxozoa								
Henneguya sp.	8	G	16	–	–	–	SA	R/D
Myxobolus lomi Azevedo et al. 2014	5	G	10	–	–	–	SA	R/D
Myxobolus sp.	29	G/H	58	–	–	–	SE	R/D
Monogenea								
Demidospermus paravalenciennesi Gutiérrez & Suriano, 1992	3	G/S	6	1–15	0.4 ± 0,04	6.67 ± 0,10	SA	D
Kritskyia boegeri Takemoto, Lizama & Pavanelli, 2002	1	S	2	5	0.1 ± 0,01	5.00	SA	D
Pavanelliella sp.	5	N/G	10	4–9	0.68 ± 0,04	6.80 ± 0.14	SA	R/D
Tereancistrum ornatus Karling et al., 2014	4	G	8	1–15	0.04 ± 0,04	5.00 ± 0.08	SA	R
Tereancistrum sp. n.	13	G	26	1–102	3.44 ± 0,29	13.23 ± 0.76	SA	R
Tereancistrum toksonum Lizama, Takemoto & Pavanelli, 2004	3	G	6	1–4	0.12 ± 0,01	2.00 ± 0.04	SA	R/D
Trinibaculum altiparanae Abdallah, Azevedo & Silva, 2013	1	G	2	1	0.02	1.00	SA	R
Phanerothecium sp.	1	S	2	3	0.06 ± 0,01	3.00	SA	R
Rhinonastes pseudocapsaloideum Kritsky, Thatcher & Boeger, 1988	7	N	14	1-–5	0.3 ± 0.02	2.14 ± 0.08	SA	R/D
Rhinoxenus curimbatae Domingues & Boeger, 2005	4	N	8	1–3	0.16 ± 0.01	2.00 ± 0.05	SA	D
Rhinoxenus sp. 1	7	S	14	1–5	0.3 ± 0.02	2.14 ± 0.08	SA	R/D
Rhinoxenus sp. 2	2	S	4	2	0,08 ± 0,01	2.00 ± 0.02	SA	R/D
Rhinoxenus sp. 3	2	S	4	1–6	0.14 ± 0.02	3.50 ± 0.03	SA	R
Digenea								
Annelamphistoma sp.	2	ST/I	4	6–40	0.92 ± 0.11	23.00 ± 0.18	SA	R
Bellumcorpus sp. (metacercarie)	6	S	12	1–6	0.3 ± 0.02	2.50 ± 0.08	SA	R
Colocladorchis sp.	4	I	8	1–6	0.56 ± 0.05	7.00 ± 0.13	SA	R/D
Diplostomidae gen. sp. (metacercarie)	14	E	28	2–16	1.1 ± 0.05	3.93 ± 0.24	SA	R/D
Digenea gen. sp.	4	I	8	1–15	0.72 ± 0.06	9.00 ± 0.16	SA	R/D
Unicoelium prochilodorum Thatcher & Dossman, 2011	4	I	8	2–21	0.7 ± 0.07	8.75 ± 0.14	SA	R
Acanthocephala								
Cistacanth	1	I	2	15	0.3 ± 0.04	15.00	SA	R
Neoechinorhynchus curemai Noronha, 1973	28	ST I	56	1–32	4.62 ± 0.15	8.25 ± 0.91	SE	R/D
Nematoda								
Contracaecum sp. 1	3	I	6	1–5	0.18 ± 0.02	3.00 ± 0.05	SA	R/D
Contracaecum sp. 2	4	S	8	1	0.08 ± 0.01	1.00 ± 0.04	SA	R/D
Procamallanus (S.) inopinatus Travassos, Artigas & Pereira, 1928	5	I	10	1–2	0.12 ± 0.01	1.20 ± 0.05	SA	R/D
Spinitectus asperus Travassos, Artigas & Pereira, 1928	10	ST I	20	1–5	0.82 ± 0.04	4.10 ± 0.18	SA	R/D
Copepoda								
Amplexibranchius bryconis Thatcher & Paredes, 1985	11	G	22	1–5	0.52 ± 0.03	2.36 ± 0.13	SA	R/D
Hirudinea								
Helobdella sp.	11	S	22	1–5	0.36 ± 0.02	1.64 ± 0.11	SA	R/D

G = Gills, H = Heart, S = Body surface, N = Nostril, E = Eyes, ST = Stomach and I = Intestine. SA = Satellite and SE = Secondary. R = Reginópolis and D = DAE.

Table 2. Dispersion index (DI), statistic test ***d*** and Green index (GI), of the parasites of *Prochilodus lineatus* from the Batalha River, Tietê-Batalha River Basin, State of São Paulo, Brazil, with prevalence of more than 10%.

Species	**DI**	***d***	**GI**
Pavanelliella sp.	6.87	16.1	0.11
Tereancistrum sp. n.	62.07	68.14	0.04
Rhinonastes pseudocapsaloideum	2.76	6.58	0.04
Rhinoxenus sp. 1	3.03	7.18	0.11
Bellumcorpus sp.	3.44	8.5	0.15
Diplostomidae gen. sp.	6.35	15.11	0.11
Neoechinorhynchus curemai	11.49	23.71	0.21
Procamallanus (S.) inopinatus	1.24	1.17	0.001
Spinitectus asperus	4.37	19.6	0.07
Amplexibranchius bryconis	3.79	9.41	0.06
Helobdella sp.	2.24	4.97	0.03

DI: > 1 = aggregate distribution; *d*: ≥ 1.96 = aggregate distribution; *d*: ≤ 1.96 = aleatory distribution; GI: > 0 = aggregate distribution (the higher the value, the greater the degree of aggregation); GI: = 0 = aleatory distribution.

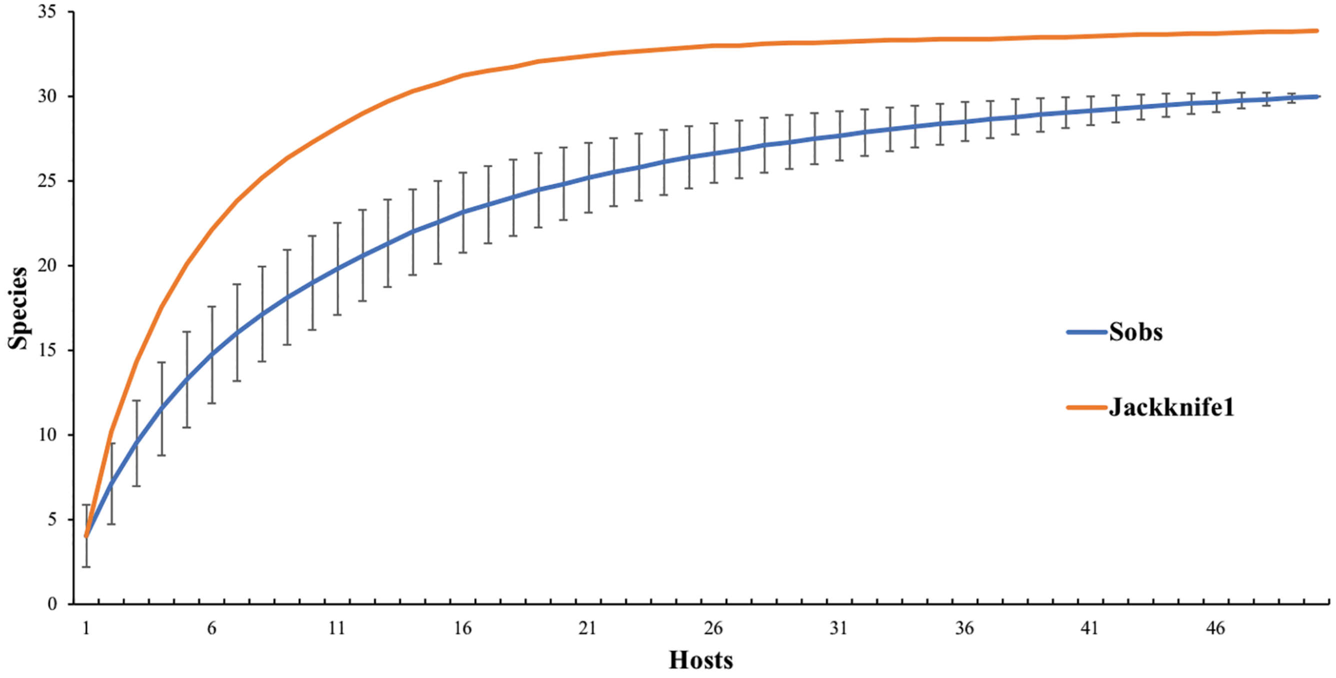

Figure 2. Accumulation curve of observed species richness (Sobs) and first-order Jackknife richness estimator (Jackknife1) of the parasites of *Prochilodus lineatus* from the Batalha River, Tietê-Batalha River Basin, State of São Paulo, Brazil.

Table 3. Averages and standard deviation of the ecological diversity indexes of the parasitic infracommunities of *Prochilodus lineatus* at both stretches (Stretch 1 – Reginópolis-SP and Stretch 2 – Piratininga-SP) sampled from the Batalha River, Tietê-Batalha River Basin, State of São Paulo, Brazil.

Indexes	**Stretch 1**	**Stretch 2**	**Stretch 1 and Stretch 2**
J’	0.93 ± 0.13	0.88 ± 0.10	0.92 ± 0.13
d	1.96 ± 0.69	1.49 ± 0.71	1.83 ± 0.72
HB	0.80 ± 0.32	0.84 ± 0.38	0.81 ± 0.33

J’ = Pielou equitability index; d = Margalef richness index; HB = Brillouin diversity index.

infection/infestation by numerous parasite species (Lizama et al. 2005), which may also explain the high parasite abundance found in this fish.

The parasitic fauna of *P. lineatus* consisted mainly of ectoparasites, particularly monogeneans. It is known that Characiformes fishes have a higher abundance of parasite species in this class (Boeger & Vianna 2006), where the presence of morphological adaptations, such as eggs with adhesive appendages that allow attachment to the substrate (Yamada et al. 2007), facilitate infestation by these parasites. Direct life cycle parasites, such as monogeneans, are most often found in lentic environments, since transmission is facilitated in low water flow environments (Lizama et al. 2006, Yamada et al. 2007, Pavanelli et al. 2008). The great majority of the *P. lineatus* specimens used in the present study were collected in a lotic stretch of the Batalha River, and those from a lentic environment (Stretch 2) presented a low prevalence of monogenetic parasitism. However, since migratory habits are a major characteristic of this species (Resende et al. 1996), it is assumed that it can move through different habitats and areas that have different structural characteristics, including wetlands and dams, and consequently is exposed to high parasitic richness (Caro et al. 1997).

The aggregate distribution pattern observed in the metazoan parasite community of *P. lineatus* in the present study followed the same pattern observed for the metazoan community of the same host species in other rivers of the Upper Paraná River Basin (Bruno 2003, Lizama et al. 2005). According to Zuben (1997), the main factors that generate an aggregate distribution of parasites in the host are mainly associated with random environmental variables, including changes in physical parameters of the environment (including both host and habitat) that, according to Anderson & Gordon (1982), includes: (1) heterogeneity in host susceptibility to infection/infestation, (2) direct reproduction of the parasite within the host and (3) heterogeneity in the hosts ability to eliminate parasites by immunological response or other types of response. Further, according to Zuben (1997), the aggregate pattern of distribution commonly found among different species of parasites contributes to community stability, minimizing the frequency of interspecific interactions between parasites, thus allowing intraspecific regulation mechanisms to dominate. Thus, competitive interactions among different species of parasites will influence only small proportions of each parasitic population present in simultaneously infected hosts.

Regarding endoparasites, digeneans constituted the second largest group of species, reflecting the already expected pattern for diversity in freshwater fish parasites in Brazil (Eiras et al. 2011, Cribb et al. 2002). Of the six species found, two were in the larval stage (metacercarie) (*Bellumcorpus* sp. and Diplostomidae gen. sp.) and only one could be identified to species level (*U. prochilodorum*). According to Niewiadomska & Niewiadomska-Bugaj (1995), the difficulty in identifying species of this subclass, particularly metacercariae, is due to variation in morphology and also to the limited number of morphological differences. Among the factors that cause variation in the morphology of these parasites are the host fish species, the density of the infection and the size and maturation stage of the host. Any of these factors can generate significant differences between individuals of the same digenean species. Another factor that may influence identification are the methodologies used for fixation, preservation and analysis of the parasites, with the use of molecular biology being a safer way to obtain accurate results regarding the identification of the species (Zago 2016). The high prevalence of Diplostomidae gen. sp. parasitizing the host's eyes has previously been reported in several species of fish in the Upper Paraná River Basin (Takemoto et al. 2009). Parasitism by diplostomids can cause severe ocular pathologies such as blindness and cataracts, impairing fish eyes and making then more susceptible to predation by piscivorous birds, which act as definitive hosts for these parasites to complete their life cycle (Pavanelli et al. 2008).

Two species of acanthocephalans were collected, but only one can be identified to the lowest taxonomic level. *Neoechinorhynchus curemai* is a parasite commonly found parasitizing *P. lineatus* (Martins et al. 2001b), and in the present study it was the second most prevalent parasite, collected in 56% of the hosts, being the only species characterized with secondary community status. It has been proven that parasitism by *N. curemai* can cause several tissue changes in the host intestine, including hyperplasia and hypertrophy of goblet cells, as well as severe inflammation and edema in the intestinal mucosa (Martins et al. 2001a).

Three of the four species of parasitic nematodes found had low prevalence and abundance; only *S. asperus* had a prevalence above 20%. The low occurrence of parasitic nematodes in *P. lineatus* has been reported previously, ranging from one to at most four species (Lizama et al. 2005, Zago 2016). High specificity with respect to the definitive host (Moravec 1998) and even competition with other parasites (Dobson 1985) may contribute to the relatively low abundance and richness of the infrapopulations of these parasites.

Despite the low prevalence and abundance of nematodes in *P. lineatus*, one should consider the zoonotic potential that some of these nematode species have, particularly anisakids, such as those in the genus *Contracaecum*. Despite the limited cases reported in humans, experimental studies in terrestrial mammals confirm the susceptibility to infection and the high zoonotic potential of the genus (Vidal-Martínez et al. 1994, Buchmann & Mehrdrama 2016). In addition, larvae of *Contracaecum* sp. have proven to be an excellent accumulator of toxic metals, and can be considered as good indicators of pollution, and therefore deserve attention (Leite et al. 2017).

Regarding copepods, *Amplexibranchius bryconis* Thatcher & Paredes, 1985 had previously been reported parasitizing *P. lineatus* (Abdallah et al. 2011), and as before, and, as before, this crustacean parasite when present was recorded as having an epibiotic association with the host. In addition, parasitism by ergasilids may cause partial or complete occlusion of the lamella blood vessel, hyperplasia and increased mucus production, causing a decrease in the respiratory capacity of the host and causing secondary infections (Pavanelli et al. 2008).

In general, relatively little is known about the biodiversity of the parasitic fauna of *P. lineatus*, with only a handful of studies conducting extensive surveys (Lizama et al. 2005, Takemoto et al. 2009, Côrrea 2014). The other existing studies on this host focus on specific taxa and the description of new species, especially for myxozoans and monogenetics (Adriano et al. 2002, Takemoto et al. 2002, Bruno 2003, Adriano 2005, Cugliana et al. 2009, Campos et al. 2011, Azevedo et al. 2014). In the present study, in addition to new records of known parasites for *P. lineatus* previously, 13 species were newly recorded parasitizing this fish (*Demidospermus paravalenciennesi* Gutiérrez & Suriano, 1992, *Pavanelliella* sp., *Tereancistrum ornatus* Karling et al., 2014, *Trinibaculum altiparanae* Abdallah, Azevedo & Silva, 2013, *Phanerothecium* sp., *Rhinoxenus* sp. 1, *Rhinoxenus* sp. 2, *Rhinoxenus* sp. 3, *Annelamphistoma* sp., *Bellumcorpus* sp., Diplostomidae gen. sp., and *P. (S.) inopinatus*), in addition to the record of a new monogenean species (*Tereancistrum* sp. n.). This expands the geographical distribution of known parasite species of *P. lineatus* and assists in increasing our knowledge of the biodiversity of these organisms in different hosts and environments.

In relation to the similarity indexes (percentage and Sorensen), the values obtained can be considered high, revealing high similarity between the parasite communities in the hosts collected in the two stretches, despite being two structurally distinct environments (lagoon = lentic and river channel = lotic). According to Poulin & Morand (2004), the geographic distance between the ichthyo parasitological communities of the same host species is often the best predictor of similarity in species composition. Although the distance between the collection sites is relatively large (135 km), *P. lineatus* is a species that performs long upstream migrations, preceded by annual floods (Stassen et al. 2010), this being the predominant factor explaining such similarity. The same can also be applied to the richness, diversity and equitability indexes, which were stable at both stretches, when it was expected that there would be significant differences between the values since these are environments with different characteristics, especially when regarding the physical and chemical characteristics of water, which, according to Galli et al. (2001), are determining factors in the composition of the parasitic communities of fish.

Conclusion

The structure and composition of the *P. lineatus* parasitic fauna did not seem to be influenced by the structural characteristics of the two stretches studied, due to the migratory habits of the host. In addition, the present work contributes significantly to the knowledge about the biodiversity of the *P. lineatus* parasites in the Batalha River, a region that has not been explored so far, helping to expand the knowledge about the parasitic fauna of Brazilian fish in different ecosystems, filling some of the existing gaps in this field and contributing to the registration of new species not yet described by science.

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Author Contributions

Lucas Aparecido Rosa Leite: 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 contente.

Larissa Sbeghen Pelegrini: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intelectual contente.

Beatriz Narciso Agostinho: Contribution to manuscript preparation.

Rodney Kozlowiski de Azevedo: Contribution to data collection. Contribution to critical revision, adding intelectual contente.

Vanessa Doro Abdallah: Substantial contribution in the concept and design of the study. Contribution to data analysis and interpretation. Contribution to critical revision, adding intelectual contente.

Conflicts of interest

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

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Short Communication

Do we underestimate the impact of roads on arboreal animals? Roadkill as an important threat to *Chaetomys subspinosus* (Mammalia: Rodentia)

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SRBEK-ARAUJO, A. C.; ALVARENGA, A. C.; BERTOLDI, A. T. **Do we underestimate the impact of roads on arboreal animals? Roadkill as an important threat to *Chaetomys subspinosus* (Mammalia: Rodentia)**. Biota Neotropica. 18(3): e20170511. http://dx.doi.org/10.1590/1676-0611-BN-2017-0511

Abstract: The Thin-spined Porcupine (*Chaetomys subspinosus*) is a medium-sized and mainly arboreal rodent, endemic to the Brazilian Atlantic Forest, and threatened with extinction. Habitat loss, hunting, forest fires, agriculture and livestock are threats identified for the species. Here we raise the alert to the impact of roads on remaining populations of *C. subspinosus* based on roadkill records from the state of Espírito Santo, southeastern Brazil. Mortality due to roadkill is likely to impact *C. subspinosus* in different regions of the state, and is a widespread problem, not unique to a single location or population. The pattern of roadkills in the studied regions suggest that the species is more susceptible to collisions with vehicles in the breeding period. Additionally, concrete barriers that divide lanes on highways seens to increase the likelihood of roadkill for Thin-spined Porcupines. We recommend that roadkill should be included in the list of threats to *C. subspinosus* in the Espírito Santo. Mortality due to roadkill is probably relevant also for populations in the states of Bahia and Sergipe, and it should be evaluated locally. The installation of road-crossing structures for wildlife, such as arboreal overpasses, is recommended on roads crossing or close to protected areas with *C. subspinosus* presence in Espírito Santo and elsewhere.

Keywords: *Atlantic Forest, protected areas, road ecology, Thin-spined Porcupine.*

Estaríamos subestimando o impacto de estradas sobre espécies arborícolas? Atropelamento de fauna como uma importante ameaça para *Chaetomys subspinosus* (Mammalia: Rodentia)

Resumo: O ouriço-preto (*Chaetomys subspinosus*) é um roedor essencialmente arborícola, de médio porte, endêmico da Mata Atlântica brasileira. Atualmente está classificado como Vulnerável à extinção. Perda de habitat, caça, incêndios florestais, agricultura e pecuária são identificados como ameaças para a espécie. A presente comunicação alerta para o impacto de estradas sobre as populações remanescentes de *C. subspinosus* com base em registros de atropelamento obtidos no estado do Espírito Santo, sudeste do Brasil. Os registros aqui apresentados indicam que a morte de espécimes devido a atropelamentos afeta populações de *C. subspinosus* em diferentes regiões do estado, sendo um problema generalizado, não consistindo em ameaça para uma única localidade ou população. O padrão observado nas regiões estudadas sugere que a espécie é mais suscetível a atropelamentos durante o período reprodutivo. Além disso, a presença de barreiras de concreto dividindo as faixas das rodovias parece aumentar a ocorrência de atropelamentos de ouriços-pretos. Recomenda-se que o atropelamento de espécimes seja incluído entre as ameaças à conservação de *C. subspinosus* no Espírito Santo, podendo representar uma ameaça relevante para a espécie também nos estados da Bahia e Sergipe, o que deve ser avaliado localmente. A instalação de estruturas para transposição rodoviária pela fauna, como passagens aéreas, é recomendada para estradas que atravessam ou que estão associadas a áreas protegidas com confirmação da presença de *C. subspinosus* no Espírito Santo e em outros estados.

Palavras-chave: *áreas protegidas, ecologia de estradas, Mata Atlântica, ouriço-preto.*

Introduction

The Thin-spined Porcupine (*Chaetomys subspinosus* Olfers, 1818) is a medium-sized, almost entirely arboreal, nocturnal and folivorous rodent in the Family Erethizontidae (Chiarello et al. 1997, Giné et al. 2010, Souto Lima et al. 2010). It is endemic to the Atlantic Forest where its historical range extends from northern Rio de Janeiro to southern Sergipe (Oliver & Santos 1991). Recent porcupine sighting records are restricted to a narrow strip along the coast from southern Espírito Santo to Sergipe (Oliveira et al. 2011). Remaining populations of *C. subspinosus* are declining (Faria et.al. 2011, Catzeflis et al. 2017) and the species is classified as Vulnerable (Brasil 2014, Catzeflis et al. 2017). The National Action Plan for the Conservation of the Thin-spined Porcupine lists its main threats as habitat loss and fragmentation due to real estate and other infrastructure development which destroy natural habitat, poaching that is common in rural areas near almost all remaining populations, forest fires that cause mortality due to mainly arboreal habits and because they sheltering in tangles of vines (limit their ability to escape from fires), and agriculture (including livestock grazing) with agrochemical use and pasture clearing at the edges of the remaining forest fragments (Faria et al. 2011).

Habitat loss and habitat fragmentation modify all aspects of the landscape (Forman & Alexander 1998, Forman & Deblinger 2000, Trombulak & Frissell 2000), and are usually associated with roads. Roads inhibit animal movement and may often be barriers (total or partial) for many animal species and thus cause isolation of populations, especially important for threatened species (Forman & Alexander 1998, Trombulak & Frissell 2000). Arboreal mammals depend on trees, rarely travel on the ground, and are vulnerable to habitat fragmentation (Lancaster et al. 2011). For these reasons, the impact of roads may be even more important for arboreal than terrestrial species, especially when causing genetic isolation of populations (Taylor et al. 2011). In addition to potential isolation of populations by roads themselves, roadkill as a consequence of crossing roads is an extremely important anthropogenic cause of mortality for vertebrates worldwide (Forman & Alexander 1998) and few species are immune to this threat (Trombulak & Frissell 2000).

Locomotion, ecology and behavior all determine wildlife vulnerability to roadkill, and slow-moving, predominantly arboreal habits (with occasional forays across open ground) and the tendency to freeze in response to approaching vehicles, along with relatively poor eyesight, are some of the characteristics that make species especially susceptible to roadkill (Laurance et al. 2009). Erethizontids hear well and have good olfaction but poor vision (Vaughan et al. 2000). *Chaetomys subspisosus* is slow-moving within the canopy, never jumping, but rather carefully traversing tree to tree (Faria et al. 2011, Oliveira et al. 2012). This porcupine rarely descends to the forest floor, using the ground to cross between trees separated by a few meters when canopies do not permit arboreal travel (Oliveira et al. 2012). Thus, *C. subspisosus* is often vulnerable to roadkill.

Here, using roadkill records from different places in Espírito Santo, we raise the alert to the impact of roads on the remaining populations of *C. subspinosus* in the Atlantic Forest of Brazil.

Material and Methods

We gathered primary (our unpublished data) and secondary (records published by other authors) data of roadkills in three regions in the state of Espírito Santo: Coastal Corridor Jucu-Setiba-Benevente (*Corredor Costeiro Jucu-Setiba-Benevente* - CCJSB; 20°23'-20°47' S and 40°19'-40°40' W), municipalities of Vila Velha, Guarapari and Anchieta, in the central-southern coast; Pedra Azul State Park region (*Parque Estadual da Pedra Azul* - PEPAZ; 20º23'-20º25' S and 41º01'-40º59' W; 1,240 ha), municipality of Domingos Martins, in the south-central mountains; and Linhares-Sooretama Block (*Bloco Linhares-Sooretama* - BLS; 18°53'-19°15' S e 39°44'-40°16' W; ~50,000 ha), between the municipalities of Linhares and Jaguaré, in the north (Figure 1).

The CCJSB comprises the Jacarenema Municipal Natural Park (*Parque Natural Municipal de Jacarenema* - PNMJ; 346 ha), the Setiba Environmental Protection Area (Área *de Proteção Ambiental de Setiba* - APA Setiba; 12,960 ha) and Paulo César Vinha State Park (*Parque Estadual Paulo César Vinha* - PEPCV; 1,500 ha), among other remnants of native vegetation (including priority areas for conservation in Espírito Santo). The PNMJ includes forest, flooded forest associated with the Jucu river, mangrove, and vegetation of restinga (relatively sparse to dense shrubs and forests found on sandy coastal plains) near the beach. The APA Setiba includes land and sea surrounding the PEPCV. The land area of APA Setiba and PEPCV are on sandy coastal plains and comprise different communities of restinga vegetation (forest, flooded and non-flooded shrub vegetation and floodplain). Records of Thin-spined Porcupine roadkills in the CCJSB region were on highway ES-060 (regionally called *Rodovia do Sol*) where it comes in contact with protected areas (~3 km in PNMJ and ~15 km in APA Setiba and PEPCV) and other remnants (Figure 1). Wildlife roadkills have been systematically monitored here daily since 2001 and is carried out by an observer traveling by car along a 67.5 km section of road that includes some urban areas. Roadkill data (from 2001 to 2015) are available at the Concessionária Rodovia do Sol (2016). The date and approximate geographic location of each roadkilled *C. subspinosus* were provided by the team of the Program for the Protection and Monitoring of Wild Animals *É o Bicho* developed by the Concessionária Rodovia do Sol S.A. This monitoring is among the legal conditions for operation of highway ES-060 (Condition n° 27 of Operating License 03/03 - IEMA).

The PEPAZ region comprises forests, including high altitude forest, and vegetation associated with rock formations. Records of *C. subspinosus* on the PEPAZ region are from our monthly surveys (primary data) carried out from January to December 2015, on the stretch of the highway BR-262 that borders the park. A 20 km section (from km 74 to 94) was traveled by car with two researchers (one driver and one observer) at an average speed of 50 km/h during five consecutive days each month.

The BLS comprises the Sooretama Biological Reserve (*Reserva Biológica de Sooretama* – RBS; 24,250 ha), the Vale Natural Reserve (*Reserva Natural Vale* – RNV; 22,711 ha) and two other nearby protected areas (Private Reserve of Natural Heritage, *Reserva Particular do Patrimônio Natural* - RPPN Recanto das Antas, of 2,212 ha, and RPPN Mutum Preto, of 379 ha). The BLS includes a mosaic of habitats in which dense lowland forest (*Tabuleiro* forest) is dominant. The BLS

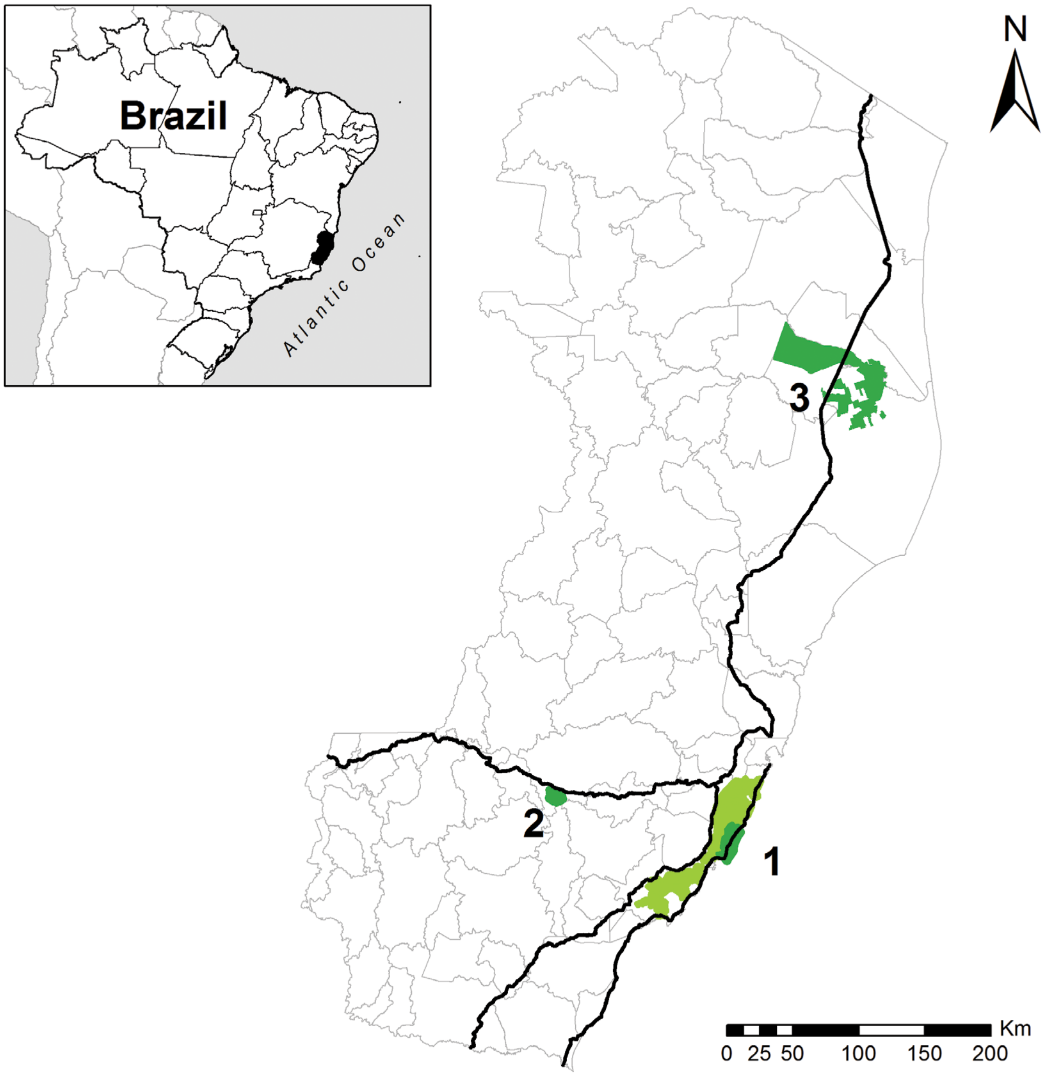

Figure 1. Location of the roadkills of the Thin-spined Porcupine (*Chaetomys subspinosus*) in Brazil (insert) and in the state of Espírito Santo: Coastal Corridor Jucu-Setiba-Benevente (light green), showing the Paulo César Vinha State Park and the land area of Setiba Environmental Protection Area (dark green; 1), Pedra Azul State Park (2) and Linhares-Sooretama Block (3). The roads associated with these regions and protected areas, where the records were obtained, are also indicated (south coast: ES-060; east-west: BR-262; north-south: BR-101).

is intersected by highway BR-101where the Thin-spined Porcupine roadkill was found (~15 km of road). Wildlife roadkills on BR-101 where it contacts the BLS are systematically monitored daily by car or on foot (A. Banhos, personal communication). A set of roadkill records from 2011 to 2014 is available in Klippel et al. (2015) and was used by us as source for roadkill in the northern part of the state. That study did not report all roadkilled mammals during the period, and so the data should be used only for qualitative purposes.

BR-262 and the BR-101 are one-lane roads with a shoulder on both sides in the region where they are monitored. ES-060 is a single lane road to km 12 where it becomes two lanes separated by a divide to km 40, followed by a concrete curb barrier between km 40 and 50, where it once again becomes single lane (Concessionária Rodovia do Sol 2016). PNMJ is between km 11 and 14, PEPCV between km 29 and 40, and APA Setiba between km 27 and 43 of ES-060. The tree canopy does not reach over the roads in any of the studied areas, and so arboreal animals must cross the road when they move from one side to the other.

Roadkills of other arboreal mammal species were available in the records we analyses and also were recorded in PEPAZ region. We include the list of species in this study to demonstrate that many additional arboreal species are affected by roads in the studied regions, and also deserve special attention in conservation and management plans as well.

Results and Discussion

Fifteen *C. subspinosus* were killed by collisions with vehicles between 2002 and 2015 on the ES-060 along the CCJSB, and roadkill rate increased since 2009 (Concessionária Rodovia do Sol 2016; Table 1). Oliveira et al. (2015) also reported a specimen found dead after being hit by a vehicle on highway ES-060, in February 2012, near PEPCV, but this animal was included in previous counts (Table 1). Roadkilled Thin-spined Porcupines in the CCJSB were only found from October to February (Table 1), and were in 13 locations in a 50 km length of road, with a distance between roadkills of 1 to 13 km. Six roadkills (~40%) occurred along the section of highway that was two-lane and separated by the concrete barrier. We found two roadkilled porcupines while sampling along the PEPAZ region: January 2015 (20°23’27” S and 41°00’36” W) and December 2015 (20°22’47” S e 41°02’19” W, Table 1). The dead animals were 3 km apart and where both sides of the road were forested. One porcupine roadkill was reported for BLS (Klippel et al. 2015), and the animal was found in October 2013 (Table 1).

The pattern of roadkills in these three regions suggest that *C. subspinosus* is more susceptible to collisions with vehicles from October to February. As such, this risk is likely to be associated with the breeding period, when animals travel in search of mates, including between forest fragments that require crossing roads. Records of infant Thin-spined Porcupines were reported from December to February in southern Bahia (Giné 2009) and in March at the PEPCV (Oliveira et al. 2012). If *C. subspinosus* is more vulnerable to roadkills in the reproductive period, mortality during breeding is likely to cause even greater population decline (loss of individuals and reduction in fecundity). If so, specific seasonal actions to protect the species during breeding when on-ground travel is most likely causing exposure to auto traffic are absolutely necessary.

The data from the CCJSB also suggest that concrete barriers that divide lanes on highways can increase the likelihood of roadkill for Thin-spined Porcupines, and so this possibility (and alternatives to reduce roadkill) should be examined in future studies. Concrete barriers impede wildlife movement, reduce landscape permeability and can trap or confuse animals as they cross highways, thereby increasing the time they spend in the roadway and the risk of roadkill (Clevenger & Kociolek 2013). We also find that roadkilled porcupines are dispersed over the landscape and not concentrated in any one location. Therefore, the elements of the landscape that favor this species attempt of highway crossing should be evaluated. Also, with the uncertainty of the exact number of animals that were hit by cars but not immediately killed and which wandered off to die in the forest (and therefore not reported), the

Table 1. Thin-spined Porcupine (*Chaetomys subspinosus*) roadkills reported in the state of Espírito Santo, Brazil. The road and the protected area or region it is associated with or near, the number of samples (NS), date and source are included.

Region	Road	Region/protected area[1]	NS	Month/Year	Source
Southern coast region	ES-060	CCJSB	1	10/2002	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - APA Setiba	1	02/2004	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - PNMJ	1	02/2009	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB	1	11/2009	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB	1	12/2009	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB	1	02/2010	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - PNMJ	1	12/2011	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - PEPCV	1	02/2012	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - PEPCV	1[2]	02/2012[3]	Oliveira et al. (2015)
Southern coast region	ES-060	CCJSB	1	12/2012	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - APA Setiba	1	01/2013	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB - PEPCV	1	12/2013	Concessionária Rodovia do Sol (2016)
Southern coast region	ES-060	CCJSB – APA Setiba	2	12/2013	Concessionária Rodovia do Sol (2016)
Northern region	BR-101	BLS	1	10/2013[3]	Klippel et al. (2015)
Southern coast region	ES-060	CCJSB - PEPCV	1	02/2014	Concessionária Rodovia do Sol (2016)
South-central mountain region	BR-262	PEPAZ	1	01/2015	This study
Southern coast region	ES-060	CCJSB	1	02/2015	Concessionária Rodovia do Sol (2016)
South-central mountain region	BR-262	PEPAZ	1	12/2015	This study

[1] Region/protected area: CCJSB = Coastal Corridor Jucu-Setiba-Benevente; PNMJ = Jacarenema Municipal Natural Park; APA Setiba = Setiba Environmental Protection Area; PEPCV = Paulo César Vinha State Park; PEPAZ = Pedra Azul State Park; BLS = Linhares-Sooretama Block. [2] This record corresponds to the specimen cited in the above-mentioned reference. [3] The month of this roadkill record was informed personally by the authors of the paper cited.

solutions to avoid roadkill should be considered in any region where roads pass through natural areas.

The Thin-spined Porcupine may have been continuously distributed throughout its original range, but Atlantic Forest has since been several subdivided by anthropic forest fragmentation, thereby causing genetic divergence between remaining isolated populations, and drastic reduction in the gene pool of the species (Oliveira et al. 2011). Thus, additional mortality associated with highways will cause further reduction of the remaining populations and reduce gene flow (and genetic variability) both on a local scale and throughout its current, fragmented, distribution. We highlight that the mortality of specimens may have more severe effects on genetic diversity (due to depletion) than does the barrier effect, and migration is usually insufficient to recoup the genetic variation lost as a result of road mortality (Jackson & Fahrig 2011). Additional negative impacts of roads is a consequence of additional forest lost and fragmentation usually accompanying roads due to human occupation, along with the introduction of invasive species and the increase in hunting pressure (Trombulak & Frissell 2000), all of which can affect porcupines (Faria et al. 2011, Oliveira et al. 2012).

The remaining area occupied by *C. subspinosus* is extremely fragmented, and only 17% of its current distribution includes intact forest and restinga vegetation (Catzeflis et al. 2017). In Espírito Santo, the porcupine has been found in only seven protected areas, including the PEPCV and BLS (Faria et al. 2011). Roadkill records in PEPAZ region are the first confirmed evidence of Thin-spined Porcupines in this protected area. Today, in addition to the presence of the species in a small number of areas, all protected areas with records of *C. subspinosus* are associated with or near roads.

Because *C. subspinosus* is almost exclusively arboreal, moving on the ground only when no options of arboreal travel are available, we might have expected a greater rate of roadkill everywhere. The few records, or absence of records in many regions is likely to be due to the lack of studies or the nature of sporadic examinations of roadkills, plus the unknown number of animals hit by cars that were not immediately killed. Regardless, the remaining populations are likely to be small (due to low abundance and habitat fragmentation, Faria et al. 2011) which further reduces the rate of roadkills when animals risk crossing roads. Furthermore, the low roadkill rate in some regions may also be due to the existence of a barrier effect caused by roads on *C. subspinosus* (less evident in the reproductive period, as suggested here). This emphasizes the isolation of remaining populations, highlighting that some regions are composed by small habitat fragments.

In addition to *C. subspinosus*, another seven arboreal mammal species were also recorded killed on the roads within the studied regions (Table 2). Arboreal animals in the genera *Callithrix* and *Coendou* were the most common roadkills. For *Callithrix*, this is likely to be due to their living in social groups (often up to 7 individuals) which habitually cross open ground (Eisenberg & Redford 1999). *Coendou* porcupines are similar in locomotion and biology to the Thin-spined Porcupine (see Introduction for details), which may explain why they are commonly found dead on roads. Additionally, they are more abundant and often common in some places (Roach & Naylor 2016a, 2016b), with ground feeding behavior for which they descend to the ground more often (Abreu et al. 2016). Among the roadkilled arboreal species, *Callithrix flaviceps* is also threatened with extinction (Endangered; Rylands et al. 2008, Brasil 2014). These data indicate that other arboreal species also can be threatened by being hit by vehicles on highways, and the impact of roads on these groups also may be underestimated, as we propose for *C. subspinosus*. We highlight that while many roadkills are likely to be associated with population density, roadkills are not good estimates of population size and should be accompanied by other measures of abundance to more accurately access risk. Additionally, the greatest number of records found in the CCJSB region should be due to the fact that this region was sampled more often, illustrating the importance of daily sampling to better estimate the impact of roadkill on wildlife.

We find that the Thin-spined Porcupine is likely to be threatened by the possibility of roadkill in different regions in the state of Espírito Santo, which is a widespread problem, not unique to a single location or population. We recommend that roadkill should be treated as an important threat to the species in this state and elsewhere. Mortality due to roadkill is probably relevant also for populations in the states of Bahia and Sergipe, and it should be evaluated locally. Future studies

Table 2. Other arboreal mammals recorded as roadkills in the state of Espírito Santo, Brazil, in addition to the Thin-spined Porcupine (*Chaetomys subspinosus*). See Material and Methods.

Species	Common name	CCJSB[1]	PEPAZ[2]	BLS[3]
Order Pilosa				
Bradypus variegatus Schinz, 1825	Brown-throated Sloth	0	0	1
Order Primates				
Alouatta guariba clamitans Cabrera, 1940 (Gregorin 2006, Rylands & Brandon-Jones 1998)	Brown Howler Monkey	1	2	0
Callithrix flaviceps (Thomas, 1903)	Buffy-headed Marmoset	0	1	0
Callithrix geoffroyi (Humboldt, 1812)	Geoffroy's Tufted-ear Marmoset	192	1	3
Sapajus nigritus (Goldfuss, 1809)	Black-horned Tufted Capuchin	2	0	0
Order Rodentia				
Chaetomys subespinosus (Olfers, 1818)	Thin-spined Porcupine	15	2	1
Coendou spinosus (F. Cuvier, 1823)	Paraguayan Hairy Dwarf Porcupine	0	22	0
Coendou insidiosus (Lichtenstein, 1818)	Bahian Hairy Dwarf Porcupine	124	0	0
Total		334	28	5

Source of records: [1] Concessionária Rodovia do Sol (2016); [2] This study; [3] Klippel et al. (2015).

should examine genetic isolation of populations on both sides of the roads to test for a barrier effect on *C. subspinosus*, as well as that of the effect of road mortality on the structure of the remaining populations. Additionally we recommend study of the biological (including behavior) and environmental factors that determine the vulnerability of *C. subspinosus* to roadkill, and the installation of road-crossing structures for wildlife, such as arboreal overpasses (such as design options proposed by Teixeira et al. 2013 and Ministry of Agriculture, Food and the Environment 2016). Arboreal overpasses should be made available immediately and especially on roads near or through protected areas where the Thin-spined Porcupine is found. Subsequent monitoring of overpasses should be carried out to confirm their use by the target species (Gregory et al. 2014). We emphasize that these arboreal overpasses are likely also to be used by other species and thereby will contribute to conservation of the arboreal community as a whole. Even if used by few individuals, those animals that use them will then contribute to the demographic and genetic connectivity of populations (Soanes et al. 2015, Soanes et al. 2018), reducing the loss of individuals by roadkill and contributing to the conservation of *C. subspinosus* (and other arboreal species) in different regions of Espírito Santo and elsewhere.

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Author Contributions

Ana Carolina Srbek-Araujo: Concept and design of the study, data collection, data analysis and interpretation, manuscript preparation. Aline de Castro Alvarenga: Data collection, manuscript preparation. Ariane Teixeira Bertoldi: 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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Native species exploited by marine aquarium trade in Brazil

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Abstract: Brazil has an important role in marine ornamental trade, exploiting native species for both international and domestic market. A few works have previously assessed wild species exploited by the Brazilian marine aquarium industry and most of them focused solely on fish. Hence, the present paper intends to address an information gap regarding the species currently traded in the country, as well as concerning their conservation statuses. Thus, different sources of information were investigated and each species was categorized in accordance with existing lists of threatened species. A wide variety of native species was identified in Brazilian marine aquarium trade, including not only fish but also invertebrates, seaweeds and macrophytes. Some of these species were legally protected, but are still commerced anyway. Such illegal exploitation of native species causes increasing concerns about the sustainability of the activity. Therefore, in order to reduce environmental impacts caused by marine ornamental trade, Brazilian authorities should encourage the implementation of eco-fees, the purchase of eco-labeled aquarium products, the development of sustainable ornamental aquaculture and ecosystem-based management initiatives.
Keywords: *Marine aquarium fish, marine invertebrates, seaweeds, marine macrophytes, illegal trade, threatened species.*

Espécies nativas explotadas pela aquariofilia marinha no Brasil

Resumo: O Brasil possui um papel importante no comércio de ornamentais marinhos, utilizando espécies tanto para exportação como para o mercado interno. Poucos trabalhos anteriores descreveram as espécies nativas utilizadas pela indústria brasileira de aquarismo marinho, e a maioria deles era focada exclusivamente no uso de peixes. Assim, o presente trabalho almeja preencher a falta de informação em relação às espécies atualmente exploradas no país, bem como relativas às suas categorias de conservação. Dessa forma, diferentes fontes de informação foram investigadas e cada espécie foi categorizada de acordo com as listas de espécies ameaçadas existentes. Uma grande variedade de espécies foi identificada no comércio do aquarismo marinho brasileiro, o que inclui não somente peixes, mas também invertebrados, macroalgas e macrófitas. Algumas dessas espécies não poderiam ser exploradas, mas mesmo assim seguem sendo comercializadas. Essa utilização ilegal de espécies nativas provoca preocupações frequentes acerca da sustentabilidade dessa atividade. Desse modo, para reduzir os impactos ambientais causados pelo aquarismo marinho, as autoridades brasileiras deveriam incentivar a implementação de taxas-ecológicas, a aquisição de produtos de aquário com selos ecológicos, o desenvolvimento sustentável da aquacultura ornamental e iniciativas de manejo baseadas no ecossistema.
Palavras-chave: *Peixes de aquário marinho, invertebrados marinhos, macroalgas marinhas, macrófitas marinhas, comércio ilegal, espécies ameaçadas.*

Introduction

Marine ornamental trade is a global multi-million dollar industry (~ US$200-300 million annually), involving the collection of more than 50 million coral reef animals (e.g. fish, corals and a wide variety of invertebrate species) to supply aquaria kept by 2 million hobbyists worldwide (Wabnitz et al. 2003, Rhyne et al. 2012a). It is estimated that the activity targets over 1,800 reef fish species from 125 families, over 150 species of stony corals and hundreds of species of non-coral invertebrates (Rhyne et al. 2012b, Rhyne et al. 2014, Leal et al. 2015).

Since both fish and invertebrates began to be exploited together in the mid 1980s, consumers gradually shifted their preference from fish-only tanks to miniature reef ecosystems (Bruckner 2005, Rhyne et al. 2009, Rhyne et al. 2012a, Murray & Watson 2014) and collectors for the aquarium trade started to act as a peculiar and unprecedented type of generalist predators, targeting both abundant and rare species, including those with critical ecological roles on the reefs (Rhyne et al. 2012b). Unlike freshwater ornamental commerce, where about 90% of fish species are produced in captivity, the great majority of marine tank species is wild-caught and, thus, elicited controversies regarding the sustainability of the activity (Wabnitz et al. 2003, Olivotto et al. 2011, Rhyne et al. 2014), as over-harvesting is among the most serious causes of coral reef degradation worldwide (Bellwood et al. 2004, Rhyne et al. 2014).

Brazil supplies significant quantities of the global marine ornamental market (Wood 2001, Bruckner 2005, Rhyne et al. 2012b) and, as in the other exporting countries, most of the exported organisms are wild-harvested, which also raised concerns about the development of this activity (Gasparini et al. 2005, Nottingham et al. 2005a).

Despite the importance of a wide variety of native organisms for both international and domestic aquarium trade, the great majority of studies available in Brazil focused on the exploitation of marine fish only (Nottingham et al. 2000, Monteiro-Neto et al. 2003, Nottingham et al. 2005a, Nottingham et al. 2005b, Ibama 2008a, Sampaio & Nottingham 2008, Sampaio & Ostrensky, 2013), and very few included the ornamental use of other marine organisms (Gasparini et al. 2005, Ibama 2008b). Thus, the goal of the present study was to list the Brazilian native species used in marine aquarium trade, providing information about their usage and conservation statuses.

Material and Methods

First, three different lists of species were compiled: (1) fish, (2) invertebrates and (3) seaweeds and aquatic macrophytes.

These inventories were based on the following sources of information: (1) scientific literature, (2) governmental lists, (3) demands of exportation sent to the Brazilian Institute of the Environment and Renewable Natural Resources (Ibama, Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis), (4) author's personal observation, (5) visits to online marine aquarium discussion forums (e.g. http://www.ipaq.org.br, http://www.reefcorner.org, http://www.reefforum.net and http://www.reefdeep.org/), (6) Brazilian pet shops websites, and (7) auction websites (http://www.mercadolivre.com.br, http://www.olx.com.br and http://www.bomnegocio.com). Searches on the literature and forums were not structured with specific keywords in order to keep it flexible enough to maximize the detection of relevant information. For instance, for pet-shops websites were used the combinations: "lojas de aquário marinho Brasil", "aquario marinho loja", "peixes ornamentais marinhos Brasil", "venda de peixes ornamentais marinhos" and "lojas de peixes marinhos Brasil".

The natural distribution for each species in Brazil was obtained using information from the following databases: http://www.fishbase.org, http://www.iucnredlist.org, http://www.marinespecies.org, as well as specific literature cited in the results section. Official data from Brazilian authorities (IN IBAMA 202/2008 and decree MMA 445/2014) and demands from export companies was also analyzed. Additionally, personal observations while visiting aquarium shops in Fortaleza (Ceará state - CE), supervision of ornamental fish unloading in Fortim (CE), visits to ornamental organisms exporting companies in Fortaleza and an aquaculture farm in Aquiraz (CE) were also used to complement the species lists.

Only species with explicit usage in aquaria were included in the inventories. Therefore, organisms exploited exclusively as handcrafts, souvenirs, curio, or for either medical or magic-religious purposes were not analyzed. The exploitation of species was analyzed concerning specific norms and the threatening statuses of each species were determined based on the Brazilian lists of threatened species and the International Union for Conservation of Nature – IUCN red list of threatened species.

Results

Exploitation allowance for all species inventoried was analyzed regarding the norms that regulate their usage in Brazil: IN Ibama 202/08 for marine fish and IN MMA 89/06 for seaweeds. As there is a paucity of specific norms for the exploitation of marine invertebrates and marine macrophytes, the only applicable rule is federal law 9,605/98.

Concerning the species' conservation statuses, fish and invertebrate were evaluated according to their classification in the Brazilian list of threatened fish and aquatic invertebrate species (decree MMA 445/14) and the IUCN red list (version 2016-3), both using the same threatening categories: (NE) Not Evaluated, (DD) Data Deficient, (LC) Least Concern, (NT) Near Threatened, (VU) Vulnerable, (EN) Endangered, (CR) Critically Endangered, (EW) Extinct in the Wild or (EX) Extinct. For the analysis of the conservation statuses of seaweeds and aquatic macrophytes, it was used the Brazilian list of threatened flora species (decree MMA 443/14) and again the IUCN red list criteria (version 2016-3).

More than 200 bone and cartilaginous fish species were identified based on 24 different sources of information (Table 1). From this total, only 136 species can be legally exploited according to IN Ibama 202/08. However, according to decree MMA 445/14, some species whose collection is not allowed by IN Ibama 202/08 may be exploited by means of specific authorizations (species categorized as VU) and others can be harvested for scientific research or conservation purposes only (species classified as EN or CR). In addition, Table 1 reports the occurrence of five fish species endemic to Brazilian oceanic islands, two new species from different genera and four updated scientific names for species reported under other synonyms in previous works.

Invertebrates were classified into seven groups: mollusks, cnidarians, crustaceans, echinoderms, polychaetes, ascidians and sponges. The first four were the most representative regarding the number of species, and some of them are included in both the Brazilian list of threatened fish and aquatic invertebrate species and the IUCN red list (Table 2).

Table 1. Marine fish species traded in Brazil for aquarium purposes and their conservation status

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Osteichthyes				
Abudefduf saxatilis[1;4;9;11;18;19;22;21;23; 24]	Allowed	NE	LC	-
Acanthostracion polygonius[4;9;11;18;19]	Allowed	NE	LC	-
Acanthostracion quadricornis[1;4;7;9; 11;18;19;24]	Allowed	NE	LC	-
Acanthurus bahianus[1;2;6;9;11;18;19;22; 23;24]	Allowed	NE	LC	-
Acanthurus chirurgus[1;2;7;9;11;18;19;22;24]	Allowed	NE	LC	-
Acanthurus coeruleus[1;2;4;6;9;11;18;19; 22;23;24]	Allowed	NE	LC	-
Achirus lineatus[11;18;19]	Allowed	NE	LC	-
Alphestes afer[9;11;18;19]	Allowed	DD	LC	-
Aluterus schoepfii[11;18;19]	Allowed	NE	LC	-
Aluterus scriptus[1;9;11;18;19]	Allowed	NE	LC	-
Amblycirrhitus pinos[1;4;9;11;18;19;22; 23;24]	Allowed	DD	LC	-
Anisotremus moricandi[4;9;21]	Prohibited	NE	LC	-
Anisotremus surinamensis[9;11;18;19]	Allowed	DD	NE	-
Anisotremus virginicus[1;4;6;8;9;11;18;19; 22;23]	Allowed	NE	LC	-
Antennarius multiocellatus[1;6;18;21]	Prohibited	DD	LC	-
Antennarius striatus[4;11;18;19;21]	Allowed	DD	LC	-
Apogon americanus[11;18;19;21;22;24]	Allowed	NE	NE	-
Apogon maculatus[1]	Prohibited	NE	LC	-
Apogon planifrons[4;11;21]	Prohibited	NE	LC	-
Apogon pseudomaculatus[1;11;18;19;21]	Allowed	NE	LC	-
Archosargus rhomboidalis[11;18;19]	Allowed	NE	LC	-
Astrapogon puncticulatus[18]	Prohibited	NE	LC	-
Aulostomus strigosus[9;11;18;19]	Allowed	NE	LC	-
Balistes vetula[1;2;4;6;9;18;21;23;24]	Prohibited	NT	NT	-
Bathygobius soporator[1;11;18;19]	Allowed	NE	LC	-
Batrachoides surinamensis[11;18;19;23]	Allowed	NE	LC	-
Bodianus insularis[12;18;20]	Prohibited	NE	LC	EI
Bodianus pulchellus[1;6;9;11;18;19;21;22; 23;24]	Allowed	NE	LC	-
Bodianus rufus[1;2;4;6;9;11;18;19;21;22;23;24]	Allowed	NE	LC	-
Bothus lunatus[4;9;11;18;19]	Allowed	NE	LC	-
Bothus ocellatus[7;9;11;18;19]	Allowed	NE	LC	-
Calamus spp.[9]	-	-	-	-
Calamus pennatula[11;18;19]	Allowed	NE	LC	-
Cantherhines macrocerus[1;4;11;18;19; 21;23; 24]	Allowed	NE	LC	-
Cantherhines pullus[1;4;9;11;18;19;21]	Allowed	NE	LC	-
Canthigaster figueiredoi[1;4;9;11;18;19;21; 24]	Allowed	NE	LC	-
Carangoides crysos[9]	Prohibited	NE	LC	-
Caranx latus[9]	Prohibited	NE	LC	-
Caranx lugubris[9]	Prohibited	NE	LC	-
Centropyge aurantonotus[1;2;4;6;7;9;11; 18;19;21;22;23;24]	Allowed	DD	LC	-
Cephalopholis fulva[1;9;18;19]	Prohibited	NE	LC	-
Chaetodipterus faber[1;6;11;18;19;23]	Allowed	NE	LC	-
Chaetodon ocellatus[1;2;4;6;7;11;18;19;21; 22;23;24]	Allowed	DD	LC	-

Continued Table 1.

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Chaetodon sedentarius[1;2;4;6;9;11;18;19; 21;22]	Allowed	NE	LC	-
Chaetodon striatus[1;2;4;6;7;9;11;18;19;21; 22;23;24]	Allowed	NE	LC	-
Chilomycterus antennatus[11;18;19;23]	Allowed	NE	LC	-
Chilomycterus antillarum[1;11;18;19]	Allowed	NE	LC	-
Chilomycterus schoepfii[6]	Prohibited	NE	LC	-
Choranthias salmopunctatus[13;18;20]	Prohibited	VU	LC	EI, DN*
Chromis flavicauda[1;4;9;21]	Prohibited	NE	DD	-
Chromis jubauna[4;9;21]	Prohibited	NE	NE	-
Chromis multilineata[1;4;9;11;18;19;21]	Allowed	NE	LC	-
Clepticus brasiliensis[9;11;18;19]	Allowed	NE	LC	-
Conodon nobilis[11;18;19]	Allowed	NE	LC	-
Coryphopterus spp.[9]	-	-	-	-
Coryphopterus glaucofraenum[11;18; 19;23]	Allowed	NE	LC	-
Cosmocampus albirostris[10;11;18;19;23]	Allowed	NE	LC	-
Cryptotomus roseus[9]	Prohibited	NE	LC	-
Cychlichthys spinosus[4;11;18;19;21]	Allowed	NE	NE	-
Dactylopterus volitans[1;2;6;9;11;18;19]	Allowed	NE	LC	-
Dermatolepis inermis[9]	Prohibited	NE	NT	-
Diodon holacanthus[4;11;18;19]	Allowed	NE	LC	-
Diodon hystrix[1;6;9;11;18;19;24]	Allowed	NE	LC	-
Diplectrum formosum[11;18;19]	Allowed	NE	LC	-
Diplectrum radiale[9;11;18;19]	Allowed	NE	LC	-
Diplodus argenteus[9]	Prohibited	NE	LC	-
Doratonotus megalepis[9;11;18;19;23]	Allowed	NE	LC	-
Dules auriga[11;18;19]	Allowed	NE	NE	-
Echeneis naucrates[1;11;18;19;23]	Allowed	NE	LC	-
Elacatinus figaro[2;4;7;8;9;14;18;20;21;22;23]	Prohibited	VU	NE	-
Emblemariopsis signifer[9]	Prohibited	NE	LC	-
Epinephelus adscensionis[18]	Prohibited	DD	LC	-
Epinephelus itajara[4;20;21]	Prohibited	CR	CR	-
Epinephelus marginatus[9]	Prohibited	VU	EN	-
Epinephelus morio[9]	Prohibited	VU	NT	-
Equetus lanceolatus[1;4;21]	Prohibited	NE	LC	-
Fistularia tabacaria[9;11;18;19]	Allowed	NE	LC	-
Gnatholepis thompsoni[9]	Prohibited	NE	LC	-
Gobiesox strumosus[11;18;19]	Allowed	NE	LC	-
Gramma brasiliensis[2;3;4;7;8;9;15;18;20; 21;23]	Prohibited	NT	NE	-
Gymnachirus nudus[11;18;19]	Allowed	NE	LC	-
Gymnothorax funebris[1;6;11;18;19;22]	Allowed	DD	LC	-
Gymnothorax miliaris[1;4;8;11;18;19;22;24]	Allowed	NE	LC	-
Gymnothorax moringa[9;11;18;19;24]	Allowed	DD	LC	-
Gymnothorax ocellatus[11;18;19]	Allowed	DD	LC	-
Gymnothorax vicinus[1;9;11;18;19]	Allowed	DD	LC	-
Haemulon aurolineatum[9]	Prohibited	NE	LC	-

Continued Table 1.

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Haemulon plumieri[1;9;22]	Prohibited	DD	NE	-
Haemulon steindachneri[9;11;18;19]	Allowed	NE	LC	-
Halichoeres bivittatus[1;4;11;18;19;21]	Allowed	NE	LC	-
Halichoeres brasiliensis[1;4;6;9;11;18;19; 21;23]	Allowed	NE	DD	-
Halichoeres dimidiatus[1;2;4;6;8;9;11;18; 19;21;22;23;24]	Allowed	NE	LC	DN**
Halichoeres penrosei[1;4;6;9;11;18;19;21]	Allowed	NE	LC	DN***
Halichoeres poeyi[1;4;9;11;18;19;21]	Allowed	NE	LC	-
Heteropriacanthus cruentatus[11;18;19]	Allowed	NE	LC	-
Hippocampus erectus[1;2;4;6;7;10;11;18;19; 21]	Allowed	VU	VU	-
Hippocampus reidi[2;4;5;7;10;11;18;19; 20;21;22]	Allowed	VU	DD	-
Holacanthus ciliaris[1;2;4;6;7;8;9;11;18;19; 21;22;23;24]	Allowed	DD	LC	-
Holacanthus tricolor[2;4;6;7;8;9;11;18;19;21; 22;23;24]	Allowed	DD	LC	-
Holocentrus adscensionis[7;9;11;18;19]	Allowed	NE	LC	-
Hypleurochilus fissicornis[9]	Prohibited	NE	LC	-
Kyphosus spp.[9]	-	-	-	-
Kyphosus incisor[11;18;19]	Allowed	NE	NE	-
Kyphosus sectatrix[11;18;19]	Allowed	NE	LC	-
Labrisomus cricota[4;21]	Prohibited	NE	LC	-
Labrisomus kalisherae[9]	Prohibited	NE	NE	-
Labrisomus nuchipinnis[9;11;18;19;21]	Allowed	NE	LC	-
Lactophrys spp.[1]	-	-	-	-
Lactophrys polygonia[6]	Prohibited	NE	NE	-
Lactophrys trigonus[11;18;19]	Allowed	NE	LC	-
Lagocephalus laevigatus[7;11;18;19]	Allowed	NE	LC	-
Liopropoma carmabi[4;21;23]	Prohibited	NE	LC	-
Lutjanus analis[1]	Prohibited	NT	VU	-
Lutjanus jocu[9]	Prohibited	NT	NE	-
Lutjanus synagris[9]	Prohibited	NT	NE	-
Malacanthus plumieri[1]	Prohibited	NE	LC	-
Malacoctenus sp. *n.*[9;18;21;22;23;24]	Prohibited	-	-	NS
Malacoctenus delalandei[9]	Prohibited	NE	LC	-
Melichthys niger[11;18]	Allowed	NE	LC	-
Menticirrhus americanus[11;18]	Allowed	DD	LC	-
Micrognathus sp.[10]	Prohibited	-	-	-
Microphis lineatus[4;21]	Prohibited	NE	NE	DN****
Micropogonias furnieri[19]	Prohibited	NE	LC	-
Microspathodon chrysurus[1;2;3;9;18;21]	Prohibited	VU	LC	-
Mugil curema[9]	Prohibited	DD	LC	-
Mulloidichthys martinicus[9;11;18;19]	Allowed	NE	LC	-
Mullus argentinae[11;18;19]	Allowed	NE	NE	-
Muraena pavonina[11;18;19]	Allowed	NE	LC	-
Mycteroperca bonaci[9]	Prohibited	VU	NT	-
Mycteroperca interstitialis[9]	Prohibited	VU	VU	-
Myrichthys breviceps[4;11;18;19]	Allowed	NE	LC	-

Continued Table 1.

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Myrichthys ocellatus[1;9;11;18;19;22]	Allowed	NE	LC	-
Myripristis jacobus[1;4;9;11;18;19]	Allowed	NE	LC	-
Ocyurus chrysurus[6;9]	Prohibited	NT	NE	-
Odontoscion dentex[9;11;18;19]	Allowed	NE	LC	-
Ogcocephalus spp.[1;6]	-	-	-	-
Ogcocephalus nasutus[1]	Prohibited	NE	LC	-
Ogcocephalus notatus[19]	Prohibited	NE	LC	-
Ogcocephalus vespertilio[1;4;9;11;18;19]	Allowed	NE	NE	-
Oligoplites saliens[11;18;19]	Allowed	NE	LC	-
Ophioblennius trinitatis[4;11;18;19;21;23; 24]	Allowed	NE	LC	-
Opistognathus sp. *n.*[1;4;18;21;23]	Prohibited	-	-	NS
Opistognathus lonchurus[4;21]	Prohibited	NE	LC	-
Orthopristis ruber[9;11;18;19]	Allowed	NE	LC	-
Parablennius marmoreus[4;9;11;18;19;21]	Allowed	NE	LC	-
Parablennius pilicornius[11;18;19]	Allowed	NE	LC	-
Paraclinus rubicundus[11;18;19]	Allowed	NE	LC	-
Paralonchurus brasiliensis[11;18]	Allowed	NE	LC	-
Paranthias furcifer[9;11;18;19]	Allowed	NE	LC	-
Pareques acuminatus[1;3;4;6;9;11; 18;19;22;23;24]	Allowed	DD	LC	-
Pempheris schomburgkii[11;18;19]	Allowed	NE	LC	-
Phaeoptyx pigmentaria[4;11;18;19;21]	Allowed	NE	LC	-
Plectrypops retrospinis[11;18;19]	Allowed	NE	LC	-
Pomacanthus arcuatus[1;2;4;6;7;9;11;18;19;21;22;23]	Allowed	DD	LC	-
Pomacanthus paru[1;2;4;6;7;9;11;18;19;21; 22;23;24]	Allowed	DD	LC	-
Pomadasys corvinaeformis[11;18;19]	Allowed	NE	NE	-
Porichthys porosissimus[11;18;19]	Allowed	NE	NE	-
Priacanthus arenatus[9]	Prohibited	NE	LC	-
Prionotus nudigula[11;18;19]	Allowed	NE	NE	-
Prionotus punctatus[1;19]	Prohibited	NE	LC	-
Prognathodes brasiliensis[4;9;11;21]	Prohibited	NE	LC	-
Prognathodes guyanensis[4;11;21]	Prohibited	NE	LC	-
Prognathodes obliquus[4;11;16;18; 20;21]	Prohibited	VU	DD	EI
Pseudocaranxs dentex[9]	Prohibited	NE	LC	-
Pseudupeneus maculatus[1;9]	Prohibited	NE	LC	-
Ptereleotris randalli[11;21]	Prohibited	NE	LC	-
Rypticus bitrispinus[9;11;18;19]	Allowed	NE	LC	-
Rypticus saponaceus[9;11;18;19]	Allowed	NE	LC	-
Scartella cristata[4;8;11;18;21;22;23;24]	Allowed	NE	LC	-
Scarus spp.[1]	-	-	-	-
Scarus trispinosus[9;20]	Prohibited	EN	EN	-
Scarus zelindae[4;9;11;18;19;21]	Allowed	VU	DD	-
Scorpaena brasiliensis[1;9;11;18;19]	Allowed	NE	LC	-
Scorpaena isthmensis[11;18;19]	Allowed	NE	LC	-
Scorpaena plumieri[1;9;11;18;19]	Allowed	NE	LC	-

Continued Table 1.

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Selar crumenophthalmus[9]	Prohibited	NE	LC	-
Selene vomer[1;2;11;18;19;22]	Allowed	NE	LC	-
Seriola spp.[9]	Prohibited	-	-	-
Serranus baldwini[1;9;11;18;19;23]	Allowed	NE	LC	-
Serranus flaviventris[1;9;11;18;19]	Allowed	NE	LC	-
Serranus phoebe[9;11;18;19]	Allowed	NE	LC	-
Sparisoma spp.[7;9]	-	-	-	-
Sparisoma amplum[9;11;18;19]	Allowed	NT	LC	-
Sparisoma axillare[9;11;18;19]	Allowed	VU	DD	-
Sparisoma frondosum[9;11;18;19]	Allowed	VU	DD	-
Sparisoma radians[9;11;18;19]	Allowed	NE	LC	-
Sparisoma tuiupiranga[4;9;21]	Prohibited	NE	NE	-
Sphoeroides greeleyi[9;11;18;19]	Allowed	NE	LC	-
Sphoeroides spengleri[1;9;11;18;19;24]	Allowed	NE	LC	-
Sphoeroides testudineus[11;18;19]	Allowed	DD	LC	-
Stegastes spp.[1]	-	-	-	-
Stegastes fuscus[3;4;9;11;18;19;21;23]	Allowed	NE	LC	-
Stegastes pictus[1;4;9;11;18;19;21]	Allowed	NE	NE	-
Stegastes rocasensis[20]	Prohibited	VU	NE	EI
Stegastes sanctipauli[18;20]	Prohibited	VU	LC	EI
Stegastes uenfi[11;18;19]	Allowed	NE	NE	-
Stegastes variabilis[1;4;9;11;18;19;21;23;24]	Allowed	NE	NE	-
Stephanolepis spp.[7]	-	-	-	-
Stephanolepis hispidus[1;9;11;18;19]	Allowed	NE	LC	-
Stephanolepis setifer[11;18;19]	Allowed	NE	LC	-
Stygnobrotula latebricola[4;11;18;19;21]	Allowed	NE	LC	-
Syngnathus sp.[10]	Prohibited	-	-	-
Synodus foetens[11;18;19]	Allowed	NE	LC	-
Synodus intermedius[7;9;11;18;19]	Allowed	NE	LC	-
Synodus synodus[9;11;18;19]	Allowed	NE	LC	-
Thalassoma spp.[1]	-	-	-	-
Thalassoma noronhanum[4;6;9;11;18;19; 21]	Allowed	NE	LC	-
Thalassophryne montevidensis[11;18; 19]	Allowed	NE	NE	-
Thalassophryne nattereri[11;18;19]	Allowed	NE	LC	-
Trachinocephalus myops[11;18;19]	Allowed	NE	LC	-
Upeneus parvus[11;18;19]	Allowed	NE	LC	-
Xyrichthys novacula[1;4;11;18;19;21]	Allowed	NE	LC	-
Xyrichthys splendens[1;11;18;19]	Allowed	NE	LC	-
Chondrichthyes				
Aetobatus narinari[8]	Prohibited	DD	NT	-
Dasyatis spp.[8]	Prohibited	-	-	-
Dasyatis guttata[1]	Prohibited	NE	DD	-
Dasyatis marianae[8]	Prohibited	DD	DD	-

Continued Table 1.

Species	Harvesting in accordance with IN 202/08	Brazilian list of threatened fish species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Ginglymostoma cirratum[1;4;20;21]	Prohibited	VU	DD	-
Narcine brasiliensis[1;4;21]	Prohibited	DD	DD	-
Pristis perotteti[17]	Prohibited	NE	NE	-
Rhinobatos spp.[1;4;21;23]	Prohibited	-	-	-
Rhinobatos percellens[1]	Prohibited	DD	NT	-
Rhinoptera bonasus[8]	Prohibited	DD	NT	-
Zapteryx brevirostris[4;21]	Prohibited	VU	VU	-

Sources of information: [1]Monteiro-Neto et al. (2003), [2]Araújo & Albuquerque-Filho (2005), [3]Ferreira et al. (2005), [4]Gasparini et al. (2005), [5]Rosa et al. (2005), [6]Nottingham et al. (2005b), [7]Nottingham et al. (2005a), [8]Sampaio & Rosa (2005), [9]Floeter et al. (2006), [10]Rosa et al. (2006), [11]IN Ibama 202/08, [12]Moura (2008a), [13]Moura (2008b), [14]Moura et al. (2008); [15]Moura & Sazima (2008); [16]Moura (2008c); [17]Charvet-Almeida & Faria (2008); [18]Sampaio & Nottingham (2008), [19]Ibama (2008a), [20]Mohr et al. (2009), [21]Sampaio & Ostrensky (2013), [22]Autor's personal observation, [23]Marine aquarium discussion forums, [24]Brazilian pet shop's websites/auction webpages. Threatening categories according to the Brazilian list of threatened fish and aquatic invertebrate species (decree MMA 445/14) and the IUCN red list of threatened species (version 2016-3): (NE) Not Evaluated, (DD) Data Deficient, (LC) Least Concern, (NT) Near Threatened, (VU) Vulnerable, (EN) Endangered, (CR) Critically Endangered – for DD and NT species recorded in Brazil see http://www.icmbio.gov.br >> biodiversidade >> fauna brasileira >> lista de espécies quase ameaçadas e com dados insuficientes. Observation: (EI) Endemic to Brazilian oceanic islands; (NS) New Species; (DN) Different Name used in references – originally mentioned as (*) *Anthias salmopunctatus*, (**) *Halichoeres cyanocephalus*, (***) *Halichoeres maculipinna* and (****) *Microphis eigenmanni*.

Table 2. Marine aquarium invertebrates traded in Brazil and their conservation status.

Species	Harvesting in accordance with the Brazilian Environmental Crime Law (9,605/98)	Brazilian list of threatened aquatic invertebrate species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Mollusks				
Anadara brasiliana[17]	Allowed	NE	NE	-
Aplysia dactylomella[17]	Allowed	NE	NE	-
Aplysia parvula[17]	Allowed	NE	NE	-
Astraea phoebia[17;20;21]	Allowed	NE	NE	-
Astraea tecta[17;20;21]	Allowed	NE	NE	-
Atrina seminuda[17]	Allowed	NE	NE	-
Berghia sp.[20]	Allowed	-	-	-
Bornella calcarata[18]	Allowed	NE	NE	-
Cassis tuberosa[17]	Allowed	NT	NE	-
Cerithium atratum[19;20;21]	Allowed	NE	NE	-
Charonia variegata[17]	Allowed	NE	NE	-
Chlamys ornata[18]	Allowed	NE	NE	-
Caribachlamys sentis[18]	Allowed	NE	NE	-
Conus spp.[2;18]	Allowed	-	-	-
Cyphoma gibbosum[18]	Allowed	NE	NE	-
Cyphoma macumba[18]	Allowed	NE	NE	-
Cypraea brasiliensis[17]	Allowed	NE	NE	-
Cypraea spurca[17]	Allowed	NE	NE	-
Elysia subornata[20]	Allowed	NE	NE	-
Euvola ziczac[17]	Prohibited	EN	NE	BL
Lima lima[18]	Allowed	NE	NE	-
Lima pellucida[18]	Allowed	NE	NE	-
Loligo plei[17]	Allowed	NE	NE	-

Continued Table 2.

Species	Harvesting in accordance with the Brazilian Environmental Crime Law (9,605/98)	Brazilian list of threatened aquatic invertebrate species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Lyropecten nodosus[18;19]	Allowed	NE	NE	-
Macrocypraea zebra[20]	Allowed	NE	NE	-
Micromelo undatus[18;20]	Allowed	NE	NE	-
Neritina virginea[19;20;21]	Allowed	NE	LC	-
Octopus vulgaris[2]	Allowed	NE	NE	-
Phidiana lynceus[18]	Allowed	NE	NE	-
Pinna carnea[17]	Allowed	NE	NE	-
Pleurobranchus sp.[18]	Allowed	-	-	-
Pteria colymbus[17]	Allowed	NE	NE	-
Rostanga byga[18]	Allowed	NE	NE	-
Spondylus americanus[18]	Allowed	NE	NE	-
Strombus pugilis[20]	Allowed	NE	NE	-
Stramonita brasiliensis[19]	Allowed	NE	NE	-
Tegula viridula[19;20;21]	Allowed	NE	NE	-
Trachycardium muricatum[17]	Allowed	NE	NE	-
Turbo canaliculatus[18]	Allowed	NE	NE	-
Crustaceans				
Acanthonix sp.[20;21]	Allowed	NE	NE	OM
Alphaeus sp.[20]	Allowed	NE	NE	-
Brachycarpus cf. *biunguinculatus*[2]	Allowed	NE	NE	-
Calcinus tibicen[2;17;19;20;21]	Allowed	NE	NE	-
Cinetorhynchus rigens[2;20;21]	Allowed	NE	NE	-
Clibanarius spp.[19;20;21]	Allowed	-	-	-
Dardanus venosus[2;20]	Allowed	NE	NE	-
Enoplometopus antillensis[2;18;20]	Allowed	DD	LC	-
Gnathophyllum americanum[18]	Allowed	NE	NE	-
Lepas anatifera[18]	Allowed	NE	NE	-
Lepas anserifera[18]	Allowed	NE	NE	-
Lysmata grabhami[2;17;19;20;21]	Allowed	NE	NE	-
Lysmata rathbunae[17;20]	Allowed	NE	NE	-
Lysmata wurdemanni[2;17;19;20;21]	Allowed	NE	NE	-
Mithrax spp.[20;21]	Allowed	-	-	-
Mithraculus forceps[20]	Allowed	NE	NE	-
Parribacus antarcticus[18]	Allowed	NE	LC	-
Periclimenes aff. *pedersoni*[2;20]	Allowed	NE	NE	-
Periclimenes aff. *yucatanicus*[2]	Allowed	NE	NE	-
Petrochirus diogenes[2;17]	Allowed	NE	NE	-
Phimochirus holthuisi[18]	Allowed	NE	NE	-
Platypodiella spectabilis[2;19;20;21]	Allowed	NE	NE	-
Scyllarides aequinoctialis[18]	Allowed	NE	LC	-
Stenopus hispidus[2;17;19;20;21]	Allowed	NE	NE	-

Continued Table 2.

Species	Harvesting in accordance with the Brazilian Environmental Crime Law (9,605/98)	Brazilian list of threatened aquatic invertebrate species (Decree MMA 445/14)	IUCN red list of threatened species	Observation
Stenopus scutellatus[2]	Allowed	NE	NE	-
Stenorhynchus seticornis[2;17;19;20;21]	Allowed	NE	NE	-
Thor aff. *amboinensis*[2;18]	Allowed	NE	NE	-
Cnidarians				
Actinoporus sp.[2]	Prohibited	-	-	-
Alicia mirabilis[2]	Prohibited	NE	NE	-
Bellactis ilkalysae[2]	Prohibited	NE	NE	-
Carijoa riisei[2]	Prohibited	NE	NE	-
Cerianthomorphe brasiliensis[3]	Prohibited	DD	NE	-
Cerianthus brasiliensis[4]	Prohibited	NE	NE	-
Condylactis gigantea[2;5]	Prohibited	EN	NE	-
Discosoma spp.[2]	Prohibited	-	-	-
Favia gravida[2]	Prohibited	NE	NE	-
Heterogorgia uatumani[2]	Prohibited	NE	NE	-
Lophogorgia punicea[2]	Prohibited	NE	NE	-
Lophogorgia violacea[2]	Prohibited	NE	NE	-
Madracis decactis[2]	Prohibited	NE	LC	-
Meandrina braziliensis[2]	Prohibited	DD	DD	-
Millepora alcicornis[2;6]	Prohibited	NE	LC	-
Millepora braziliensis[2]	Prohibited	DD	DD	-
Montastrea cavernosa[2]	Prohibited	NE	NE	-
Muricea flamma[2]	Prohibited	NE	NE	-
Muriceopsis sulphurea[2]	Prohibited	NE	NE	-
Mussismilia braziliensis[2]	Prohibited	VU	DD	-
Mussismilia harttii[2]	Prohibited	EN	DD	-
Mussismilia hispida[2]	Prohibited	NE	DD	-
Palythoa caribaeorum[2]	Prohibited	NE	NE	-
Phyllogorgia dilatata[2;7]	Prohibited	DD	NE	-
Plexaurella grandiflora[2]	Prohibited	NE	NE	-
Plexaurella regia[2]	Prohibited	NE	NE	-
Porites branneri[2]	Prohibited	NE	NT	-
Scolymia wellsi[2]	Prohibited	NE	DD	-
Siderastrea stellata[2]	Prohibited	NE	DD	-
Zoanthus spp.[2]	Prohibited	-	-	-
Echinoderms				
Asterina stellifera[8;16]	Prohibited	NE	NE	-
Astropecten brasiliensis[16]	Prohibited	VU	NE	-
Astropecten marginatus[16]	Prohibited	NE	NE	-
Astrophyton sp.[2]	Prohibited	-	-	-
Echinaster spp.[2]	Prohibited	-	-	-
Echinaster (Othilia) brasiliensis[2;9]	Prohibited	NE	NE	-

Continued Table 2.

Species	**Harvesting in accordance with the Brazilian Environmental Crime Law (9,605/98)**	**Brazilian list of threatened aquatic invertebrate species (Decree MMA 445/14)**	**IUCN red list of threatened species**	**Observation**
Echinaster (Othilia) echinophorus[2;10]	Prohibited	NE	NE	-
Echinaster (Othilia) guyanensis[2]	Prohibited	NE	NE	-
Echinometra lucunter[20]	Prohibited	NE	NE	-
Eucidaris tribuloides[2;11;20]	Prohibited	NE	NE	-
Linckia guildingii[2;12;19]	Prohibited	VU	NE	-
Lytechinus variegatus[20]	Prohibited	VU	NE	-
Luidia clathrata[16]	Prohibited	NE	NE	-
Luidia senegalensis[16]	Prohibited	VU	NE	-
Narcissia trigonaria[2;13]	Prohibited	NE	NE	-
Ophioderma spp.[2]	Prohibited	-	-	-
Oreaster reticulatus[14]	Prohibited	VU	NE	-
Tropiometra carinata[20]	Prohibited	NE	NE	-
Polychaetes				
Eurythoe complanata[15]	Prohibited	NE	NE	-
Spirobranchus spp.[2]	Prohibited	NE	NE	-
Ascidians				
Botrylloides nigrum[20]	Prohibited	NE	NE	IE
Polycarpa insulsa[20]	Prohibited	NE	NE	IE
Styela plicata[20]	Prohibited	NE	NE	-
Sponges				
Aplysina fulva[1]	Prohibited	NE	NE	-
Axinyssa sp.[1]	Prohibited	-	-	-
Dragmacidon reticulatum[1;22]	Prohibited	NE	NE	-
Tethya sp.[1]	Prohibited	-	-	-

Source or information: [1]Sampaio et al., (2004); [2]Gasparini et al. (2005); [3]Pires & Castro (2008a); [4]Pires & Castro (2008b); [5]Pires & Castro (2008c); [6]Pires & Castro (2008d); [7]Castro & Pires (2008); [8]Brites et al. (2008a); [9]Ventura et al. (2008a); [10]Ventura et al. (2008b); [11]Ventura et al. (2008c); [12]Brites et al. (2008b); [13]Brites et al. (2008c); [14]Brites et al. (2008d); [15]Amaral et al. (2008); [16]Amaral et al (2010); [17]Authorization of exportation issued by Ibama; [18]Authorization of exportation requested but not issued by Ibama; [19]Author's personal observation; [20]Marine aquarium discussion forums; [21]Brazilian pet shop's websites/auction webpages; [22]Hajdu et al (2011). Threatening categories according to the Brazilian list of threatened fish and aquatic invertebrate species (decree MMA 445/14) and the IUCN red list of threatened species (version 2016-3): (NE) Not Evaluated, (DD) Data Deficient, (LC) Least Concern, (NT) Near Threatened, (VU) Vulnerable, (EN) Endangered. Observations: (BL) Although law 9,605/98 allows collection of mollusks, the species cannot be harvested since it is classified as EN in the Brazilian List of threatened fish and aquatic invertebrate species, (OM) Originally Misidentified as *Xenocarcinus* sp. or *Macropodia longirostris*, (IE) Incidental Exploitation attached to "liverocks".

Even though law 9,605/98 allows exploitation of mollusks and crustaceans (because they are defined as fishing resources), it prohibits the usage of species that figure in the Brazilian List of threatened fish and aquatic invertebrate species, as well as of those other invertebrates not defined as fishing resources (cnidarians, echinoderms, polychaetes, ascidians and sponges). Hence, exploitation of the bivalve *Euvola ziczac* (Linnaeus, 1758) is forbidden because it is classified as EN, according to decree MMA 445/14.

Table 2 also presents species that were misidentified in marine aquarium discussion forums and Brazilian pet shop's websites or auction webpages, besides organisms that were incidentally exploited attached to liverocks.

It was also recorded the use of seaweeds and saltwater macrophytes in marine tanks throughout the country (Table 3) and the great majority of species is neither cited in the Brazilian list of threatened flora species (decree MMA 443/14) nor in the IUCN red list of threatened species. The only exception is *Halophila decipiens* Ostenfeld, which is categorized as Least Concern (LC) solely in the IUCN red list.

Table 3. Seaweeds and aquatic macrophytes used in marine aquarium trade in Brazil

Species	Harvesting in accordance with IN 89/06	Brazilian list of threatened flora species (Decree MMA 443/14)	IUCN red list of threatened species
Green seaweeds (Chlorophyta)			
Acetabularia calyculus[2]	Allowed	NE	NE
Bryopsis sp.[2]	Allowed	-	-
Caulerpa prolifera[2]	Allowed	NE	NE
Caulerpa racemosa[2]	Allowed	NE	NE
Caulerpa sertularioides[2]	Allowed	NE	NE
Caulerpa taxifolia[2]	Allowed	NE	NE
Chaetomorpha linum[2;4]	Allowed	NE	NE
Chaetomorpha sp.[2;3]	Allowed	-	-
Codium sp.[2]	Allowed	-	-
Halimeda sp[2]	Allowed	-	-
Udotea sp.[2]	Allowed	-	-
Red seaweeds (Rodophyta)			
Acanthophora sp.[2]	Allowed	-	-
Ceramium sp.[2]	Allowed	-	-
Chondria sp.[2]	Allowed	-	-
Gracilaria sp.[2]	Allowed	-	-
Jania sp.[2]	Allowed	-	-
Lithothamnium spp.[1]	Allowed	-	-
Brown seaweeds (Phaeophyta)			
Dictyota cervicomis[2]	Allowed	NE	NE
Lobophora sp.[2]	Allowed	-	-
Padina sp.[2]	Allowed	-	-
Macrophytes (Sea grasses)			
Halophila decipiens[2]	Not applicable	NE	LC
Halodule sp.[2]	Not applicable	-	-

Source of information: [1]Ibama (2008a); [2]Marine aquarium discussion forums; [3]Author's personal observation; [4]Brazilian pet shop's websites/auction webpages. Threatening categories according to the Brazilian list of threatened flora species (decree MMA 443/15) and the IUCN red list of threatened species (version 2016-3): (NE) Not Evaluated, (LC) Least Concern.

Discussion

Web surveys have been successfully used to investigate aquarium trade worldwide (Kay & Hoyle 2001; Walters et al. 2006; Keller & Lodge 2007) and specifically in Brazil, this tool has been used to access the commerce of freshwater species (Magalhães & Jacobi 2010; Magalhães et al 2017).

Many native species traded by the marine aquarium industry in Brazil figure in Brazilian lists of threatened species and, currently, it is much easier to compare these species with those categorized in the IUCN red list. Preceding Brazilian lists of threatened species (IN MMA 05/04 and IN MMA 52/05) had their own categories and classification criteria, but most recent Brazilian lists (decree MMA 443/14 and decree MMA 445/14) followed the IUCN red list patterns, which allow more reliable comparisons and avoid mismatches already detected - agreements regarding categories increase credibility of red lists, while desagreemends can either do the opposite or demonstrate that in particular cases a species may locally present a distinctive threatening degree compared to the general reality along its whole distribution (Bender et al. 2012). Bony fishes represent the great majority of the exploited species. Despite only 136 species can be legally commercialized according to IN Ibama 202/08, about 70 others are illegally traded in Brazil. This situation not only demonstrates a lack of more effective control and inspection by the Brazilian authorities (e.g. Ibama) but also indicates that many dealers and tank owners (i.e., aquarium hobbyists) simply either ignore or unknow the norms that regulate the exploitation of marine aquarium organisms. Such illegal trade is specially worrying because some organisms are included in the

Brazilian list of threatened species under really threatening conservation statuses (e.g. EN or CR categories) or maybe worse, whose statuses are simply unknown (e.g. NE or DD categories).

Among many fish species, the barber goby *Elacatinus figaro* Sazima, Moura & Rosa, 1997 *(*VU, in accordance with decree MMA 445/14) and the Brazilian basslet *Gramma brasiliensis* Sazima, Gasparini & Moura, 1998 (whose harvesting was prohibited until December 2014, for being categorized as threatened with extinction by an older norm - IN MMA 05/04) were frequently cited by different sources of information investigated, indicating that, despite the prohibition of exploitation imposed by IN Ibama 202/08, both species are commonly found in ornamental trade.

This statement is corroborated through the seizure by Ibama of 18 *E. figaro* specimens, that were being illegally traded by means of the Brazilian postal service in 2010 (Gurjão et al. 2017), and another confiscation of *E. figaro* and *G. brasiliensis* specimens, at Guarulhos international airport, during the 2014 Fifa World Cup in Brazil. (http://www.ibama.gov.br/publicadas/pagina-19-20). Another aspect that deserves special attention regarding the exploitation of the *E. figaro* is the potential negative ecological effect in reef areas, since it is a recognized cleaner species that plays an important role at cleaning stations and thus, in maintaining the functioning of the marine ecosystem (Sazima et al. 2000, Campos & Sá-Oliveira 2011). Considering the Brazilian list of threatened species and the distribution of the fish traded, it must be highlighted that *Choranthias salmopunctatus* (Lubbock & Edwards, 1981), *Prognathodes obliquus* (Lubbock & Edwards, 1980)*, Stegastes rocasensis* (Emery, 1972) and *S. sanctipauli* Lubbock & Edwards, 1981 are endemic to Brazilian oceanic islands (e.g. Rocas Atoll and St Peter and St Paul's Archipelago - SPSPA) and, hence, their populations are more vulnerable to exploitation due to isolation (Mohr et al. 2009). Even considering the fragile aspects of these isolated populations and the prohibition of harvesting individuals at Brazilian oceanic islands by IN Ibama 202/08, almost all of them were already recorded as being captured for the aquarium industry – the only exception is *C. salmopunctatus*, which, despite never observed in the Brazilian ornamental market, is a desired species, specially by the millionaire Asian commerce, due to its unique characteristics (e.g. attractive color, rarity: low density/absolute number, and is the only species of the genus in Brazil) and extremely restricted geographic distribution (endemic to SPSPA: very limited horizontal and depth ranges) (Luiz-Júnior et al. 2007, Sampaio & Nottingham 2008).

Some authors mention the aquarium trade of certain fish that could not be identified further than the genus level, but that comprise species listed in decree MMA 445/14: *Micrognathus* (*M. erugatus* – CR), *Scarus (S. trispinosus* – EN and *S. zelindae* – VU), *Sparisoma* (*S. axillare* – VU, *S. frodosum* – VU and *S. rocha* – VU), *Stegastes* (*S. rocasensis* – VU, *S. sanctipauli* – VU and *S. trindadensis* – VU), *Dasyatis* (*D. centroura* – CR and *D. colarensis* – VU) and *Rhinobatos* (*R. horkelli* – CR and *R. lentiginosus* – VU) (Monteiro et al. 2003, Gasparini et al. 2005, Nottingham et al. 2005a, Sampaio & Rosa 2005, Rosa et al. 2006), thus, it is possible that other threatened species have been exploited by the Brazilian marine aquarium industry.

Other important threatened species are the longsnout (*Hippocampus reidi* Ginsburg, 1933) and lined (*Hippocampus erectus* Perry, 1810) seahorses. These species have received particular attention from the scientific community and Brazilian governmental authorities, who decided to keep them with the lowest exportation quota (250 specimens of each species/exporter/year). This is because populations pressed by aquarium harvesting activities had shown lower densities and smaller individuals (Ibama 2007, 2008a). However, the effectiveness of such measure is questionable since untrained and ill-intentioned dealers used to mislabel specimens of either *H. erectus* or *H. reidi* as they were *Hippocampus kuda* Bleeker, 1852 (Monteiro-Neto et al. 2003), while field surveys demonstrated that only *H. reidi* was actually exported, and the given quota could be doubled if 250 *H. reidi* were traded under the name of H. *erectus* (Rosa et al. 2011). Furthermore, there is still controversy about the distribution and taxonomy of Brazilian seahorses. Despite most authors state that *H. reidi* has a wider distribution along the Brazilian coast, while *H. erectus* is more restricted to southeastern and southern regions. However, evidences suggest that both species may have a continuous distribution along the Brazilian coast (Silveira 2011). Moreover, while *H. reidi* and *H. erectus* are the only valid names for the Brazilian seahorses (Fishbase 2017), a revision of the genus *Hippocampus* not only revealed that individuals identified in Brazil as *H. erectus* are morphologically and genetically similar to *Hippocampus patagonicus* Piacentino & Luzzatto, 2004 (Silveira et al. 2014), but also indicated the existence of a highly population limited to northeastern Brazil, distinguishable from these two previously mentioned species (Ibama 2009, Rosa et al. 2011).

Taxonomic problems are also on traded labrid, opistognatid and labrosomid fish. After revalidation of some Brazilian wrasse species and reevaluation of their distribution (Rocha & Rosa 2001, Rocha 2004), it is likely that specimens referred as *Halichoeres radiatus* (Linnaeus, 1758), *Halichoeres cyanocephalus* (Bloch, 1791) and *Halichoeres maculipinna* (Müller & Troschel, 1848) in previous works were actually misidentified, and should be, in fact, the labrids *Halichoeres brasiliensis* (Bloch, 1791), *Halichoeres dimidiatus* (Agassiz, 1831) and *Halichoeres penrosei* Starks, 1913, respectively. Another possible mistake occurred for *Opistognathus aurifrons* (Jordan & Thompson, 1905), which shall be in fact a new species of the same genus - *Opistognathus* sp. n. - (Sampaio & Nottingham 2008) and a third taxonomic incongruity is related to the forbidden exploitation of a new labrosomid species - *Malacoctenus* sp. n. (Floeter et al. 2003) -, which have been erroneously commercialized as a blenid, called 'red blenny'. Additionally, preceding articles also recorded the presence of *Microphis eigenmanni* in the Brazilian ornamental trade*,* which is a not valid synonym of *Microphis lineatus* (Kaup, 1856) (Fishbase 2017)*.* Similarly, *Canthigaster figueiredoi* Moura & Castro, 2002, used to be referred as *Cantigaster rostrata* (Bloch, 1786) in previous works (Sampaio & Nottingham 2008). Therefore, some fish scientific names recorded here may be different from those reported on original papers, but are in accordance with the most recent synonyms used (Fishbase, 2017)*.*

With regards to unthreatened species, angelfish have been systematically recorded among the most exploited species by the Brazilian marine aquarium industry (Nottingham et al. 2000, Monteiro-Neto et al. 2003, Gasparini et al. 2005, Nottingham et al. 2005A, Feitosa et al. 2015) and despite the paucity of updated information about the exploitation of marine fish, the most recent official data available indicate that (Linnaeus, 1758)*, Holacanthus tricolor* (Bloch, 1795), *Pomacanthus paru* (Bloch, 1787)*, Pomacanthus arcuatus* (Linnaeus, 1758) and *Centropyge aurantonotus* Burgess, 1974, are still the most targeted species (Ibama 2008a). Another fact that corroborates this statement is the growing demand for pomacanthids in the international market throughout the years, which lead the Brazilian authorities to attribute differentiated exportation quotas to them - substantially higher than the ones given to the other species by means of the IN Ibama 202/08. Additionally, the illegal exploitation of rare specimens from isolated populations of *H. ciliaris* (e.g. wholly yellow, blue or white morphs and other unique color variants, endemic to SPSPA) (Feitoza et al. 2003, Luiz-Júnior 2003,), whose individual prices in the Japanese market can achieve up to US$8.900,00, can decrease the genetic diversity (Gasparini et al. 2005) or even put these oddities in risk of extinction by means of an Anthropogenic Allee Effect (Courchamp et al. 2006).

None of the cartilaginous fish identified could be exploited according to IN Ibama 202/08, but such restriction is not entirely complied by the Brazilian aquarium industry. The clandestine harvest of these species is especially serious due to the fact that some sharks (*Ginglymostoma cirratum* (Bonnaterre, 1788), *Zapteryx brevirostris* (Müller & Henle, 1841) and rays (*Rhinobatus horkelii* Müller & Henle, 1841, *Rhinobatus lentiginosus* Garman, 1880, *Dasyatis centroura* (Mitchill, 1815) and *Dasyatis colarensis* Santos, Gomes & Charvet-Almeida, 2004) are listed in decree MMA 445/14. Illegal collections of *G. cirratum* and rhinobatids for the ornamental trade are not uncommon (Monteiro-Neto et al. 2003, Gasparini et al. 2005, Mohr et al. 2009). On the other hand, the harvest of sawfish for the same purpose seems to be rarer, despite newborn individuals be ordered by the aquarium industry (Charvet-Almeida & Faria, 2008). Regarding the trade of unthreatened sharks and rays, most species are sporadically harvested, with exception of *Narcine brasiliensis* (Olfers, 1831) and *Rhinobatos percellens* (Walbaum, 1792), whose captures involve a great number of newborn individuals and possibly are concentrated at a nursery site in Todos os Santos Bay, Bahia state, Northeastern Brazil (Sampaio & Rosa 2005).

Concerning invertebrates, the exploitation of bivalves for marine aquarium purposes seems to be negligible in Brazil, when compared to other organisms. However, in 2005, one of the Brazilian most famous aquarium company requested Ibama's authorization to export these organisms. The company granted the demand for six species (*Anadara brasiliana* (Lamarck, 1819), *Atrina seminuda* (Lamarck, 1819), *Euvola ziczac* (Linnaeus, 1758), *Pinna carnea* Gmelin, 1791, *Pteria colymbus* (Roding, 1798) and *Trachycardium muricatum* (Linnaeus, 1758)) - noting that exports of *E. ziczac* occurred prior to its inclusion as EN in the Brazilian list of threatened fish and aquatic invertebrate species in 2014-, but despite export of other species were not authorized for different reasons, they are still legally exploitable for the domestic market accorting to Federal Law 9,605/98.

Distinct groups of gastropods are explored by the Brazilian aquarium industry. The prosobranchs *Cerithium atratum* Born, 1778*, Neritina virginea* Linnaeus, 1758 and *Tegula viridula* (Gmelin, 1791) are widely commercialized as aquarium 'clean-up crew' or 'algae cleaners', due to their feeding habit of grazing on unwanted algae. Other prosobranchs are not frequently traded, but Brazilian aquarium dealers requested Ibama to give them authorization to export *Cassis tuberosa* (Linnaeus, 1758)*, Charonia variegata* (Lamarck, 1816)*, Conus* spp., *Cyphoma gibbosum* (Linnaeus, 1758)*, Cyphoma macumba* Petuch, 1979*, Cypraea brasiliensis* Lorenz & Hubert, 1993*, Cypraea spurca* Linnaeus, 1758 and *Turbo canaliculatus* Hermann, 1781. The harvest of *Macrocypraea zebra* (Linnaeus, 1758) and *Strombus pugilis* Linnaeus, 1758 for marine tanks was mentioned at discussion forums and one of the authors observed a *Stramonita brasiliensis* Claremont & Reid, 2011 specimen being sold at an aquarium pet shop, in Ceará state, Northeastern Brazil. However, since *S. brasiliensis* is a predatory sea snail and may feed on other mollusks inside marine tanks, it is possible that the species was mistakenly harvested and unintentionally being sold as a hermit crab carrying a mollusk empty shell.

Concerning opistobranchs, it shall be highlighted not only the maintenance of *Elysia subornata* (Verrill, 1901) individuals by aquarium hobbyists but also spawnings of the species inside tanks, discribed in discussion forums. In addintion, nudibranchs of the genus *Berghia* are wanted in marine aquariums to eradicate the undesirable sea anemone *Aiptasia* sp.

In spite of only two cephalopod species were recorded in the present inventory, it must be considered the possibility of exploitation of a third species, *Octopus insularis* Leite, Haimovici, Molina & Warnke, 2008 - a recently described species from the *O. vulgaris* complex that might have been misidentified as the latter, due to their pattern of distribution along the Brazilian coast and other similarities (Leite et al. 2008).

At discussion forums, unidentified chitons (Polyplacophora) were also mentioned as being kept in marine aquariums either for controlling excessive growth of algae or for revolving sediments. In many cases, these organisms were reported to be collected incidentally, attached to fouled rocks placed into tanks.

None of the crustaceans recorded here figure in decree MMA 445/14. The hermit crabs *Calcinus tibicen* (Herbst, 1791) and *Clibanarius* spp. are widely commercialized as members of the aforementioned 'clean up crew', while *Dardanus venosus* (H. Milne Edwards, 1848) is wanted for aesthetic reasons, since the species often has a sea anemone attached to its shell. Other uncommon hermit crabs are wanted by marine tank owners because of their unique size and beauty, e.g. the giant hermit crab *Petrochirus diogenes* (Linnaeus, 1758) and the red-strip hermit crab *Phimochirus holthuisi* (Provenzano, 1961), respectively.

Concerning other crabs, while *Platypodiella spectabilis* (Herbst, 1794) and *Stenorhynchus seticornis* (Herbst, 1788) are traded mainly for their color pattern and unique features, respectively – in spite of the latter also act as a cleaner of reef fish (Medeiros et al. 2011), the algae-eating crabs *Mithrax* spp. and *Mithraculus forceps* (Milne-Edwards, 1875) (Olivotto et al. 2011), are desired to control the growth of unwanted bubble algae *Valonia* spp. inside tanks. It was recorded a probable taxonomic mistake in the identification of the decorator crab mentioned at discussion forums and sold online through pet shop websites. The species is mentioned as 'gorgonian spider-crab' or simply as 'gorgonian spider', under the scientific names *Xenocarcinus* sp. or *Macropodia longirostris* (Fabricius, 1775). However, as both genuses are not reported for Brazil (L. E. A. Bezerra pers. comm.) and the crab advertised is very cheap and, so, presumably not imported, it is more likely to be another majiid crustacean, the Brazilian decorator crab *Acanthonyx* sp.

Besides their beauty, shrimps *Stenopus hispidus* (Olivier, 1811) and *Lysmata grabhami* (Gordon, 1935) are known for removing ectoparasites from reef fish, while *Lysmata wurdemanni* (Gibbes, 1850) and *Lysmata rathbunae* Chace, 1970 are wanted to control population of *Aiptasia* sp. inside tanks. Gasparini et al (2005) also reported the trade of the gold coral banded shrimp, *Stenopus scutellatus* Rankin, 1898, but the occurrence of the species was not mentioned at any other source of information investigated here. The other shrimps *Cinetorhynchus rigens* (Gordon, 1936)*, Thor* aff. *amboinensis*, *Periclimenes* aff. *yucatanicus* and *Periclimenes* aff. *pedersoni* are unusually sold in Brazilian market, despite their conspicuous body shape, color pattern, and behavioral characteristics, including the known cleaning activities of the latter (Floeter et al. 2007). The snapping shrimp *Alphaeus* sp., also infrequently traded, is kept specially to control flatworm populations inside marine tanks. Since there are 29 species of the genus *Alphaeus* in Brazil, including *A. rudolphi* spec. nov. - a new snapping shrimp of the *Alpheus armatus* species complex (Almeida & Anker 2011), – it was not possible to determine whether one or more species of the genus is traded.

Despite unattractive featured for ornamental purposes, the potential exploitation of the barnacles *Lepas anatifera* Linnaeus, 1758 and *Lepas anserifera* Linnaeus, 1767*,* and the lobsters *Gnathophyllum americanum* Guérin-Méneville, 1855, *Parribacus antarcticus* (Lund, 1793) and *Scyllarides aequinoctialis* (Lund, 1793) was also recorded, since authorization from Ibama to export these organisms alive was also requested. The dwarf reef lobster, *Enoplometopus antillensis* Lütken, 1865, also had its request of authorization for exportation denied by Ibama, but, differently from the other lobsters, this species is attractive to aquarium hobbyists at discussion forums due to its bright color and small size and, so, is still traded inside the country.

The recorded cnidarians belonged to distinct subgroups (sea anemones, octocorals, fire corals, besides other hard and soft corals) and among this wide variety of organisms, only three species are considered threatened in Brazil: *Condylactis gigantea* Weinland, 1860 (EN)*, Mussismilia braziliensis* (Verrill 1868) (VU) and *Mussismilia harttii* (Verrill, 1868) (EN). Special attention shall be given to the illegal exploitation of *C. gigantea*, due to its intensive harvest by the ornamental industry in southeastern Brazil and its local extinction at Arraial do Cabo region, Rio de Janeiro state (Gasparini et al. 2005). Through discussion forums it was observed the illegal trade of sea whips, usually called 'monkey-tail gorgonian' and 'fox-tail gorgonian'. Despite forum members refer to them as members of the family Plexauridae, not only the precise identification of these two gorgonians is impossible based exclusively on common names, but also it is unknown whether or not they are recorded here, since this inventory mentions the plexaurid species *Plexaurella grandiflora* Verrill, 1912 *and Plexaurella regia* Barreira & Castro, 1986.

The echinoderms listed were clearly dominated by sea stars, demonstrating the importance of a wide variety of species to the Brazilian aquarium trade. It is worth notice that some echinoderm species are threatened with extinction in Brazilian waters and authorities should give special attention to ban the harvest of two species: *Linckia guildingii* Gray, 1840, which have been traded illicitly through the Brazilian postal service (Gurjão et al. 2017) and *Eucidaris tribuloides* (Lamarck, 1816), which is constantly mentioned at discussion forums as being used in marine aquariums. Although sea cucumbers are not listed in the tables presented here, because it was not possible to identify the species traded, it was recorded the illegal selling of holothurians at discussion forums, under the common names 'giant sea cucumber', 'detritivorous sea cucumber' and 'burrowing sea cucumber'.

Differently from the other polychaetes identified here (*Spirobranchus* spp. and *Eurythoe complanata* (Pallas, 1766)), desired because of their beauty, *Diopatra cuprea* (Bosc, 1802) (categorized as VU in the Brazilian official list of threatened species) is indirectly used for ornamental purposes, as a source of food for some marine fish species (Steiner & Amaral, 2008). In addition, exploitation of other unreported native polychaetes is likely to happen in Brazil, since the country supplies the UK ornamental market with such worms (Murray et al. 2012).

Tunicates were also recorded among organisms used in marine aquariums in Brazil. While *Botrylloides nigrum* Herdman, 1886, *Styela plicata* Lesuer, 1823 and possibly one unidentified didemnid seems to be unintentionally harvested adhered to live rocks taken from the wild and set into marine tanks, other species sold as 'black ascidian' and 'red ascidian' are deliberately traded by discussion forum members. As there was no photo of the black ascidian advertised, species could not be surely identified. However, due to its wide distribution throughout tropical waters, including Brazilian coast (Lotufo 2002), the possibility of the latter be the solitary *Phallusia nigra* Savigny, 1816 cannot be disregarded. Concerning the red ascidian, photos resembled *Polycarpa insulsa* (Sluiter, 1898). Although uncommon, previous studies had already recorded the presence of tunicates, as well as sponges, on other marine ornamental foreign markets (Wabnitz et al. 2003, Murray & Watson 2014).

The four sponge species identified here, *Aplysina fulva* (Pallas, 1766)*, Axinyssa* sp.*, Dragmacidon reticulatum* (Ridley & Dendy, 1886) and *Tethya* sp. are usually called 'yellow sponge', 'finger', 'red ball' and 'yellow ball', respectively (Sampaio et al. 2004). Discussion forums showed that sponges known as 'red ball' and 'yellow ball' are traded through the internet, however it was not possible to assure the species' identity based exclusively on common names. Additionally, the exploitation of a subespheric morphotype of *D. reticulatum*, called 'sponge-ball' and previously cited as *Pseudaxinella reticulata* (Ridley & Dendy, 1886), by the Brazilian aquarium industry have already been reported (Hajdu et al. 2011).

In Brazil, aquarium trade of seaweeds is extremely rare, since traditionally the co-habitation of corals and macroalgae is not wanted because seaweeds are avid competitors inside tanks, limiting coral growth. In most cases, macroalgae, e.g. *Chaetomorpha* spp., are kept solely in the aquarium's sump, aiming nitrogen and phosphorus removal. However, a few tank owners share their experience at discussion forums, keeping 'marine planted aquariums' rather than coral reef systems and, thus, seaweeds had been used associated to the sea grasses, e.g. *Halophila decipiens* Ostenfeld and *Halodule* sp. Due to the low relevance of 'marine planted aquariums' compared to 'mini reef systems' little attention has been paid to the exploitation of those resources, with exception of Ibama (2008a), which briefly mentioned the usage of macrophytes and *Lithothamnium* spp. in Brazilian aquarium trade.

The wide variety of native species inventoried in the present work demonstrates that Brazil is following the global trend of keeping diversified marine life in aquaria, which have caused increasing concern about the sustainability of reef ecosystems' exploitation (Wabnitz et al. 2003, LeGore et al. 2005, Calado 2006, LeGore et al 2008, Smith et al. 2010, Murray et al. 2012, Reynoso et al. 2012, Rhyne et al. 2012b).

The indiscriminate removal of pomacanthids (a true 'keystone guild') from reef ecosystems, for instance, might have serious negative reflexes (e.g. excessive sponge growth and less juveniles serving as 'cleaners') on community structure and these impacts caused to the reef might be greater than their abundance suggest (Gasparini et al. 2005). Similarly to other fish kept in aquaria, such as surgeonfish (mainly living plant consumers) and parrotfish (primarily detrital aggregates feeders), angelfish perform vital ecological roles in coral reef trophodynamics (e.g. controlling sponge and tunicate densities) (Hourigan et al. 1989, Hill 1998, Sazima et al. 1999, Andréa et al. 2007, Konow & Bellwood 2011, Batista et al. 2012, Reis et al. 2012) and, thus, their overexploitation and inter-specific relationships had also being object of concern (Hill 1998, Comeros-Raynal et al. 2012). Nevertheless, the potential impact of fisheries targeting aquarium reef fish in Brazil is difficult to be evaluated, because little is known about the distribution of this type of fishing effort throughout the country and the actual level of threat to reef fish is hard to be assessed (Floeter et al. 2006).

Invertebrate grazers are also being collected at an increasingly rapid pace, mostly to control algal growth in home aquaria, but, as they play a corresponding role in the wild, their removal may strongly impact their native reefs (Rhyne et al. 2009). On healthy reefs, for instance, both the establishment and the survival of corals depend on high rates of herbivory to suppress macroalgae and reduce competition with cnidarians (Bonaldo & Hay, 2014).

Another interspecific association, the cleaning activity, may be negatively affected by continuous harvesting of fish and shrimps, intensified by the high turnover in ornamental trade, since specialized cleaners generally have a short life in aquariums due to their distinctive feeding habits (Gasparini et al. 2005). Hence, since the influence of the species abundance on cleaning interactions is modulated by the trophic habits and social behavior of the interacting species, the removal of a single cleaner species from a reef will deeply affect the ecosystem functioning, as there seems to be little redundancy on this role when pairs of species are concerned (Floeter et al. 2007).

Not only Brazilian coral reefs but also estuaries (Nottingham et al., 2005b) and sponge reefs (Rocha et al., 2000; Andréa et al., 2007) may be affected by uncontrolled ornamental fisheries. Additionally, large endangered vertebrates can also be harmed by indiscriminate collection of invertebrates and depletion of banks of either macroalgae or macrophytes, e.g. marine turtles (*Eretmochelys imbricata* (Linnaeus, 1766) and *Chelonia mydas* (Linnaeus, 1758)), that feed on a wide variety of invertebrates or graze the substratum (Stampar et al., 2007; Goatley et al., 2012), and the Brazilian marine manatee, *Trichechus manatus* Linnaeus, 1758, whose diet is composed of seaweed and sea grass species identified here (Borges et al., 2008).

Hence, in the face of such concerning scenario, marine aquarium consumers have an important role requiring species from regulated fisheries and shipped in accordance with well-established guidelines (eco-labeled products) and Brazilian authorities must seek environment friendly measures (e.g. implementation of eco-fees to support research on marine ornamental fisheries and mariculture (Leal et al. 2015)).

Aquaculture initiatives must be incentivated, since they might considerably reduce collecting pressure over populations of targeted species (Calado et al. 2003, Pomeroy et al. 2006, Olivotto et al. 2011, Murray & Watson 2014), specially because almost the totality of the native marine aquarium organisms exploited in Brazil are wild-caught and captive breeding of native marine ornamentals is restricted to a few species (e.g. *Elacatinus figaro* and *Hippocampus reidi*) (Meirelles 2008, Hora & Joyeux 2009, Ibama 2009, Côrtes & Tsuzuki 2010). However, captive breeding shall not entirely substitute wild-caught species, because many people depend on the harvesting of aquarium species to survive (Rhyne et al. 2014).

Another way of preventing or reducing overexploitation, would be through ecosystem-based management initiatives (Tissot et al. 2010, Rhyne et al. 2014), as the creation of new marine reserves and the adequate management of the existing ones, in order to promote recovery of stocks of heavily exploited species by the aquarium trade (Friedlander 2001, Tissot et al. 2004, Tissot et al. 2009, Stevenson & Tissot 2013). Such initiatives, instead of preserving a particular species, aim not only the protection of the whole ecosystem but also assure the continuity of inter and intra-specific associations, including the safety of important spawning aggregation sites that are crucial for the survival of some aquarium traded species (Friedlander 2001, Gerhardinger et al. 2009, Comeros-Raynal et al. 2012, Feitosa et al. 2015).

In addition, IBAMA must intensity inspections driven to aquarium trade; IN Ibama 202/08 and decree MMA 445/14 must be reviewed by specialists to identify prohibited species; and educational campaigns explaining the dangers of overexploitation of marine life, involving aquarium stores, importers, wholesalers, retailers and aquarium hobbyists should be carried out.

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Autor Contributions

Lívio Moreira de Gurjão: conceived the work, obtained and analyzed the data and wrote the manuscript;

Tito Monteiro da Cruz Lotufo: contributed to analysis and interpretation and also wrote the manuscript.

Conflicts of interest

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

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The effects of fish feeding by visitors on reef fish in a Marine Protected Area open to tourism

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Abstract: Coral reef-based tourism has risen sharply across the globe, coupled with an increase in fish feeding by visitors. Studies indicate that fish feeding is one of the leading causes of changes in distribution patterns, abundance, the structure of marine fish communities, and fish behavior. The aim of this study was to determine the effect of human presence and fish feeding on the behavior of reef fish by conducting in situ experiments in tide pools in a Marine Protected Area located at the northern limit of the Abrolhos Bank in the South Atlantic Ocean. Eight feeding sessions were conducted and filmed, resulting in a total of 160 minutes of video footage. Each filming session recorded four different experimental conditions alternating between human presence, human absence, and fish feeding. Our findings suggest that fish feeding may cause changes in fish behavior, such as habituation to human presence, conditioning to fish feeding, increased aggressiveness, attacks on humans, and short-term changes in species distribution. The continuation of fish feeding over time can cause an increase in the size of the populations of species that consume food provided during feeding and consequently trigger changes in the structure of communities.
Keywords: *Abudefduf saxatilis, behavior, fish feeding, in situ experiment, South Atlantic, tide pools.*

O efeito da alimentação artificial em peixes recifais em uma Área Marinha Protegida com atividade turística

Resumo: O uso turístico de recifes de corais tem aumentado fortemente em todo o mundo, e com ele o fornecimento de alimento aos peixes por humanos. Porém, estudos indicam essa atividade como causa de distúrbios nos padrões de distribuição, abundância, estrutura da comunidade e comportamento de peixes marinhos. O objetivo deste estudo foi determinar o efeito da presença humana e da alimentação artificial no comportamento dos peixes recifais, através de experimentos *in situ* realizados em piscinas de maré de uma Área Marinha Protegida no limite Norte do Banco dos Abrolhos, Atlântico Sul. Para isso foram realizadas 8 sessões (160 min) de filmagem remota do experimento de alimentação artificial. Cada sessão de filmagem registrou quatro tratamentos, os quais alternavam momentos de presença e ausência humana, e de alimentação dos peixes.Os resultados encontrados indicam que a alimentação artificial tem provocado alterações comportamentais nos peixes como a habituação à presença humana, condicionamento à oferta de alimento, aumento da agressividade, ataques a humanos e alterações na distribuição das espécies em curto prazo. A permanência da atividade de alimentação artificial, ao longo do tempo, pode provocar o aumento das populações que consomem o alimento oferecido e consequentemente, desencadear alterações na estrutura da comunidade.
Palavras-chave: *Abudefduf saxatilis, alimentação de peixes, Atlântico Sul, comportamento, experimento in situ, piscinas de maré.*

Introduction

Coral reef ecosystems harbor rich biodiversity and are crucially important both from a socioeconomic and ecological standpoint, providing various environmental services and benefits, such as coastal protection, maintenance of fish stocks, ecotourism and species with pharmaceutical potential (Brander *et al.* 2007). However, coral reefs have been severely impacted by climate change (Mumby & Anthony 2015), ocean acidification (Comeau *et al.* 2015), overfishing (McClanahan *et al.* 2015), sewage and industrial waste (Wear & Thurber 2015), and tourism (Pereira *et al.* 2014).

Marine Protected Areas (MPAs) are areas set aside to protect marines ecosystems, including reef ecosystems, and play a critical role in conserving marine biodiversity and natural resources used by coastal populations (Giglio *et al.* 2015). The implementation of protective measures helps to keep reef communities healthy, thus maintaining their tourism attractiveness (Green & Donnelly 2003) and contributing to the growth of tourism in these areas (Milazzo *et al.* 2002). However, studies demonstrate that poorly planned or intensive tourist use can adversely affect marine ecosystems and associated marine life (Creed & Amado-Filho 1999, Eckrich & Holmquist 2000, Albuquerque *et al.* 2014, Giglio *et al.* 2016). Several researchers have investigated the impacts of tourism on reef environments, including trampling (Sarmento & Santos 2012, Giglio *et al.*, 2017, Williamson *et al.* 2017), boat anchoring (Saphier & Hoffmann 2005, Beeden *et al.* 2014, Kininmonth *et al.* 2014), free and autonomous diving (Lamb *& True* 2014, Hein *et al.*2015), and fish feeding (Milazzo 2011, Feitosa *et al.* 2012, Bookhouse *et al.* 2013).

Fish feeding is a popular tourist attraction at coral reefs around the world (Giglio *et al.* 2015) and is used by tourism operators to lure specific species and give visitors an opportunity to observe marine life up close. However, research shows that this activity may disrupt distribution patterns, abundance and the structure of marine fish communities (Brunnschweiler & Barnett 2013, Brunnschweiler *et al.* 2014), as well as affecting fish health by increasing fat deposition and vulnerability to diseases caused by microorganisms and ectoparasites (Semeniuk & Rothley 2008).

One of the main impacts of this activity is changes in the natural behavior of fish. For example, by becoming used to the presence of humans, species that otherwise would not approach humans freely gather around bathers to seek supplementary feeding (Albuquerque *et al.* 2014). Furthermore, fish can also become dependent upon fish feeding (Ilarri *et al.* 2008) and, in the long term, the acquired behavior of obtaining food from human hands may reduce their ability to obtain food by themselves in the wild. Another behavioral change reported in the literature is increased aggressiveness during feeding, both in fish competing among themselves for food and towards the people who are feeding them (Milazzo 2011). Fish feeding may also lead to shifts in circadian rhythms, as observed in the naturally nocturnal southern stingray (*Hypanus americanus*), which has become diurnal due to daytime fish feeding activities (Corcoran *et al.* 2013).

Despite studies showing the negative impacts of artificial feeding, few MPAs have implemented fish feeding rules (Corcoran *et al.* 2013). There is also divergence of opinion among MPA managers in relation to fish feeding, given the trade-offs between financial sustainability and the negative impacts of the activity on the conservation of marine life (Hémery & McClanahan 2005, Milazzo *et al.* 2005). In the Great Barrier Reef Marine Park in Australia, for example, fish feeding is permitted provided that the total food used does not exceed 1 kg per day (GBRMPA 2000). In contrast, in the Booderee National Park, also in Australia, feeding marine life is prohibited. This divergence of opinion is also manifested in Kenya, where fish feeding is permitted in certain areas in the Malindi, Watamu, and Mombasa MPAs while being prohibited in the Kisite MPA. In Brazil, fish feeding was permitted in the Abrolhos Marine National Park up to 2003, when the activity was banned in the area.

Coral reef-based tourism has risen sharply across the globe (Milazzo *et al.* 2002), coupled with an increase in the amount of food fed to fish by visitors. Concerns over the negative impacts of fish feeding have led to an increase in studies assessing the effects of this activity on these unique ecosystems (Medeiros *et al*., 2007). However, only a few studies have assessed the impacts of fish feeding on coral reefs in the South Atlantic Ocean (Medeiros *et al.* 2007, Ilarri *et al.* 2008, Feitosa *et al.* 2012, Pereira *et al.* 2014, Albuquerque *et al.* 2014). Thus, detailed studies concentrating on this region are needed to gain a deeper understanding of the effects of fish feeding on the biology of reef fish, which range from physiological and behavioral aspects to impacts on fish communities, in order to improve the management and conservation of these areas.

The species belonging to the genus *Abudefduf* (Family: Pomacentridae) are omnivorous and may be considered generalists. This flexibility allows them to employ opportunistic feeding strategies, making them particularly susceptible to the effects of fish feeding. Studies have reported changes in behavior and the spatial distribution in three species – *A. saxatilis* (Medeiros *et al.* 2007, Ilarri *et al.* 2008, Feitosa *et al.* 2012, Albuquerque *et al.* 2014), *A. sexfasciatus*, and *A. sparoides* (Hémery & McClanahan 2005). It is believed that these changes could have damaging long-term ecological and economic impacts.

In light of the above, the aim of this study was to determine the effect of human presence and fish feeding on the behavior of reef fish by conducting in situ experiments in a MPA northern limit of the Abrolhos Bank located in the South Atlantic.

Material and Methods

1. *Study area*

The Recife de Fora Marine Park (RFMP) is a MPA located at the northern limit of the Abrolhos Bank, approximately 4 kilometers from the coast and the city of Porto Seguro in the State of Bahia, Brazil. The park has an area of approximately 17.5 km^2 and is located between parallels 16°23‟30”/16°25‟06” S and meridians 38°58‟30”/38°59‟18” W (Porto Seguro 2016) (Figure 1). The depth in the middle of the central plateau of the park varies from 6 to 8 meters in the internal portion to a maximum of 20 meters in its western portion (Costa Jr. *et al.* 2002). The RFMP is one of the region's main tourist attractions and received 50,000 visitors in 2015.

The park harbors all 16 coral reef builder species described in Brazil (Castro & Pires 2001) and 43 fish species have been recorded in the area to date (Chaves *et al.* 2010), including *Gramma brasiliensis* Sazima, Gasparini & Moura 1998, *Sparisoma amplum* (Ranzani 1841), *Elacatinus figaro* Sazima, Moura & Rosa 1997, and *Scarus trispinosus*

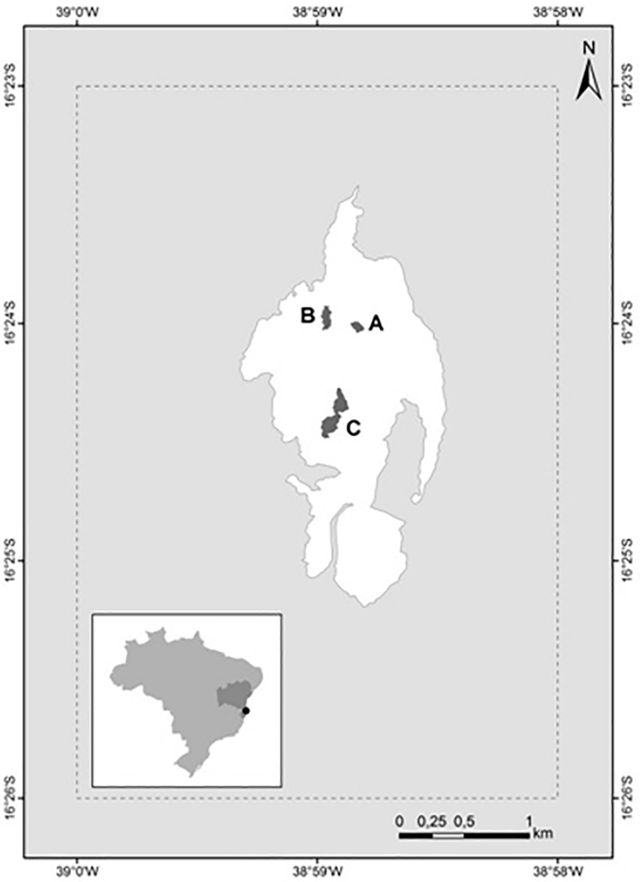


Figure 1. Location of the of Recife de Fora Marine Park (Porto Seguro, Bahia, Brazil). The letters indicate the tide pools: "A" = Dolphin pool, "B" = Visitors' pool, "C" = *Panam* pool, not included in this study.

(Valenciennes, 1840), which are endemic to Brazil (Floeter *et al.* 2008), and the endangered species *Mycteroperca bonaci* (Poey 1860) (Ferreira *et al*. 2008) and *Epinephelus itajara* (Lichtenstein 1822) (Padovani-Ferreira *et al*. 2012).

Depressions in the plateau of the reef form tide pools at low tide, including the *Piscina da Visitação* (the visitors' pool) and *Piscina do Golfinho* (dolphin pool), which have historically been the park's main tourist attraction. The visitors' pool has an area of approximately 9,000 m^2 and a depth of 1.4 meters. Organized visitation to this area began over three decades ago. Although the current visitor limit is 400 visitors/day, this number peaked at 1,200 visitors/day in the past (Porto Seguro 2016). Visitation occurs at low tide, when visitors are led to the pool by park guides, when the bathers, guides and photographers feed the fish using bread, biscuits, dry fish and dog food, and shredded shrimp (Porto Seguro 2016). The dolphin pool has an area of 4,500 m^2 and roughly the same depth as the visitors' pool, but has been closed to visitors since 2002. For the purposes of this study, the visitors' pool was called the "Feeding Area" (FA) and the dolphin pool "Control Area" (CA).

A manipulative experiment was conducted in each area under the following different experimental conditions: 1) "Pre-bather" – comprising a period of five minutes before the bather entered the area, seeking to reproduce natural conditions without any disturbance; (2) "Bather presence" – comprising the first five minutes after the bather entered the area, to identify changes in fish behavior in the presence of a human; (3) "Post-bather" - comprising the 5-minute period immediately after the bather left the area, to determine how the fish reacted after the end of visual stimulation and the length of time it took for them to begin stabilizing their behavior; and (4) "Feeding" - with the presence of the bather feeding the fish for a period of 5 minutes, to assess changes caused by the presence of a person offering food (Figure 2). Fish behavior and abundance under each condition was assessed using remote filming sessions.

Sampling was conducted between March and April 2014 during the daytime at low tide before the arrival of tourists. During sampling, horizontal visibility was at least 4 meters in both the FA and CA. A GoPro Hero 3 Silver Edition (GoPro Inc.) digital camera was used to record fish behavior and quantify abundance installed at a fixed point at the edge of each tidal pool and 60 cm from the bottom.

Recording started as soon as the camera was installed. The first 8-10 minutes of the recording were discarded to allow the fish to stabilize their behavior after the researcher left the water. The Pre-bather condition comprised the 5-minute period after stabilization. At the end of this period, the bather entered the water and remained in front of the camera at a distance of exactly 1.5 meters for 5 minutes. It is important to note that, although it is probable that the changes caused by the presence of only one bather do not fully represent those caused by the 400 bathers that visit the FA each day, the identification of significant changes in the presence of one person provides an indication of the intensity of the changes caused by a larger number of people. At the end of this period, the bather left the tidal pool and the Post-bather condition began. At the end of this period, the bather entered the pool again and remained in the same position as in the Bather presence condition, this time offering food to the reef fish for a period of 5 minutes.

The above process was repeated 4 times in each area on separate occasions. For each of the four filming sessions in each area, a one-minute frame was selected from each of the 5-minute periods, giving a total of five frames per experimental condition per session and a overall total of 20 frames per condition for each area (n = 80 for both FA and CA). For each one-minute frame, the specific abundance of fish within a distance of 1.5 meters from the camera (distance between the camera and the bather) was quantified. Species were identified according to Humann & Deloach (2002) and Sampaio & Notthingham (2008).

The bather was represented by a researcher using the type of clothing and behavior used by photographers working for tour operators. The food used was the same shredded shrimp used by the tour operators, which is the most commonly used food during visitation. A total of 250 grams of food were gradually fed to the fish over the 5-minute period under the feeding conditions. This quantity corresponds to the amount fed by photographers and was based on information obtained through personal communications with the tourist agents.

To complement the experiments and to help identify possible changes in behavior, fish behavior in the two areas was recorded not only during the filming sessions, but also between experiments during normal group visits to the park, focusing on, but not limited to, the following aspects of behavior: agonistic interactions, interaction with the bather, attacks on the bather, reaction time to in-water food stimulation, and opportunistic feeding (for example, herbivores feeding on shrimp).

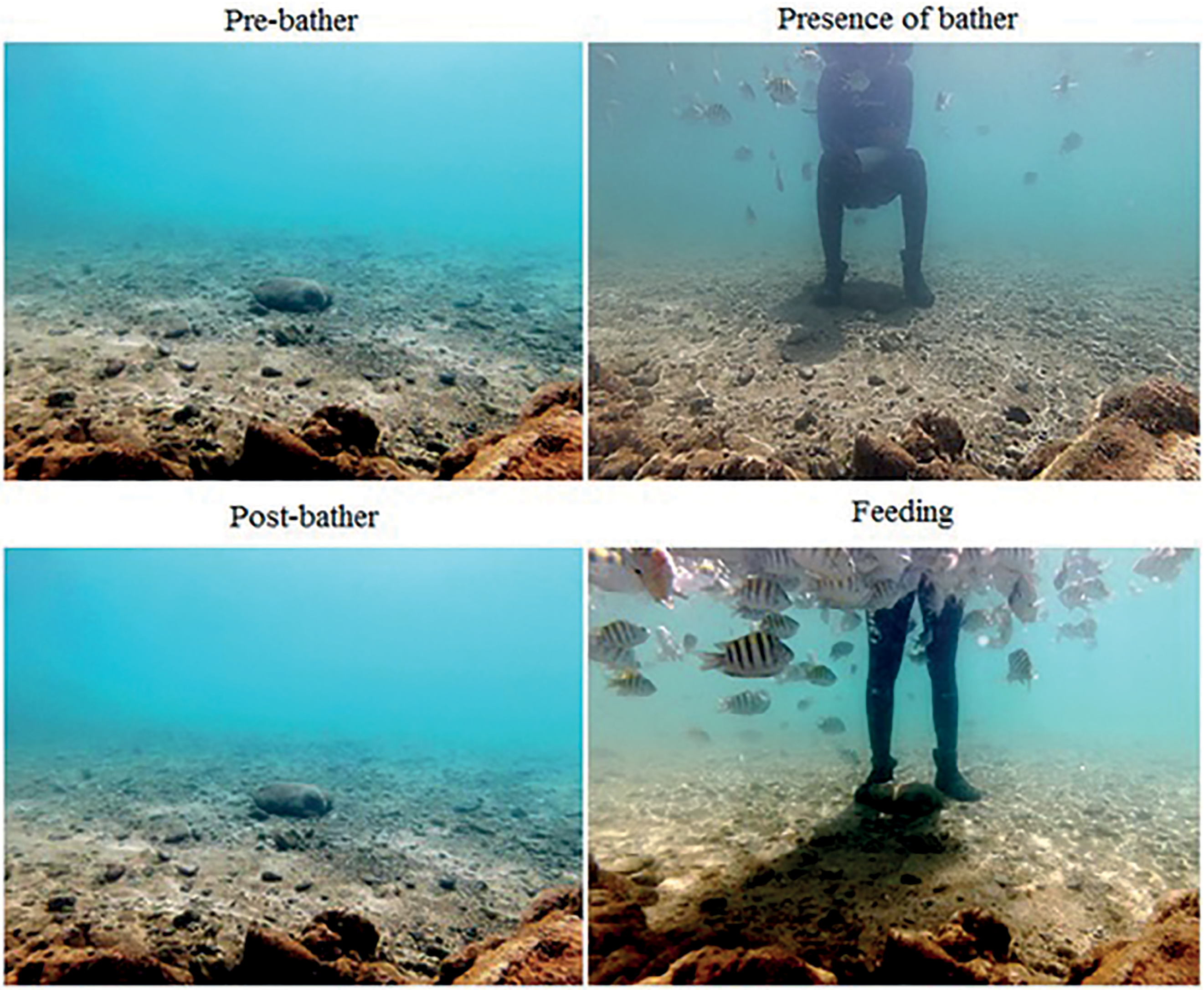


Figure 2. Experimental conditions in the Recife de Fora Marine Park.

2. *Statistical analysis*

A two-way nested ANOVA was used to compare fish abundance between different experimental conditions in both areas, followed by Tukey's post hoc test when a statistically significant result was obtained. Abundance data was log (x + 1) transformed to meet ANOVA assumptions. The analyses were performed using the software package Statistica 8.0 (StatSoft 2007), adopting a 5% significance level.

Results

A total of 2,816 individuals comprising nine families and 17 species were identified from the 160 frames, which is equivalent to 32.6% of the 43 species registered in the RFMP. Fourteen of these species consumed food in the CA, compared to only four in the FA (Table 1).

The most abundant species under all conditions in the Feeding Area and Control Area were *Abudefduf saxatilis* and *Haemulon aurolineatum*, respectively (Figure 3).

In the FA, the abundance of *A. saxatilis* differed significantly between different experimental conditions (F = 80.89, df = 3, p <0.05) and areas (F = 76.62, df = 1, p <0,05). Species abundance was significantly greater under feeding conditions in both the FA and CA. The density of *A. saxatilis* was significantly greater in the presence of the bather than in the absence of a bather in the FA. Densities did not differ significantly between the other conditions (Figure 4, Table 2).

The abundance of *A. saxatilis* in the FA was shown to significantly greater under experimental conditions involving human presence (Figure 4, Table 2). Abundance in the FA was over twice that in the CA in presence of feeding.

Opportunistic behavior was observed in herbivorous species such as *Stegastes fuscus, Scarus trispinosus, Sparisoma axillare, Acanthurus chirurgus, A. coeruleus,* and *A. bahianus,* which consumed shrimp. In the FA, in the presence of a bather (without feeding) and in the presence of feeding, large numbers of *A. saxatilis* approached the bather, migrating from different areas of the pool to the experiment site. In contrast, in the CA, besides not attracting this species, the presence of the bather scared away other species.

Table 1. Fish species registered under feeding conditions in the Feeding Area and Control Area. Recife de Fora Marine Park, Bahia, Brazil. "Consumed" indicates species that consumed the food provided.

Family	Species	Trophic group	Feeding Area	Control Area
Acanthuridae	*Acanthurus bahianus* Castelnau 1855	Herbivorous	Present/Did not consume	Present/Consumed
	Acanthurus chirurgus (Bloch 1787)	Herbivorous	Present/Did not consume	Present/Consumed
	Acanthurus coeruleus Bloch & Schneider 1801	Herbivorous	Absent	Present/Consumed
Carangidae	*Caranx bartholomaei* Cuvier 1833	Carnivore	Present/Did not consume	Absent
Haemulidae	*Anisotremus virginicus* (Linnaeus 1758)	Invertivore	Present/Did not consume	Present/Consumed
	Haemulon aurolineatum Cuvier 1830	Invertivore	Absent	Present/Consumed
	Haemulon parra (Desmarest 1823)	Invertivore	Absent	Present/Consumed
	Haemulon plumierii (Laceepède 1801)	Invertivore	Absent	Present/Consumed
Labridae	*Halichoeres brasiliensis* (Bloch 1791)	Invertivore	Absent	Present/Consumed
	Scarus trispinosus Valenciennes 1840	Herbivorous	Present/Consumed	Present/Consumed
	Sparisoma axillare (Steindachner 1878)	Herbivorous	Present/Consumed	Present/Consumed
Lutjanidae	*Ocyurus chrysurus* (Bloch 1791)	Carnivore	Present/Did not consume	Present/Consumed
Mullidae	*Mulloidichthys martinicus* (Cuvier 1829)	Carnivore	Absent	Present/Consumed
	Pseudupeneus maculatus (Bloch 1793)	Invertivore	Present/Did not consume	Absent
Pomacanthidae	*Pomacanthus paru* (Bloch 1787)	Omnivorous	Present/Did not consume	Absent
Pomacentridae	*Abudefduf saxatilis* (Linnaeus 1758)	Omnivorous	Present/Consumed	Present/Consumed
	Stegastes fuscus (Cuvier 1830)	Herbivorous	Present/Consumed	Present/Consumed

Aggressive behavior was observed in *A. saxatilis* during feeding in the FA, consisting of agonistic interactions (biting and chasing other fish) with conspecific individuals and other species, such as *Stegastes fuscus, Acanthurus chirurgus*, and *Scarus trispinosus*. *A. saxatilis* also directed attacks at the bather, biting his hands during feeding. This type of aggressive behavior did not occur in the CA.

The grouping of *A. saxatilis* promoted by feeding in the FA may lead to "thefts" from *S. fuscus'* algae farms. It is possible that the abundance of *A. saxatilis* leads to increased agonistic behavior from individuals of the species *S. fuscus*, which leave their algae farms unprotected when they chase intruders. In such situations, conspecific individuals and other species, such as *A. saxatilis, Acanthurus bahianus*, and *A. chirurgus*, feed on the cultivated algae. In some cases, individuals of the territorial species *S. fuscus* stopped defending their territories to consume the food provided by the bather, thus enabling other fish to "steal" from their farms.

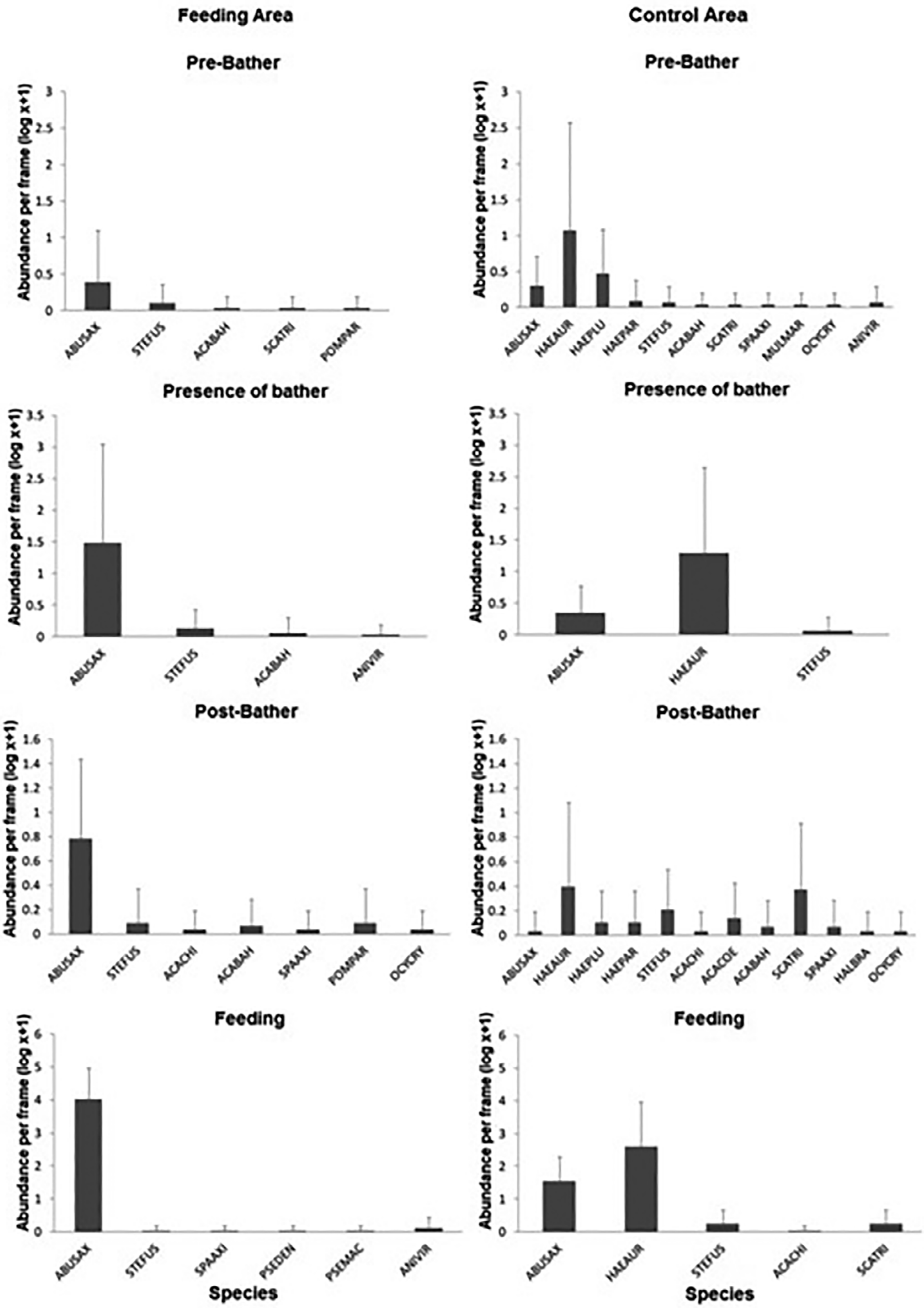
Discussion

The number of species observed consuming food shows that fish feeding directly affects one third of the species of reef fish found in the RFMP. In a study conducted in MPAs in the southeast of Kenya, the number of species that consumed bread fed by tourists (14 species) was identical to that found by the present study (Hémery & McClanahan 2005). The fish identified by this study also included individuals from the families Pomacentridae, Acanthuridae and Labridae, suggesting that fish feeding can affect ichthyofauna in different geographic regions in similar ways. However, the lack of experimental studies does not allow comparisons to be made with other regions.

The low species richness among individuals that consumed food provided by the bather in the FA is probably due to the high abundance and aggressive nature of *A. saxatilis* in this area. It is also interesting to note that four of the species that consumed food in the CA did not consume food in the FA, despite being present in both areas. This finding corroborates the findings of other studies that suggest aggressive species tend to benefit more from fish feeding by excluding nonaggressive species (Perrine 1989, Orams 2002).

The results also suggest that feeding promoted an increase in the abundance of *A. saxatilis* in the FA. Although studies conducted prior to the introduction of tourism to this tidal pool do not exist, this hypothesis is supported by the behavioral changes observed in this study and by the findings of other studies (Medeiros *et al.* 2007, Ilarri *et al.* 2008, Feitosa *et al.* 2012). *A. saxatilis* is an abundant species in tropical reefs in the Atlantic Ocean (Humann & Deloach 2002) and is considered to be a generalist and opportunistic due to its omnivorous diet (Deloach 1999). *A. saxatilis* has also been reported to be the most abundant species in feeding areas in other reefs in the northeast of Brazil, including Picãozinho (Medeiros *et al.* 2007, Ilarri *et al.* 2008) and Maragogi (Feitosa *et al.* 2012), where feeding was pointed out by the authors to be the primary cause of the increased abundance of this species. Two species of the genus *Abudefduf* (*A. sexfasciatus* and *A. sparoides*) were also found to be the most abundant species in feeding areas in the Malindi, Watamu, and Mombasa MPAs in southeastern Kenya (Hémery & McClanahan 2007).

Our findings indicate that the intensification of fish feeding activities in MPAs can lead to an increase in the size of populations of *Abudefduf* and other generalist fish species, leading to a corresponding increase in the number of omnivores in areas where feeding activity is frequent. It is known that fish feeding is associated with higher fish survival and reproduction rates (Sweatman 1996), favoring an increase in the size of populations of fish that consume the food provided during feeding. However, the impact of these increases in population size on the structure of reef communities remains unclear. Therefore, monitoring these fish populations is essential to ensuring the effective management of MPAs.


Feeding Area

Control Area

Pre-Bather

Presence of bather

Post-Bather

Feeding

Figure 3. Average Abundance (± SD) of fish species under the four experimental conditions in the Feeding and Control Areas in the of Recife de Fora Marine Park. Data log (x + 1) transformed. ABUSAX = *Abudefduf saxatilis*, ACABAH = *Acanthurus bahianus*, ACACHI = *A. chirurgus*, ACACOE = *A. coeruleus*, ANIVIR = *Anisotremus virginicus*, HAEAUR = *Haemulon aurolineatum*, HAEPAR = *H. parra*, HAEPLU = *H. plumieri*, HALBRA = *Halichoeres brasiliensis*, MULMAR = *Mulloidichthys martinicus*, OCYCRY = *Ocyurus chrysurus*, POMPAR = *Pomacanthus paru*, PSEMAC = *Pseudupeneus maculatus*, SCATRI = *Scarus trispinosus*, SPAAXI = *Sparisoma axillare*, STEFUS = *Stegastes fuscus*.

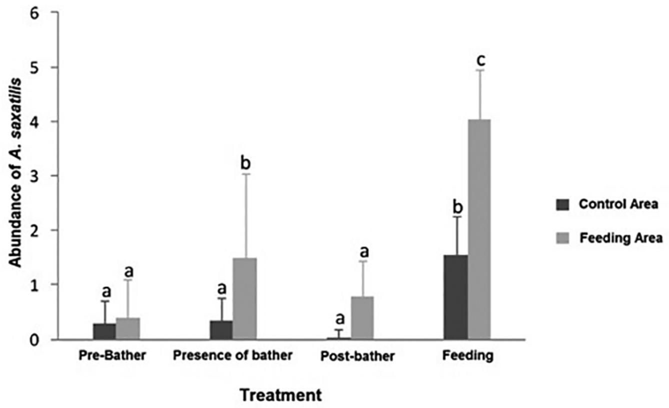


Figure 4. Average abundance of *Abudefduf saxatilis* (± SD) under different experimental conditions in the Feeding and Control Areas of Recife de Fora Marine Park. Different letters indicate a significant difference between treatments according to the results of the Tukey post hoc test.

Our results support the theory proposed by other authors that suggests that fish feeding is the leading cause of the increased abundance of omnivores associated with increases in the size of the population of *A. saxatilis* (Medeiros *et al.* 2007, Ilarri *et al.* 2008, Feitosa *et al.* 2012). The results of this study should therefore be used to inform tourism management planning in the RFMP and in other MPAs where fish feeding is permitted.

The behavioral findings of this study suggest that fish feeding also causes changes in the natural behavior of certain species. *A. saxatilis* showed a change in behavior in the FA, approaching the bather even in the absence of feeding. This suggests a change in behavior conditioned by human presence (visual stimulus) and the presence of food (chemical stimulus). According to Bond (1979), behavioral changes can be caused by both visual and olfactory stimuli and may lead to differences in fish distribution patterns. The behavior of *A. saxatilis* towards people has been studied in feeding areas in reefs in the states of Paraiba (Medeiros *et al.* 2007, Ilarri *et al.* 2008, Feitosa *et al.* 2012) and Pernambuco (Feitosa *et al*., 2012) in the Northeast Region of Brazil. Furthermore, *A. sexfasciatus* showed the same pattern of habituation to the presence of humans in feeding areas in MPAs in Kenya (Hémery & McClanahan 2007). The conditioning of *A. saxatilis* may be explained by the learning ability of fish, as reported by Shettleworth (1984). In extreme cases, conditioning of the behavior of fish to the presence of humans can lead to dependence on fish feeding (Harriot 2002).

We also observed aggressiveness among individuals of the species *A. saxatilis* in the FA, evident in frequent agonistic interactions in competition for food. It is believed that fish feeding may cause increased aggression and changes in the genetic structure of populations due to the possible natural selection of more aggressive fish (Moribe 2000, Semeniuk & Rothley 2008, Hammerschlag *et al.* 2012). Furthermore, biting during agonistic interactions may cause skin lesions, making the injured fish more susceptible to infections (Brookhouse *et al.* 2013).

Changes in fish behavior due to intense fish feeding activities have been reported by other studies. At the Shark Reef Marine Reserve in Fiji, fish feeding has been shown to cause shifts in the movement patterns of the bull shark (*Carcharhinus leucas*), which attended the feeding area with increasing frequency over time, leading to changes in habitat loyalty (Brunnschweiler & Barnett 2013). In the Cayman Islands, daytime fish feeding activities led to a reversal of the feeding patterns of the naturally nocturnal southern stingray (*Hypanus americanus*) and affected their spatial distribution, leading to unnatural grouping (Corcoran *et al.* 2013). Thus, our findings are consistent with the behavioral changes observed in other studies conducted with other species in other regions around the world.

Our findings also show that species that are endemic to Brazil, such as *S. trispinosus*, *S. axillare, H. brasiliensis*, and *S. fuscus*, consumed the food provided. Besides being endemic, *S. trispinosus* is an endangered species from the family Labridae found in the South Atlantic (Padovani-Ferreira et al. 2012) and is considered extinct in other parts of Brazil, such as Arraial do Cabo in the State of Rio de Janeiro (Floeter *et al.* 2007). Labridae also plays an important role in controlling macroalgae populations in reef environments (Francini-Filho & de Moura 2008, Bonaldo *et al.*, 2014) and thus affects the balance between coral reefs and macroalgae, which is highly important to the maintenance of healthy coral reefs (Ainsworth & Mumby 2015). Given the conservation status and role played by this family of fish, PMA management and conservation strategies should take into consideration the possible effects of fish feeding on the biology of these fish.

The change in the feeding behavior of the herbivores *S. fuscus, Sc. trispinosus, S. axillare, A. chirurgus, A. coeruleus* and *A. bahianus*, indicates that fish feeding may cause changes in food preferences. Herbivorous fish are essential for maintaining the resilience of reef environments (Bellwood *et al.* 2004, Mumby *et al.* 2006). Therefore, considering the importance of the ecosystem services provided by these herbivores, further research should be conducted into the effects of fish feeding on the food preferences of these species.

Table 2. Tukey's post hoc test results for *Abudefduf saxatilis* showing the association between abundance and the factors experimental condition and area. Recife de Fora Marine Park, Bahia, Brazil.

	Area	Treatment	1	2	3	4	5	6	7
1	FA	Pre-Bather							
2	FA	Bather presence	<0,05						
3	FA	Post-Bather	NS	NS					
4	FA	Feeding	<0,05	<0,05	<0,05				
5	CA	Pre-Bather	NS	<0,05	NS	<0,05			
6	CA	Bather presence	NS	<0,05	NS	<0,05	NS		
7	CA	Pos-Bather	NS	<0,05	NS	<0,05	NS	NS	
8	CA	Feeding	<0,05	NS	<0,05	<0,05	<0,05	<0,05	<0,05

FA = Feeding Area; CA = Control Area; NS = not statistically significant

Fish feeding in RFMP may also be affecting the health of these animals by increasing fat deposition around vital organs (Moribe 2000, Orams 2002), susceptibility to microbial infections, stomach ulcers and ectodermal parasites, and skin lesions (Brookhouse *et al.* 2013; Semeniuk & Rothley 2008). Furthermore, the grouping of animals caused by fish feeding may favor the spread of diseases among conspecific individuals and other species (Orams 2002). These and other effects of fish feeding on fish health should be taken into account to ensure the effective management of MPAs.

Our findings suggest that changes have occurred to the population of *A. saxatilis*, which are likely to resonate throughout the entire structure of the ichthyofauna. The present study highlights behavioral changes among reef fish resulting from fish feeding activities in the RFMP. These changes include habituation to human presence, conditioning to fish feeding, increased aggressiveness, attacks on humans, and short-term changes in species distribution. To prevent the intensification of changes caused by fish feeding, the competent authorities should take appropriate steps to control the quantity and frequency of feeding and monitor the dynamics of affected fish populations.

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Author Contributions

Yuri Cruz de Paula: 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;

Alexandre Schiavetti: 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.

Cláudio L. S. Sampaio: contribution to manuscript preparation; contribution to critical revision, adding intelectual content.

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

Conflicts of interest

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

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Inventory

Ichthyofauna of the Ribeirão Frutal and tributaries, upper Rio Paraná basin, Minas Gerais, Southeastern Brazil

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Abstract: The Ribeirão Frutal basin is located within the upper Rio Paraná system, in the Frutal municipality, Minas Gerais State, Southeastern Brazil. Until now, there was no complete ichthyological survey published for this small basin. Therefore, here we provided a list of fish species from the Ribeirão Frutal and some of its tributaries. We found 31 species of fishes representing five orders and ten families. We identify a likely undescribed and five non-native species in the Frutal basin. Lastly, we recommend new surveys of fishes in small hydrographic basins within upper Rio Paraná system.

Keywords: *Ichthyology, inventory, Neotropical region, Rio Grande, survey.*

Ictiofauna do Ribeirão Frutal e afluentes, bacia do alto Rio Paraná, Minas Gerais, Sudeste do Brasil

Resumo: A bacia do Ribeirão Frutal está localizada no sistema do alto Rio Paraná, no município de Frutal, Estado de Minas Gerais, Sudeste do Brasil. Até o momento, não existe publicação de um levantamento ictiológico completo para essa bacia. Portanto, aqui fornecemos uma lista das species de peixes do Ribeirão Frutal e alguns de seus tributários. Nós encontramos 31 espécies de peixes representando cinco ordens e 10 famílias. Identificamos uma provável espécie não descrita e cinco não nativas na bacia do Frutal. Por fim, recomendamos novos levantamentos de peixes em pequenas bacias hidrograficas do sistema do alto Rio Paraná.

Palavras-chave: *Ictiologia, inventário, levantamento, região Neotropical, Rio Grande.*

Introduction

Ichthyological surveys, also referred to in scientific literature as inventories (e.g., Giongo et al. 2011; Vidotto-Magnoni et al. 2015), are performed in order to assess the biodiversity of a stream, river or lake (Silveira et al. 2010). Consequently, these studies may result in new discoveries (e.g., undescribed species) and can be implemented as the basis for conservation actions (e.g., Castro et al. 2004, Serra et al. 2007, Raiol et al. 2012, Ohara & Loeb 2016, Silva-Oliveira et al. 2016, Melo et al. 2016, Ferreira et al. 2017).

The Ribeirão Frutal basin is a tributary of the Rio Grande, in the upper Rio Paraná system, Minas Gerais State (*Triângulo Mineiro*), Brazil. This small basin (with just over 120 Km^2) (Machado & Audino 2017) has been impacted by several anthropogenic disturbances (see Machado & Audino 2017; and Brito et al. 2017 and references therein). Recently, a truck leaked a substantial volume of hydrochloric acid into one of its tributaries, the Vertente Grande, killing a large number of its aquatic organisms (Azevedo-Santos & Coelho, pers. obs.). Another threat, for instance, is the advancement of the urban area of the Frutal municipality, which may compromise the water quality of the lower Ribeirão Frutal. Despite being located in a relatively well-populated region, there has been no comprehensive survey of its fish fauna until now. In order to understand the ichthyofauna from this small basin facing numerous threats due to anthropogenic development, we here provided a survey of fish species that occur in the Ribeirão Frutal and some of its tributaries.

Material and methods

Samplings were carried April, May, August, and October 2017, using handnets (mesh of 1.5 mm), a small trawl (mesh of 1.5 mm), and gill nets (10 and 20 mm between opposite nodes). Collections involved the efforts of two researchers during daytime and, when possible, in the evening. A total of 10 localities were sampled in the Ribeirão Frutal basin (Figure 1-2; Table 1). Immediately after removal from wild, individuals of several species were photographed alive in an aquarium. Vouchers were euthanized in anesthetic (i.e., benzocaine), fixed in 10% formalin, transferred to 70% alcohol and subsequently deposited in Brazilian collections: LBP (Laboratório de Biologia de Peixes, Botucatu, Botucatu, SP, Brazil); NUP (Coleção Ictiológica do Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura, Universidade Estadual de Maringá, Maringá, PR, Brazil); and UNT (Coleção de Peixes do Laboratório de Ictiologia Sistemática da Universidade Federal do Tocantins, Porto Nacional, TO, Brazil). Identifications were carried out using published literature and, when possible, with the help of a specialist in each group (see Acknowledgements section). All collections for this study were performed with permission of IBAMA (SISBIO - Number 58612-1).

Results

Samples resulted in 765 individuals distributed in five orders, ten families, and 31 fish species (Table 2). Among families, Characidae was the most represented, followed by Cichlidae, and Loricariidae (Figure 3). Site four (S4) presented the highest species-richness, with 16 species (Table 3). At site nine (S9), a small tributary entering the Ribeirão Frutal near its confluence with the Rio Grande, we recorded one likely undescribed species (i.e., *Satanoperca* sp.; Figure 4j). Finally, we found five fish species believed to be non-native to the Ribeirão Frutal basin: *Cichla* cf. *piquiti* Kullander & Ferreira 2006, *Knodus moenkhausii* (Eigenmann & Kennedy 1903), *Metynnis lippincottianus* (Cope 1870), *Oreochromis niloticus* (Linnaeus 1758), and *Poecilia reticulata* Peters 1859.

Discussion

Most species found in our survey (about 87.1 %) were recorded by Langeani et al. (2007) for the upper Rio Paraná basin. However, these authors do not provide the distribution of species within the basin (see Langeani et al. 2007). Therefore, we contribute to the knowledge of the distribution of species into the Ribeirão Frutal basin, in the upper Rio Paraná system.

Recently, Santos et al. (2017) provided a list of species from two regions of the lower rio Preto, Rio Grande basin, in the upper Paraná system; relatively near the Ribeirão Frutal basin. These authors found representatives of the order Characiformes, Gymnotiformes, Cyprinodontiformes, Perciformes (=Cichliformes herein), Siluriformes, and Synbranchiformes (see Santos et al. 2017 for more details). In this work, we did not find any representatives of Gymnotiformes. As species of this order occur in the Rio Grande basin (Santos et al. 2017; see also Castro et al. 2004), it is possible that species of this order also occur at the present study location, but were merely not sampled.

Langeani et al. (2007) showed that Characidae and Loricariidae, in general, are the two most species-rich families of the upper Rio Paraná basin. In addition, several ichthyofaunal surveys (e.g., Pavanelli et al. 2007, Smith et al. 2007, Oliveira et al. 2009, Fagundes et al. 2015, Hoffmann et al. 2015, Cardoso et al. 2016, Frota et al. 2016, Santos et al. 2017) show this pattern in tributaries of the basin. However, in our work Cichlidae was the second most represented family (Fig. 3); probably due to the presence of two non-native species of this family (i.e., *Cichla* cf. *piquiti* and *Oreochromis niloticus*).

Satanoperca sp. (Fig. 4j) is probably the undescribed species previously mentioned by Ota (2013). According to Ota (2013), this species occurs in the Tocantins/Araguaia and upper Rio Paraná basins, but there is no certainty as to whether or not it is native to the upper Paraná basin. On the other hand, *Astyanax* sp. is very similar to

Table 1. Sampled localities in the Ribeirão Frutal basin, upper Rio Paraná system, Brazil.

Site	Coordinates	Remarks
S1	20° 0'14.97"S, 48°55'33.76"W	A tributary of left side of the Ribeirão Frutal; known as "Córrego do Jaó". It presents sections with riparian vegetation. A stretch upstream is dammed and forms a small reservoir. Near to the tributary there is an expansion of the urban perimeter.
S2	19°56'13.50"S, 48°56'56.50"W	Site in the upper portion of main channel of the Ribeirão Frutal.
S3	20°00'36.1"S, 48°56'39.3"W	A small tributary of right side of the Ribeirão Frutal. Unknown name.
S4	20°00'19.9"S, 48°56'37.2"W	Site in the middle portion of main channel of the Ribeirão Frutal. Region subject to the expansion of the urban perimeter.
S5	20°03'50.2"S, 48°56'49.6"W	Lower portion of left tributary of the Ribeirão Frutal; known as "Córrego do Marianinho". Its headwater is the target of the expansion of the urban perimeter.
S6	20°01'13.25"S, 48°56'25.08"W	A tributary of left side of the Ribeirão Frutal; known as "Vertente Grande". This tributary has much of its course within the urban perimeter (with sections channeled by cement). There are evidences that the tributary receives domestic effluents.
S7	19°55'41.5"S, 48°56'07.5"W	Headwater of the Ribeirão Frutal. Portions with presence of riparian vegetation and lotic habitats in which the water flows on rocky substratum.
S8	19°55'52.0"S, 48°56'05.1"W	The first tributary of the Ribeirão Frutal, left side. Unknown name.
S9	20°06'11.6"S, 48°57'32.2"W	Left side tributary of the Ribeirão Frutal. Unknown name. Probably one of the most heavily impacted tributaries with dams (forming small reservoirs) in different stretches. We observed the cultivation of oranges near to the affluent.
S10	20°04'25.3"S, 48°57'02.4"W	Left side tributary of the Ribeirão Frutal; known as "Córrego do Sal".

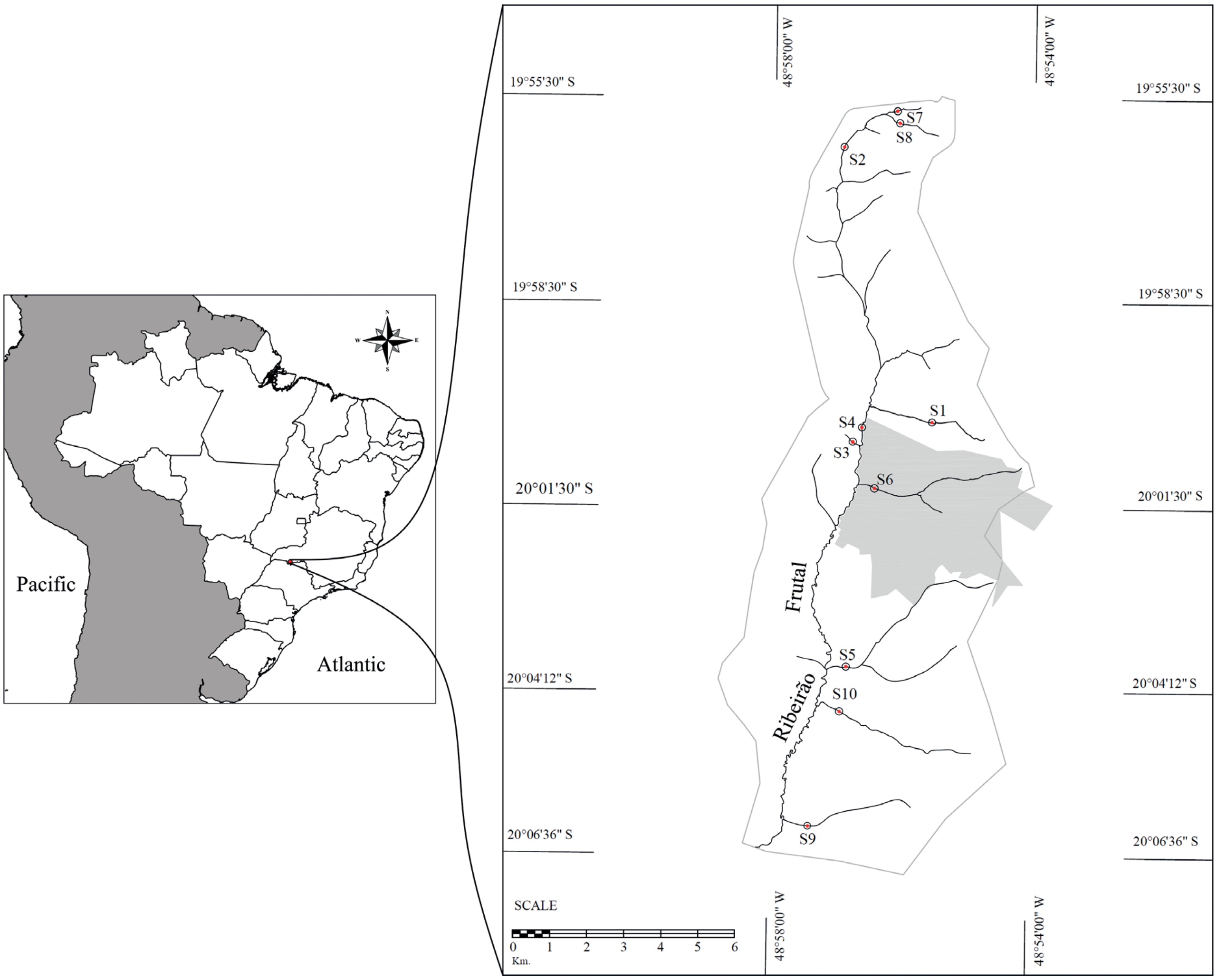

Figure 1. Map showing the Ribeirão Frutal basin with the sites sampled in the basin, upper Rio Paraná system, Brazil. Shaded area represents the urban perimeter of the Frutal municipality.

A. paranae Eigenmann, 1914. In addition, the species collected seems to have two morphotypes in the body shape. Only with further analysis, preferably including osteological and molecular data, can we certain of its specific identification.

We found a total of five non-native species in Ribeirão Frutal basin: *Oreochromis niloticus*, native to the Afrotropical region (Welcomme 1988), *Poecilia reticulata*, described based on material from "Caracas" (Peters 1859, p. 412), in Venezuela, *Metynnis lippincottianus* and *C.* cf. *piquiti*, both native to the Amazon basin (Jégu 2003, Kullander & Ferreira 2006), and *Knodus moenkhausii*, which is not native to the upper Rio Paraná basin according to Langeani et al. (2007). *Hyphessobrycon eques* was found only in two tributaries of the Ribeirão Frutal basin (see Tab. 3). Castro et al. (2004) were not certain whether this species was native to the Rio Grande. With no evidence to suggest recent introduction of this species to the Ribeirão Frutal, we will tentatively consider it to be native. All of the five aforementioned non-native species seem to be fully-established and reproducing at the collecting sites in Ribeirão Frutal basin.

Numerous small basins of the upper Rio Paraná system have been impacted by anthropogenic activities (e.g., Santos et al. 2017, this work). Therefore, modern and comprehensive ichthyological surveys are needed to effectively document the fish fauna of these small tributary basins. Obviously, together with these surveys, measures to avoid or minimize negative impacts to these environments should be adopted. For the Ribeirão Frutal basin, in particular, we recommended three first actions: (i) management of the non-native fish species mentioned here and initiatives to avoid new introductions (e.g., Azevedo-Santos et al. 2015); (ii) avoid urban sprawl over the basin; and (iii) remove the dams (i.e., small reservoirs) in the headwater streams of the basin.

Figure 2. Photos showing stretches of each sampling site (S1 to S10) from the Ribeirão Frutal basin, upper Rio Paraná system, Brazil.

Table 2. Fish species collected in the Ribeirão Frutal basin, upper Rio Paraná system, Brazil. Classifications follow Eschmeyer and Fong (2018)

Species	Voucher
CHARACIFORMES	
Crenuchidae	
Characidium zebra Eigenmann, 1909[1]	LBP 24213
Parodontidae	
Apareiodon ibitiensis Campos, 1944[1]	LBP 24406; LBP 24989
Parodon nasus Kner, 1859[1]	LBP 23623; LBP 24412; LBP 24415; LBP 24429; LBP 24988
Serrasalmidae	
Metynnis lippincottianus (Cope, 1870)*	NUP 19142; LBP 24996
Characidae	
Astyanax bockmanni Vari & Castro 2007[1]	LBP 25000
Astyanax lacustris (Lütken, 1875)[1]	LBP 23611; LBP 23616; LBP 24225
Astyanax sp.	NUP 19125; LBP 23609; NUP 19139; NUP 19131; NUP 19140; LBP 24402; LBP 24419; LBP 24420; LBP 24990; LBP 24995
Bryconamericus turiuba Langeani, Lucena, Pedrini & Tarelho-Pereira, 2005[1]	NUP 19128; NUP 19134; LBP 24404; LBP 24407; LBP 24986
Hyphessobrycon eques (Steindachner, 1882)[1]	LBP 23604; LBP 24423; LBP 24980
Knodus moenkhausii (Eigenmann & Kennedy, 1903)[1,*]	NUP 19129; NUP 19132; NUP 19136; LBP 24217; NUP 19144; LBP 24403; LBP 24411; LBP 24425; LBP 24428; LBP 24982; LBP 24987; LBP 24999; LBP 25001
Moenkhausia cf. *intermedia* Eigenmann, 1908[1]	LBP 23606
Oligosarcus pintoi Campos, 1945[1]	LBP 23603; LBP 23675; LBP 23618
Piabina argentea Reinhardt, 1867[1]	LBP 23612; LBP 24405; LBP 24413
Serrapinnus notomelas (Eigenmann, 1915)[1]	LBP 23607
SILURIFORMES	
Heptapteridae	
Cetopsorhamdia iheringi Schubart & Gomes, 1959[1]	LBP 24408
Imparfinis borodini Mees & Cala, 1989[1]	LBP 24410
Rhamdia quelen (Quoy & Gaimard, 1824)[1]	LBP 23622; LBP 24417; LBP 24983
Callichthyidae	
Aspidoras fuscoguttatus Nijssen & Isbrücker, 1976[1]	NUP 19124; NUP 19127; NUP 19133; NUP 19137; LBP 23677; NUP 19138; LBP 24339; LBP 24401; LBP 24414; LBP 24418; LBP 24991
Loricariidae	
Hypostomus ancistroides (Ihering, 1911)[1]	NUP 19126; NUP 19130; NUP 19135; NUP 19141; LBP 23676; LBP 24215; LBP 24219; LBP 24223; LBP 24340; LBP 24424; LBP 24984; LBP 24998
Hypostomus cf. *iheringii* (Regan, 1908)[1]	LBP 24218; LBP 24993; LBP 25002
Hypostomus nigromaculatus (Schubart, 1964)[1]	LBP 23608; LBP 24220; LBP 24409; LBP 24427; LBP 24985
Hypostomus cf. *topavae* (Godoy, 1969)[1]	LBP 24416
SYNBRANCHIFORMES	
Synbranchidae	
Synbranchus marmoratus Bloch, 1795[1]	LBP 25003
CICHLIFORMES	
Cichlidae	
Cichla cf. *piquiti* Kullander & Ferreira, 2006[1,*]	LBP 23619
Cichlasoma paranaense Kullander, 1983[1]	LBP 23605; LBP 23620; LBP 24979; LBP 24997
Crenicichla britskii Kullander, 1982[1]	LBP 23617; LBP 24421
Geophagus brasiliensis (Quoy & Gaimard, 1824)[1]	LBP 23610; LBP 23674; LBP 23621; LBP 24422
Oreochromis niloticus (Linnaeus, 1758)[1,2,*]	LBP 23614; LBP 24222
Satanoperca sp.	NUP 19143; LBP 25426
CYPRINODONTIFORMES	
Poeciliidae	
Phalloceros harpagos Lucinda, 2008	UNT 016907; UNT 016908; UNT 016909; UNT 016910; UNT016911; LBP 24214; LBP 24216; LBP 24224; LBP 24981
Poecilia reticulata Peters, 1859[1,2,*]	LBP 23613; LBP 23615; LBP 24221; LBP 24226; LBP 24992; LBP 24994

[1] Species recorded by Langeani et al. (2007) for the upper Rio Paraná basin. [2] Species recorded (without voucher) by Azevedo-Santos & Coelho (2017) for the Vertente Grande, Ribeirão Frutal basin. * Non-native species in the Ribeirão Frutal basin.

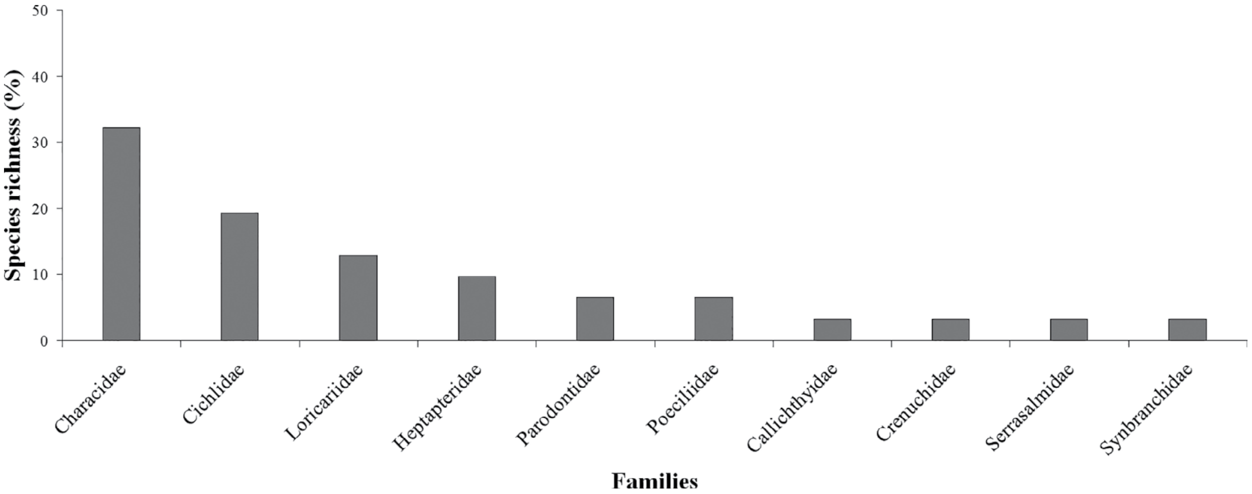

Figure 3. Species richness (%) of each fish family collected in Ribeirão Frutal basin, upper Rio Paraná system, Brazil.

Table 3. Species collected (X) in each site (S1 to S10) of the Ribeirão Frutal basin, upper Rio Paraná system, Brazil.

Species	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Apareiodon ibitiensis				X						
Aspidoras fuscoguttatus	X	X		X	X	X	X			
Astyanax bockmanni										X
Astyanax lacustris				X					X	
Astyanax sp.	X	X	X	X			X	X		
Bryconamericus turiuba		X		X						
Cetopsorhamdia iheringi				X						
Characidium zebra	X									
Cichla cf. *piquiti*									X	
Cichlasoma paranaense			X						X	
Crenicichla britskii									X	
Geophagus brasiliensis				X		X			X	
Hyphessobrycon eques			X						X	
Hypostomus ancistroides	X	X		X	X	X			X	
Hypostomus cf. *iheringii*					X					X
Hypostomus nigromaculatus				X	X					X
Hypostomus cf. *topavae*						X				
Imparfinis borodini				X						
Knodus moenkhausii		X	X	X	X				X	X
Metynnis lippincottianus									X	
Moenkhausia cf. *intermedia*			X							
Oligosarcus pintoi			X			X			X	
Oreochromis niloticus						X				
Parodon nasus				X	X					X
Phalloceros harpagos		X	X	X	X			X		X
Piabina argentea		X		X						
Poecilia reticulata				X	X	X				X
Rhamdia quelen				X		X				X
Satanoperca sp.									X	
Serrapinnus notomelas			X							
Synbranchus marmoratus										X
Species richness	4	7	8	16	8	8	2	2	11	9

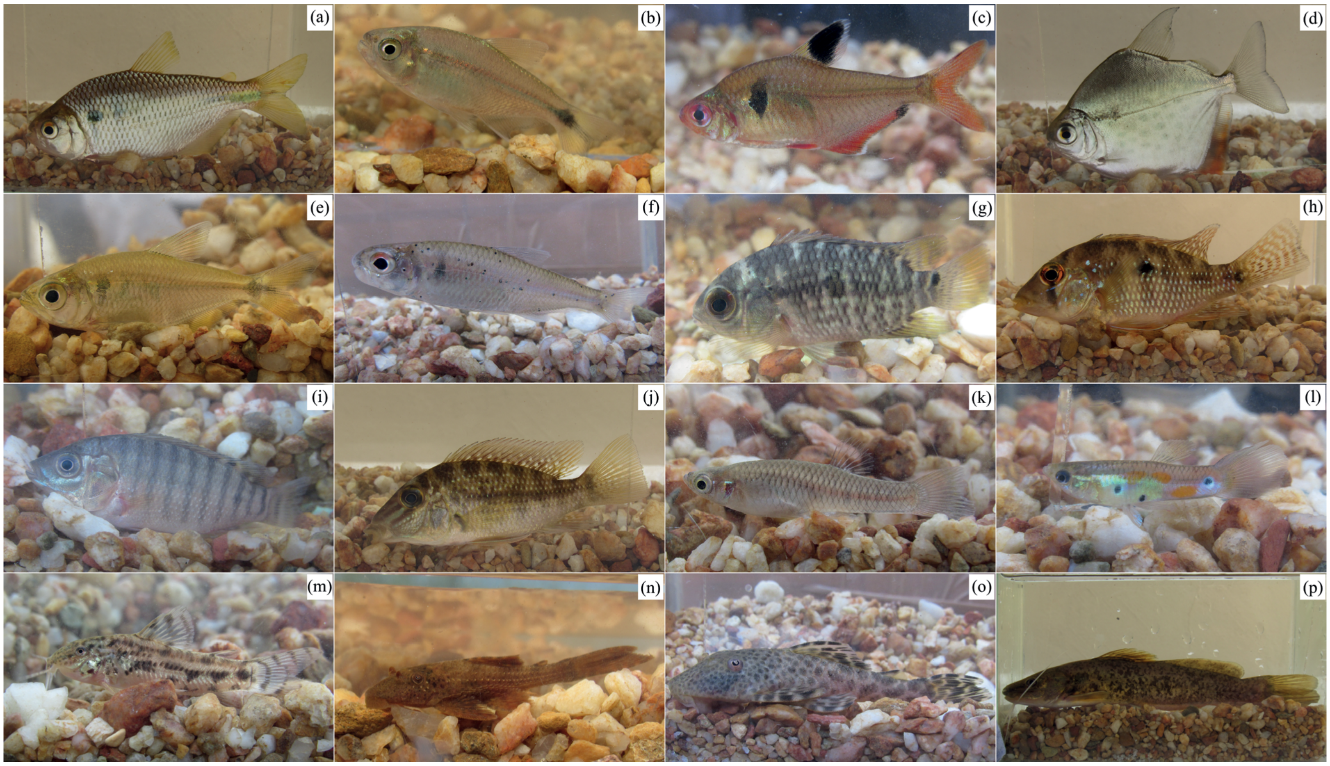

Figure 4. Live specimens representing several of the species collected in Ribeirão Frutal basin: (a) *Astyanax lacustris*, LBP 23616; (b) *Astyanax* sp., NUP 19125; (c) *Hyphessobrycon eques*, LBP 23604; (d) *Metynnis lippincottianus*, NUP 19142; (e) *Oligosarcus pintoi*, LBP 23603; (f) *Piabina argentea*, LBP 23612; (g) *Cichlasoma paranaense*, LBP 23605; (h) *Geophagus brasiliensis*, LBP 23621; (i) *Oreochromis niloticus*, LBP 23614; (j) *Satanoperca* sp., NUP 19143; (k) *Phalloceros harpagos*, UNT 016907; (l) *Poecilia reticulata*, LBP 23615; (m) *Aspidoras fuscoguttatus*, NUP 19127; (n) *Hypostomus ancistroides*, NUP 19130; (o) *Hypostomus nigromaculatus*, LBP 23608; (p) *Rhamdia quelen*, LBP 23622.

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Authors' Contributions

Valter M. Azevedo-Santos formulated the idea. Valter M. Azevedo-Santos and Paula N. Coelho performed the field work including collection of all the specimens. All authors collaborated with the identification, data analysis, and writing of the manuscript.

Conflicts of Interest

We, the authors, declare that we have no conflicts of interest related to the publication of this article.

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Thematic Review

A synopsis of fern galls in Brazil

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Abstract: Galls are neo-formed plant structures induced by species-specific interaction between an inducing organism and a host plant. Lycophytes and ferns are two distinct plant lineages historically lumped together as pteridophytes. A number of authors suggest low gall frequency in lycophytes and ferns, compared to angiosperms. This study aimed at compiling an updated overview of fern galls in Brazil, providing information on hosts, gall-inducing organisms and associated fauna. The synopsis was compiled using existent data and by updating scientific names and gathering new information obtained by the authors in fieldwork. To date, galls have been recorded on 16 fern species but none on lycophytes. However, the inducer was identified at species level in only three gall morphotypes from three fern species, with a doubtful inducer for *Pteridium* sp. Galls are induced by mites (Eriophyidae) and insects of the orders Diptera, Lepidoptera, Thysanoptera, and Hemiptera, Cecidomyiidae (Diptera) being the most frequent galler insect.

Keywords: *Pteridophytes, lycophytes, galls, Neotropical, Cecidomyiidae.*

Sinopse de galhas em samambaias no Brasil

Resumo: Galhas são estruturas vegetais neoformadas induzidas por interações espécie-específicas entre um organismo indutor e uma planta hospedeira. Licófitas e samambaias são duas linhagens de plantas historicamente classificadas juntas como pteridófitas. Diversos autores sugerem que há uma baixa frequência de galhas em licófitas e samambaias, em comparação com as angiospermas. Este trabalho tem como objetivo fornecer um panorama atualizado sobre as galhas em samambaias no Brasil, disponibilizando informações sobre espécies hospedeiras, galhadores e fauna associada. A sinopse foi realizada compilando-se os dados existentes, atualizando os nomes científicos e agregando informações novas obtidas pelos autores em trabalhos de campo. Até o momento, foram registradas galhas em 16 espécies de samambaias e nenhum registro em licófitas. Contudo, em apenas três morfotipos de galhas, de três espécies de samambaias, o indutor foi identificado a nível de espécie, com um indutor duvidoso para *Pteridium* sp. As galhas são induzidas por ácaros (Eriophyidae) e insetos das ordens: Diptera, Lepidoptera, Thysanoptera, Hemiptera, sendo Cecidomyiidae (Diptera) o galhador mais frequente.

Palavras-chave: *Pteridófitas, licófitas, galhas, Neotropical, Cecidomyiidae.*

Introduction

Lycophytes and ferns are two distinct plant lineages historically lumped together under various terms, such as “pteridophytes” or “ferns and allied plants” (Smith et al. 2006, PPG I 2016). Interactions between lycophytes, ferns and arthropods, especially in the Neotropical region, are poorly documented, little known and even neglected.

Galls, characteristic neo-formed plant structures created by hyperplasia and cellular hypertrophy that can occur in different plant organs, are generated by species-specific interactions between an inducing organism and a host plant (Mani, 1964; Isaias et al. 2013). While some authors claim that galls can be induced by bacteria, fungi, nematodes, mites and insects (Mani, 1964), others state that their induction is restricted to nematodes, insects and mites (Raman, 2007). Inducers establish a parasitic relationship and manipulate the metabolism of the host plant (Mani 1964, Raman, 2007).

A number of authors suggest low gall frequency in lycophytes and ferns, compared with angiosperms (Mehltreter et al. 2010). The chapter entitled Zoocecidium, published in 1938 in the Manual of Pteridology, is an important review of lycophyte and fern galls (Docters van Leeuwen 1938). Recently, for the Neotropical region, Hanson & Gómez-Laurito (2005) recorded 18 species of ferns with galls in Costa Rica.

The aim of this study was to perform an updated overview of fern galls in Brazil, providing information on host species, gall-inducing insects and associated fauna.

Material and Methods

The synopsis was compiled using existing data, and by updating scientific names and gathering new information obtained by the authors in fieldwork.

Data concerning lycophyte and fern galls were collected from historic reference studies (Houard 1933), in the catalogue of Cecidomyiidae of the world (Gagné & Jaschhof 2017); in 55 articles from inventories of insect galls in Brazil published between 1988 and 2017 and studies carried out exclusively with fern galls (Kraus et al. 1993, Martins & Pimenta 1988, Brown et al. 2004, Maia & Santos, 2011, 2015, Farias et al. 2018).

Lycophyte and fern nomenclatures were updated using the Flora do Brasil 2020 database and the PPG I classification system (2016).

Complementary data were obtained in fieldwork aimed at collecting fern galls in the Atlantic Forest in the following locations: Itatiaia National Park, Serra dos Órgãos National Park, Maricá Environmental Protection Area, Serra do Barbosão Municipal Nature Park (all in Rio de Janeiro state), Campos do Jordão (São Paulo state) and Tiradentes (Minas Gerais state). A total of 12 collections were carried out between 2011 and 2017. All the gall morphotypes were photographed, collected and taken to the laboratory where part of the sample was dissected, in order to obtain immature gall-inducing insects and determine the inducer taxon. Another part was packed in covered plastic pots, labeled and inspected daily until gall rot occurred, to obtain adult gall-inducing insects. Gall shapes were standardized in accordance with Isaias et al. (2013). All the biological material was deposited in the entomological collection of the National Museum (MNRJ) of the Federal University of Rio de Janeiro (UFRJ) and Herbarium of the Teacher Training Faculty (FFP) of Rio de Janeiro State University (UERJ).

Results and Discussion

The first records of fern galls in Brazil were made by Houard (1933). This author reported six gall morphotypes in five fern species (Table 1). In none of these the inducer was identified at a specific level, one gall was mite-induced (Eriophyidae) and five by the orders Diptera, Hemiptera and Thysanoptera. In *Niphidium crassifolium*, Houard (1933) found that the inducer is a Coccidae (Hemiptera); Maia & Mascarenhas (2017) also recorded globoid gall morphotype on the adaxial side of the leaves of this fern (Figues. 1 E-F). However, other insects, including inquilines (Psocoptera, Sciaridae: Diptera and *Corythaica cyathicollis* (Costa, 1864) Tingidae: Hemiptera), parasitoids (Platygastridae: Hymenoptera) and an unidentified Diptera larva (likely the galler) were found in recent field studies, which raises doubts about the true identity of the inducer. Some species of Platygastridae (Hymenoptera) parasitize Cecidomyiidae gallers (Johnson et al. 2013). Our group is conducting more detailed analyses in an attempt to clarify this issue.

According to Martins & Pimenta (1988), *Dolichophaonia gallicola* (Diptera, Muscidae) is the gall-inducing insect of *Pteridium aquilinum* (Table 1). However, Monteiro & Oda (1999) question whether this muscid is the gall inducer. These authors argue that the inducer is an inquiline and not a cecidogenous species, since it exhibits the buccal apparatus of a predator.

Kraus et al. (1993) conducted anatomical studies on stem galls of *Microgramma squamulosa* induced by a microlepidoptera (Gelechiidae: Lepidoptera). Later, Brown et al. (2004) described it as a new species: *Tortrimosaica polypodivora* (Tortricidae: Lepidoptera). (Figure 1L).

More recently, Maia & Santos (2011, 2015) recorded two stem gall morphotypes in *Microgramma vacciniifolia*, one ellipsoid-shaped (fusiform) induced by the microlepidoptera *Tortrimosaica polypodivora* (Figure 1A), and the other spherical (globoid) and induced by the Cecidomyiidae: Diptera *Primadiplosis microgrammae* (Figure 1B). Two species of parasitoid wasps (Hymenoptera) were also observed: Torymidae and Tetrastichinae (Eulophidae). In microlepidoptera-induced galls a parasitoid wasp Cheloninae (Braconidae) was also recorded, in addition to an inquiline species of *Dasineura* sp. (Cecidomyiidae: Diptera). In recent fieldwork, a new gall morphotype was recorded for species of *Microgramma squamulosa* and *M. vacciniifolia*. It is a conical and lenticular leaf gall respectively, with a white waxy layer at the opening on the abaxial side of the leaf, whose inducer remains undetermined (Figures I-J).

Maia et al. (2008) recorded a leaf gall on *Cyathea* sp., without identifying its inducer. In field studies conducted in Itatiaia National Park, a globoid leaf gall not yet identified, induced by Cecidomyiidae (Diptera), was found on *Cyathea dichromatolepis* (Figure 1C). A lenticular leaf gall induced by a new species of Cecidomyiidae was recorded on *Cyathea phalerata* in the Atlantic Forest of Pernambuco state, Brazil (Farias et al. 2018).

Leaf galls were recorded on *Pleopeltis hirsutissima* (Figure 1D), *Pleopeltis minima*, *Serpocaulon catharinae* and *Campyloneurum nitidum* (Figures 1G-H). In the first two species, the morphotype is globoid and induced by Cecidomyiidae (Diptera). In the third fern species the gall is conical and in the last lenticular, both with a white waxy layer at the opening on the abaxial side of the leaf. However, we were unable to identify the inducers. Witches' broom galls on leaves of *Dicranopteris flexuosa* was registered, probably induced by mites.

The leaves are the most frequently attacked organ and the shape of the gall is predominantly globoid. The galls are induced by mites (Eriophyidae) and insects of the orders Diptera, Lepidoptera, Thysanoptera and Hemiptera, Cecidomyiidae (Diptera) being the most frequent gall-inducing insect. In other parts of the world, there are records of fern galls induced by Hymenoptera (Balick et al. 1978, Houard 1908, 1933, Docters van Leeuwen, 1938, Bera et al. 2003) and Coleoptera (Docters van Leeuwen 1938, Bera et al. 2003).

There are no specific inventories for the lycophyte and fern galls of different ecosystems worldwide. The most important data are from Costa Rica, where galls were recorded on only 18 of the 1,120 fern species cataloged, that is a ratio of 0.016 (no. of ferns with galls/no. of ferns species) (Hanson & Gómez-Laurito 2005). In Brazil, galls have been recorded on 16 of the 1,144 fern species cataloged (Flora do Brasil 2020), a ratio of 0.013. However, the inducer was identified at species level in only three gall morphotypes from three fern species (Maia & Santos 2011, 2015). To date, no lycophyte species have been recorded as hosting galls in Brazil and Costa Rica. However, there are records of five Selaginellaceae species from India, New Guinea, Germany and the Malay Peninsula (Alston 1945, Docters van Leeuwen 1938, Mani 1964, Patra et al. 2009).

Table 1. Synopsis of fern galls in Brazil. MG: Minas Gerais state, RJ: Rio de Janeiro state, SP: São Paulo state.

Family	**Host species**	**Organ with gall**	**Gall shape**	**Gall-inducing insect**	**Location**	**Reference**
Blechnaceae	*Salpichlaena volubilis* (Kaulf.) J.Sm. *1	Leaf	Leaf roll	Eriophyidae	Not indicated	Houard (1933)
Cyatheaceae	*Cyathea dichromatolepis* (Fée) Domin	Leaf	Globoid	Cecidomyiidae (Diptera)	Itatiaia National Park, RJ	This publication
	Cyathea phalerata Mart.	Leaf	Lenticular	Cecidomyiidae (Diptera)	Pernambuco state	Farias et al. (2018)
	Cyathea sp.	Leaf	---	Not identified	Restinga de Bertioga State Park, SP	Maia et al. (2008)
Dennstaedtiaceae	*Pteridium* sp.*2	Leaf (Rachis)	Fusiform	*Dolichophaonia gallicola* (Albuquerque, 1958) (Diptera, Muscidae)*7	Belo Horizonte, MG	Martins & Pimenta (1988)
Gleicheniaceae	*Dicranopteris flexuosa* (Schrad.) Underw.	Leaf	“Witches’ broom”	Mite?	Tiradentes, MG	This publication
Hymenophyllaceae	*Hymenophyllum hirsutum* (L.) Sw.*3	Stem (Rhizome)	Globoid	Diptera	Not indicated	Houard (1933)
	Hymenophyllum pulchellum Schltdl. & Cham. *4	Stem (Rhizome)	Globoid	Diptera	Not indicated	Houard (1933)
		Leaf	Globoid	Diptera	Not indicated	Houard (1933)
Polypodiaceae	*Campyloneurum nitidum* (Kaulf.) C. Presl	Leaf	Lenticular	Not identified	Serra dos Órgãos National Park, RJ	This publication
	Microgramma squamulosa (Kaulf.) de la Sota	Stem (Creeping)	Fusiform	*Tortrimosaica polypodivora* Brown &. Baixeras, 2004 (Tortricidae, Lepidoptera)	São Paulo, SP	Kraus et al. (1993) Brown et al. (2004)
					Nova Friburgo, RJ	This publication
		Leaf	Conical	(Cecidomyiidae, Diptera)	Campos do Jordão, SP Nova Friburgo, RJ	This publication
	Microgramma vacciniifolia (Langsd. & Fisch.) Copel.	Stem (Creeping)	Globoid	*Primadiplosis microgrammae* Maia, 2011 (Cecidomyiidae, Diptera)	Maricá Environmental Protection Area, RJ	Maia & Santos (2011, 2015) Gagne & Jaschhof (2014)
		Stem (Creeping)	Fusiform	*Tortrimosaica polypodivora* (Tortricidae, Lepidoptera)	Maricá Environmental Protection Area, RJ	Maia & Santos (2015)
		Leaf	Lenticular	Cecidomyiidae, Diptera	Serra do Barbosão, RJ	This publication
	Niphidium crassifolium (L.) Lellinger *5	Leaf	Clavate	Coccidae (Hemiptera)	Not indicated	Houard (1933)
				Not identified	Itatiaia National Park, RJ	Maia & Mascarenhas (2017)
				Not identified (Diptera?)	Itatiaia National Park, RJ Serra dos Órgãos National Park, RJ	This publication
	Pleopeltis hirsutissima (Raddi) de la Sota	Leaf	Globoid	Cecidomyiidae, Diptera	Serra dos Órgãos, RJ	This publication
	Pleopeltis minima (Bory) J. Prado & R.Y. Hirai	Leaf	Globoid	Cecidomyiidae, Diptera	Niterói, RJ	This publication
	Serpocaulon catharinae (Langsd. & Fich.) A.R. Sm.	Leaf	Conical	Not identified	Serra dos Órgãos National Park, RJ Itatiaia National Park, RJ	This publication
					Minas Gerais, MG	Rosy Isaias (Personal communication)
	Serpocaulon sp. *6	Leaf	Lenticular	Thysanoptera*8	Not indicated	Houard (1933)

Name used in the original reference: *1-*Blechnum volubile* Kaulf.; *2-*Pteridium aquilinum* (L.) Kuhn; *3-*Hymenophyllum ciliatum* (Sw.) Sw.; *4-*Hymenophyllum lineare* Sw. var. *brasiliense* Rosenstock; *5-*Polypodium crassifolium* L. (em Houard 1933); *6-*Polypodium* sp.; *7-*Phaonia gallicola* Albuquerque, 1958; *8-*Thripsidae.*

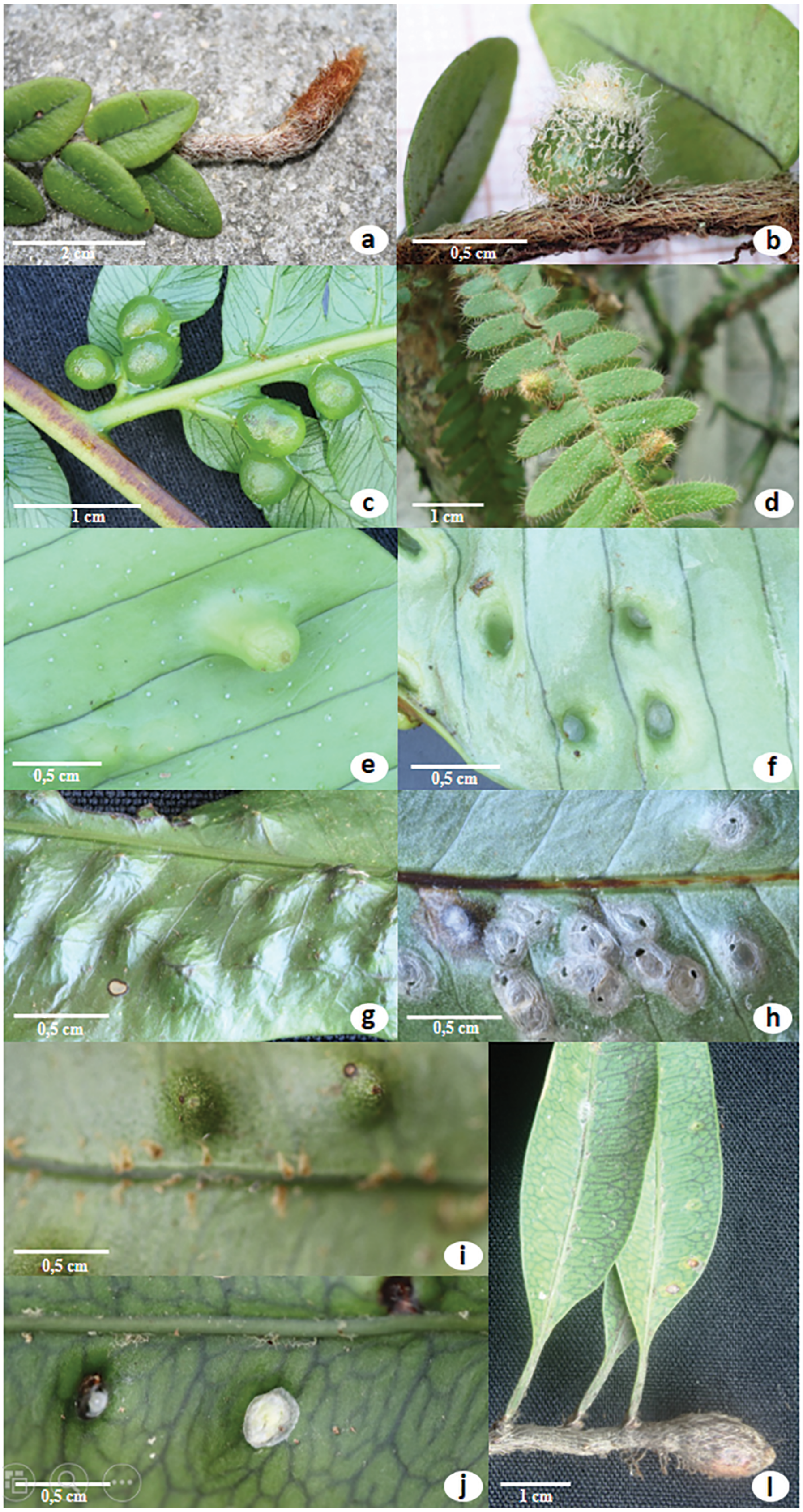

Figure 1. Fern galls in Brazil. a-b *Microgramma vacciniifolia*. a - Gall induced by *Tortrimosaica polypodivora* (Lepidoptera). b - Gall induced by *Primadiplosis microgrammae* (Cecidomyiidae-Diptera). c - *Cyathea dichromatolepis*. d - *Pleopeltis hirsutissima*. e-f *Niphidium crassifolium*, e - adaxial side of the leaf, f - abaxial side of the leaf. g-h *Campyloneurum nitidum*. g - adaxial side of the leaf, h - abaxial side of the leaf. i-l *Microgramma squamulosa*. i-j-gall induced by Cecidomyiidae(Diptera). i - adaxial side of the leaf, j - abaxial side of the leaf. l- gall induced by *Tortrimosaica polypodivora* (Lepidoptera). All photos by Marcelo Guerra Santos except photo “d” by Alene Ramos Rodrigues.

The estimated number of ferns worldwide is 10,578 species (PPG I). In line with the methodology used by Espírito-Santo & Fernandes (2007) and the data collected to date from Brazil and Costa Rica, we estimate an average of 153 fern species with galls worldwide. According to Espírito-Santo & Fernandes (2007), the Neotropical region is the least studied for galls, despite its substantial plant richness. As such, these values are likely underestimated.

Espírito-Santo & Fernandes (2007) suggest that for angiosperms there is a positive correlation between gall-inducing insects and plant richness. These authors also underscore the presence of "superhost plants", which may confirm a high richness of gall-inducing insects. In Brazil, the greatest gall richness was recorded for the genus *Cyathea*, with three species and the family Polypodiaceae, with 10 morphotypes in eight fern species. Three gall morphotypes were recorded in *Microgramma vacciniifolia* (Table 1).

The present synopsis systematized and broadened the records of fern galls in Brazil, thereby contributing to the knowledge of these interactions worldwide. The biological difficulties in obtaining adult insects precluded identifying their specific level or order. In some cases, the identitity of the galler remains unknown. However, the information presented here is important in designing future studies on the interactions between ferns and insects, especially those involving galls.

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Author's Contribution

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

Valéria Cid Maia: 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

Simple does not mean poor: grasslands and forests harbor similar ant species richness and distinct composition in highlands of southern Brazil

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Abstract: Several studies addressed ant communities in the dense Atlantic Forest that runs along the Brazilian coast. However, comparatively little is known about the mixed forests and grasslands that occur in the southern range of the Atlantic Forest domain. In this study we performed the first standardized assessment of ants in the forest-grassland mosaic found in the highlands of the state of Santa Catarina. We aimed to investigate and compare ant richness and composition between mixed forests and grasslands in the main mountain range of south Brazil. Ants were collected in two years with ground pitfalls, tree pitfalls and litter samples. Sixty ant species were recorded, resulting in 22 new records for "Planalto Serrano" region and three for the state of Santa Catarina: *Eurhopalothrix depressa*, *Pheidole radoszkowskii* and *Wasmannia williamsoni*. There was significant dissimilarity in ant species composition between grasslands and forests, but no difference in ant species richness, even considering the higher number of strata in mixed forests. Similar richness and low number of arboreal species suggest that this ant community is structured similarly to temperate ones. Both habitats presented a large proportion of exclusive species. The fact that species composition between grassland and forest areas differed, coupled with the similarity in species richness between habitats and the record of new ant species for the region, calls for strong conservation efforts in grasslands of southern Brazil, which still are little protected by conservation areas.

Keywords: *Formicidae, Atlantic Forest, Araucaria Forest, PPBio da Mata Atlântica, Eurhopalothrix depressa, Wasmannia williamsoni.*

Simples não significa pobre: campos e florestas abrigam riqueza similar e composições distintas de espécies de formiga em regiões altas do sul do Brasil

Resumo: Diversos estudos já foram realizados com comunidades de formigas nas regiões de Floresta Ombrófila Densa que ocorrem ao longo da costa do Brasil. Comparativamente menos é conhecido sobre as Florestas Ombrófilas Mistas e campos que ocorrem na parte meridional do domínio da Mata Atlântica. Neste estudo nós realizamos o primeiro levantamento padronizado de formigas no mosaico de florestas e campos que ocorre nas regiões altas de Santa Catarina. Nosso objetivo foi investigar e comparar a riqueza e composição de espécies entre florestas e campos na principal cordilheira do sul do Brasil. Formigas foram coletadas em dois anos com armadilhas de queda no solo e nas árvores, e com amostras de serapilheira. Sessenta espécies foram registradas, constituindo 22 novos registros para a região do Planalto Serrano e três para o estado de Santa Catarina: *Eurhopalothrix depressa*, *Pheidole radoszkowskii* e *Wasmannia williamsoni*. A composição de espécies diferiu significativamente entre florestas e campos, mas não houve diferença em riqueza de espécies, mesmo considerando o maior número de estratos nas florestas. Riqueza similar e pequeno número de espécies arbóreas sugere que esta comunidade é estruturada de modo semelhante às comunidades de regiões temperadas. Ambos os habitats apresentaram alta proporção de espécies exclusivas. O fato de que a composição foi diferente, somado à similaridade em riqueza de espécies entre habitats e ao registro de novas espécies, indica a necessidade de maiores esforços conservacionistas nos campos do Sul do Brasil, que ainda são pouco protegidos por unidades de conservação.

Palavras-chave: *Formicidae, Mata Atlântica, Floresta de Araucária, PPBio da Mata Atlântica, Eurhopalothrix depressa, Wasmannia williamsoni.*

Introduction

Brazil is one of the most biodiverse countries in the world, due to its high diversity of ecosystems, many containing a high number of endemic species (Lewinsohn & Prado 2003). The Atlantic Forest runs from northeast to south Brazil and its considered one of the world's eight main hotspots of biodiversity (Myers et al. 2000). It harbors a multitude of vegetation types under its domain, such as highland mixed forests on the southern border of its distribution (IBGE 2012). Highland mixed forests, or "*Araucaria* forests", are constituted by a mixture of tropical and temperate flora and are characterized by the occurrence of the endemic *Araucaria angustifolia* (Bertol.) Kuntze, one of the few gymnosperm trees native to Brazil (Gasper et al. 2013). However, in areas of high altitude, forests are sharply interrupted by grasslands patches, resulting in mosaics of variable habitat complexity (Klein 1978). Here, we define habitat complexity as the vertical variation in habitat physiognomy (August 1983). Forests can be considered habitats with higher degree of complexity in relation to grasslands, due to the presence of several strata (ground, litter, understory, canopy) and more heterogeneous distribution of abiotic conditions and resources.

Grasslands occurring in highlands of southern Brazil are considered a remnant of the Glacial, Early and Mid-Holocene period, when the cold weather favored grasslands instead of forests (Behling & Pillar 2007). Nowadays, forests have been slowly expanding over grasslands, but these persist because of a complex set of drivers (Silva & Anand 2011). Even though grasslands are considered important sources of regional biodiversity, they are frequently neglected on the conservation agenda (Overbeck et al. 2007, Overbeck et al. 2015). In addition, out of the few studies about conservation biology directed to grasslands in the highlands of southern Brazil, most have addressed the protection of endemic grassland plants (e.g. Overbeck et al. 2007). Less works called attention to animal species, which also occur as endemic in grasslands around the world (Bond & Parr 2010). Thus, surveys of arthropods richness and abundance can provide interesting data about the value of such grasslands for the conservation of animal diversity (Albuquerque & Diehl 2009, Azcárate & Peco 2012, Rosado et al. 2012, Diehl et al. 2014).

Ants are an important component of most terrestrial ecosystems, showing high richness and abundance, and displaying many ecological associations with plants and other animals (Ness et al. 2010). Because ant colonies are sessile, habitat structure is fundamental to determine food resources and availability of nesting sites (Blüthgen & Feldhaar 2010), which results in ants being highly dependent on microhabitat conditions. Indeed, this strong relationship imply that ant species composition can be dissimilar between distinct phytophysiognomies that co-occur at the same site, such as in forest-grassland mosaics (Gibb & Parr 2010).

Several studies addressed ant communities in the dense Atlantic Forest that runs along the Brazilian coast (a.k.a. Atlantic Forest *stricto sensu* or Atlantic Rainforest; e.g. Silva & Lopes 1997, Freitas et al. 2014, Silva & Brandão 2014). Comparatively little is known about highland mixed forests and grasslands (Ulysséa et al. 2011), which only recently started to be systematically investigated (Pinheiro et al. 2010, Dröse et al. 2017, Franco & Feitosa 2018). We contributed to fill this knowledge gap with the first standardized assessment on the forest-grassland mosaic found in the highlands of the state of Santa Catarina, as a part of the "Programa de Pesquisa em Biodiversidade (PPBio) da Mata Atlântica" (Atlantic Forest Research Program on Biodiversity). Additionally, we compared ant species composition and richness between mixed forest and grasslands patches, with the hypothesis that ant distribution would be affected by habitat structure, thus leading to distinct assemblages coexisting in this mosaic.

Methods

1. *Study Area*

Fieldwork was carried out in "Parque Nacional de São Joaquim (PNSJ)" (28°8'53.07"S and 49°36'34.61"O), a national park which lies on the "Serra Geral" formation, the main mountain range of southern Brazil. The geology of this region is a result of volcanic sheds of basaltic lava that started ~127 million years ago (Milani et al. 2007). The climate in the region is Cfb following Köppen's classification (Alvares et al. 2014), with mean annual precipitation ~1,400 mm and mean annual temperature ~14ºC (Fernandes & Omena 2015). On winter, temperatures commonly fall below 0ºC with occasional occurrence of snow (Nimer 1989).

The PNSJ has a total area of 49,300 ha and it is within the Atlantic Forest domain. Altitude of sampled plots ranged from 1,462 m to 1,671 m. Samples were collected in two vegetation types, namely mixed forest ("Floresta Ombrófila Mista Alto-Montana") and grassland ("Campos do Planalto"). Grasslands are predominantly covered by a dense layer of herbaceous stratum, with occasional shrub patches and small isolated trees. Mixed forests have a canopy height of ~8 m, with emergent *Araucaria angustifolia* trees typically reaching 13 m (Rafael Barbizan Sühs, personal communication).

2. *Field Procedures*

Two field campaigns were conducted during summer, one in March 2014 and other in February 2015. Sampling was carried out on a rectangular grid of 5 × 1 km with 10 plots, 1 km apart from each other (Figure S1 in Supplementary information). Each plot was 250 m long and followed the isocline of the ground. This design follows the RAPELD method for biodiversity surveys (Magnusson et al. 2005), implemented in the "PPBio Mata Atlântica". Five sampling points were established at each plot, 50 m apart from each other, totaling 50 sampling points. Nineteen sampling points were located in grasslands (n_g) and 31 in forests (n_f).

Ants were sampled with up to three methods, depending on the habitat structure of each sampling point (see Figure S2 in Supplementary information for detailed schematics). Ground pitfall traps were installed on all sampling points and retrieved after 48 hours. Each pitfall trap consisted on 300 ml plastic cups with diameter of 7.2 cm, buried at ground level, with a solution of water, detergent and salt as killing agent. Arboreal pitfalls followed the same design and were tied to trees at 1.5 m high, only in sampling points where there was at least one tree with more than 15 cm of diameter at breast height (DBH). Because of the low number of ants collected on arboreal pitfall traps in 2014, we added sardine baits to these traps in 2015. Additionally, in all sample points with accumulated leaf litter, we collected 1 m^2 of it, three meters away from the ground pitfall. The litter was sifted on the field and later placed into Winkler extractors for 48 hours. Tree pitfalls and litter samples were collected mainly in forests, but also in a few grassland points whenever isolated trees/litter were available. All collected material was stored in ethanol 95%.

3. *Laboratory Procedures*

For each sample, ants were initially sorted to morphospecies and at least one individual per morphospecies was mounted on paper triangles fixed in entomological pins. Ants were identified at genus level according to Palacio & Fernández (2003). Species were identified with taxonomic keys and further compared with reference collections and AntWeb images (AntWeb 2017). Genus and species names were updated with Bolton (2017). When no taxonomic resources were available, they remained as morphospecies. All species identifications were revised by taxonomists of the "Laboratório de Sistemática e Biologia de Formigas" (Universidade Federal do Paraná) (see Acknowledgements). Voucher specimens are deposited in the reference collection of the "Laboratório de Biologia de Formigas" (ECZ, Universidade Federal de Santa Catarina).

The sources used for species-level identification were: *Acromyrmex* – Gonçalves (1961); *Anochetus* – Fernández (2008); *Eurhopalothrix* – Longino (2013a); *Gnamptogenys* – Lattke et al. (2007); *Heteroponera* – Arias-Penna & Fernández (2008); *Linepithema* – Wild (2007); *Neivamyrmex* – Watkins (1976); *Octostruma* – Longino (2013b); *Oxyepoecus* – Albuquerque & Brandão (2004), Albuquerque & Brandão (2009); *Pachycondyla* – Fernández (2008); *Strumigenys* – Bolton (2000); *Trachymyrmex* – Mayhé-Nunes & Brandão (2005); *Wasmannia* – Longino & Fernández (2007).

New species records were checked against the most recent checklist for Santa Catarina (Ulysséa et al. 2011) and the literature published afterwards.

4. *Data Analysis*

Data from the two field campaigns were merged for each sample point. We excluded from quantitative analyses data from one plot that was relocated between years. Therefore, the effective number of sampling points used in the analyses was 45, 19 in grassland (n_g) and 26 in forest areas (n_f), including 45 ground pitfalls ($n_g = 19$, $n_f = 26$), 29 tree pitfalls ($n_g = 6$, $n_f = 23$) and 27 litter samples ($n_g = 1$, $n_f = 26$).

We calculated species frequency (number of records divided by total number of sampling points, calculated separately for each habitat) using presence-absence data, which is preferred to represent ant abundance (Longino 2000). To compare species richness between grassland and forest samples, we fitted curves of interpolation and extrapolation of Hill numbers to the split dataset, following the method developed by Chao et al. (2014) with order q = 0, which is the Hill number where there is zero weight for species abundances and thus stands for species richness. We built curves for ground pitfalls (the sampling method shared for all sampling points) and for all methods combined, which represents the sum of the strata available at each sample point. Interpolation and extrapolation curves were calculated with the R package "iNEXT" (Hsieh et al. 2016) and 95% confidence intervals were estimated based on 1000 permutations. Comparison of confidence intervals was used to test for species richness differences between vegetation types. We compared curves both within the observed range of data and after extrapolating up to 50 sampling units within each vegetation type. Interpolated curves are important to smooth sampling effects in unbalanced designs as ours, while extrapolation allows the use of all sample units, and point out the expected behavior of the curves if a larger proportion of the community were sampled (Colwell et al. 2012).

To visualize differences in ant species composition among sampling points, we ran a Principal Coordinate Analysis (PCoA), using the package "vegan" (Oksanen et al. 2016). The PCoA was based on Jaccard similarities, using only ground pitfall records. To test the differences in composition between the two habitats, we calculated a Generalized Linear Model for multivariate response data (GLM_{mv}) using the package "mvabund" (Wang et al. 2012). The GLM_{mv} allows for choosing a family for the distribution of residuals. For species presence-absence data, the best option is to use the binomial distribution because it accounts for data with both lower and upper boundaries. Choosing an adequate distribution has the advantage that the GLMmv avoids confounding differences in location (true differences in species composition) and dispersion (differences due to changes in beta diversity; Wang et al. 2012). Specifically, the GLM_{mv} has been shown to be more reliable to point out only location differences than traditional approaches, such as permutational multivariate analysis of variance – PERMANOVA, which can point out significant differences because of either location, dispersion, or even both patterns (Wang et al. 2012). Because GLM_{mv} are more specific in terms of what their results tell, we can also be more confident in answering more specific questions, here whether there are differences in species composition, not beta diversity, between grassland and forest. We analyzed all data in R, version 3.3.2 (R Core Team 2016).

Results

We recorded 60 ant species belonging to nine subfamilies and 23 genera. Our survey adds 22 new species to the "Planalto Serrano" region list, including three new to Santa Catarina territory: *Eurhopalothrix depressa*, *Pheidole radoszkowskii* and *Wasmannia williamsoni*. Forty-six species were sampled in forests and 34 in grasslands (Table 1). Twenty-six species were exclusive to forests (56% of the total for this vegetation), while 14 were exclusive to grasslands (41%). The most frequent species in mixed forest was *Heteroponera dentinodis* (relative frequency = 0.81), followed by *Oxyepoecus crassinodus* (0.73). In grassland, the most frequent species was *Pheidole* sp.5 (0.68), followed by *Camponotus rufipes* (0.53). Compared to ground pitfalls (40 spp.) and litter samples (42 spp.), tree pitfalls collected fewer species (11 spp.), and only two species were exclusively recorded by this method.

The interpolation curves based only on ground pitfalls showed a higher number of observed species in grasslands (Figure 1). However, with extrapolation, the curves tend to overlap, suggesting no actual difference between vegetation types. This richness equivalence is highlighted when we added data from leaf litter and tree pitfalls. With pooled data from all strata, the curves were similar and confidence intervals largely overlapped (Figure 2).

The GLM_{mv} results showed a difference in species composition between grasslands and forests (Wald-value = 4.159; p = 0.034). Most sampling points were separated in two groups corresponding to the two habitats, although there were intermediate sites that shared species from both habitats (Figure 3).

Table 1. Ant species recorded in "Parque Nacional de São Joaquim", southern Brazil. Frequencies represent number of records relative to number of sample points for each habitat (grasslands = 19, forest = 26). For methods, it is indicated whether the species was recorded or not with the method.

Species	**Habitat**		**Method**		
	Grassland	**Forest**	**GP**	**TP**	**LL**
Acromyrmex crassispinus (Forel, 1909)		0.27	+		+
Anochetus altisquamis Mayr, 1887		0.08	+		+
Brachymyrmex sp.1		0.19	+		+
Brachymyrmex sp.2	0.11	0.12	+		+
Camponotus alboannulatus† Mayr, 1887		0.12		+	+
Camponotus crassus† Mayr, 1862	0.16		+		
Camponotus fastigatus† Roger, 1863	0.32		+		
Camponotus melanoticus† Emery, 1894	0.21	0.12	+	+	+
Camponotus rufipes (Fabricius, 1775)	0.53	0.35	+	+	+
Camponotus sp.	0.05			+	
Crematogaster sp.1		0.12			+
Crematogaster sp.2		0.08		+	+
Crematogaster sp.3	0.11		+		
Crematogaster sp.4	0.05			+	
Crematogaster sp.5		X			+
Discothyrea sp.		0.04			+
Eurhopalothrix depressa‡ Ketterl, Verhaagh e Dietz, 2004		0.08			+
Gnamptogenys striatula† Mayr, 1884	0.42		+		
Heteroponera dentinodis† (Mayr, 1887)		0.81	+		+
Hypoponera sp.1		0.69	+		+
Hypoponera sp.2	0.05	0.27			+
Hypoponera sp.3		0.19			+
Linepithema angulatum† (Emery, 1894)	0.11		+		
Linepithema gallardoi† (Brèthes, 1914)	0.37	0.23	+		+
Linepithema micans (Forel, 1908)	0.21	0.27	+		+
Myrmelachista gallicola† Mayr, 1887		0.04			+
Neivamyrmex hetschkoi† (Mayr, 1886)		X			+
Neivamyrmex punctaticeps† (Emery, 1894)		X			+
Nylanderia sp.		0.08	+		+
Octostruma rugifera† (Mayr, 1887)		0.04			+
Oxyepoecus crassinodus† Kempf, 1974		0.73	+	+	+
Oxyepoecus plaumanni† Kempf, 1974		0.04			+
Oxyepoecus vezenyii† (Forel, 1907)		0.31			+
Pachycondyla striata Smith, 1858	0.26	0.08	+		
Pheidole aberrans† Mayr, 1868	0.05		+		
Pheidole avia† Forel, 1908		X			+
Pheidole radoszkowskii‡ Mayr, 1884	0.21		+		
Pheidole rosae† Forel, 1901	0.21	0.04	+		+
Pheidole aff. *lutzi*	0.11	0.04	+	+	
Pheidole sp.1		0.31	+		+
Pheidole sp.3	0.16	0.35	+		+
Pheidole sp.4		0.12	+		+
Pheidole sp.5	0.68	0.23	+		+

Continued Table 1.

Species	Habitat		Method		
	Grassland	Forest	GP	TP	LL
Pheidole sp.9	0.05	0.04	+		
Pheidole sp.11	0.16	0.08	+		+
Pseudomyrmex termitarius (Smith, 1885)		0.12		+	+
Solenopsis sp.1	0.16	0.69	+		+
Solenopsis sp.2	0.16	0.12	+		+
Solenopsis sp.4	0.05		+		
Solenopsis sp.5		X			+
Solenopsis sp.6		0.04		+	
Solenopsis sp.7	0.16	0.08	+	+	+
Solenopsis sp.8	0.05		+		
Strumigenys louisianae† Roger, 1863	0.05		+		
Strumigenys aff. *louisianae*	0.11	0.04	+		+
Trachymyrmex holmgreni† Wheeler, 1925	0.05		+		
Trachymyrmex sp.	0.05		+		
Wasmannia affinis† Santschi, 1929	0.05	0.65	+		+
Wasmannia auropunctata† (Roger, 1863)	0.11	0.04	+		+
Wasmannia williamsoni‡ Kusnezov, 1952	0.16	0.04	+		+
TOTAL	34	46	40	11	42

X = the species was recorded only on the plot removed from quantitative analyses. GP = ground pitfall traps. TP = tree pitfall traps. LL = leaf-litter samples. † = new records for the Planalto Serrano region. ‡ = new records for the state of Santa Catarina.

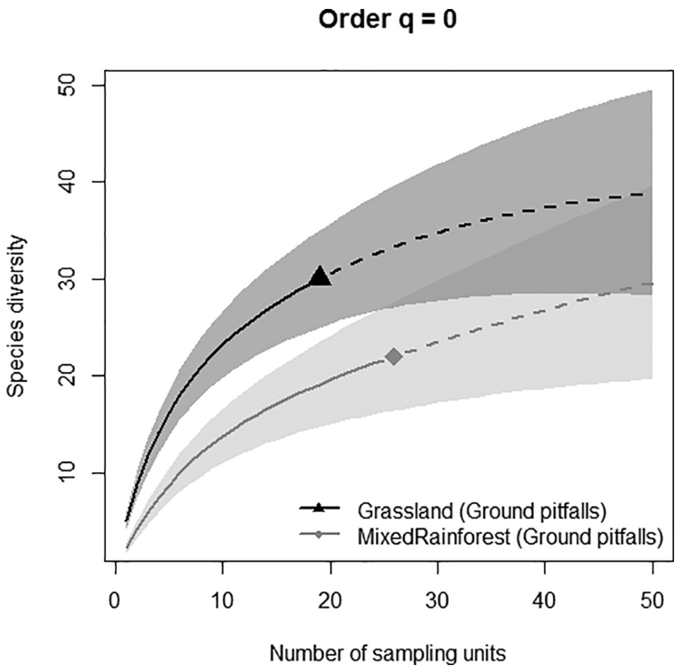

Figure 1. Interpolation, extrapolation and 95% confidence intervals of species richness in grassland and forest, using data from ground pitfall traps. Interpolation – solid lines; extrapolation – dashed lines.

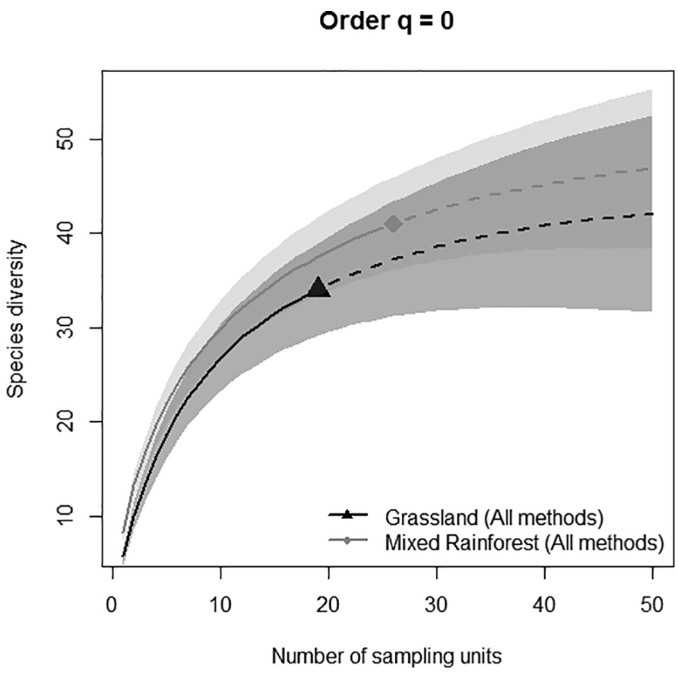

Figure 2. Interpolation, extrapolation and 95% confidence intervals of species richness in grassland and forest, using pooled data from all sampling methods. Interpolation – solid lines; extrapolation – dashed lines.

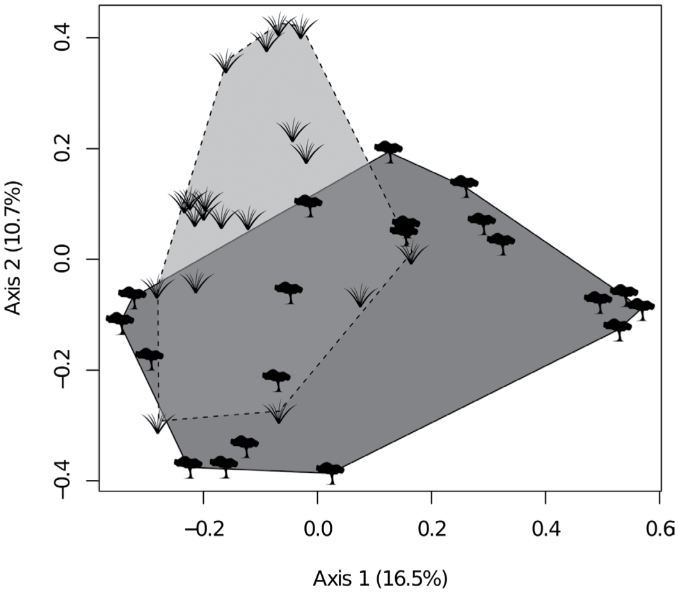

Figure 3. Principal Coordinate Analysis showing the dissimilarity of ant species composition between sites on grasslands or forests using data from ground pitfalls. Lines indicate convex hulls encompassing sites from each type of habitat (forests – solid line; grasslands – dashed line).

Discussion

1. New regional records

Santa Catarina is one of the few Brazilian states with a recent checklist of ant species recorded in its territory (Ulysséa et al. 2011). The "Planalto Serrano" region, where the PNSJ is located, was deemed as a research priority, with only 12 species recorded at the time (Ulysséa et al. 2011). The great number of new ant species in "Planalto Serrano" recorded here mainly is a result of this lack of knowledge, but also can be related to the complementarity of our sampling effort. The combination of pitfalls and leaf litter collection on the ground is strongly advised for biodiversity assessments (Alonso & Agosti 2000, Bestelmeyer et al. 2000, King & Porter 2005, Lopes & Vasconcelos 2008), since the first method catch larger and more active species (Sabu et al. 2011), while the second is efficient to sample smaller and less active ants, including cryptic species (Fisher 1999). Finally, our sampling effort in grasslands sites, a poorly surveyed physiognomy until now in Santa Catarina, is another important source of those new records, a trend that is increasingly highlighted in other grasslands surveys of South Brazil recently published (Pinheiro et al. 2010, Rosado et al. 2012, Dröse et al. 2017, Franco & Feitosa 2018).

With regard to the new records for the state of Santa Catarina, this is only the third known record for *Eurhopalothrix depressa*, previously found once in mixed forest, further south of our location, and once in dense Atlantic Forest (Ketterl et al. 2004). Considered a highly distinctive species of the genus (Longino 2013a), these few records suggest rarity and endemic distribution inside the Atlantic Forest domain. Little information is available about its biology, but most representatives of the genus are predators inside the leaf litter, where they are more often recorded (Longino 2013a). *Wasmannia williamsoni* is considered a grassland-associated species (Cuezzo et al. 2015), and indeed we found it more often in this vegetation. Although the previous authors suggested it to be a relict endemic lineage of the central region of Argentina, it was found in the Pampa region of southern Brazil (Rosado et al. 2012), and our record expands its distribution by 600 km further north. Finally, *Pheidole radoszkowskii* is a widespread species – or species complex – found all over the Neotropical region (Wilson 2003). Contrary to *E. depressa*, this record is likely result of the taxonomic uncertainty associated with most species of *Pheidole*.

2. Species richness and composition

A positive relationship between species richness and habitat complexity often is found in tropical and subtropical ant communities (Majer et al. 1997, Vasconcelos & Vilhena 2006, but see Lassau & Hochuli 2004). In lowland forests with higher canopy and strong presence of epiphytes, arboreal ants largely contribute to forest richness (Vasconcelos & Vilhena 2006, Neves et al. 2013). However, this relationship might be found even when only the ground stratum is considered (Andersen 1986). The ground of a forest can be considered more complex and heterogeneous (Farji-Brener et al. 2004) than a grassland because of a litter layer with variable depth, more heterogeneous distribution of plants in the understory, and availability of tree-related resources (e.g. fallen fruits). In our study, ant richness was similar between habitats. This similarity was observed both when we compared pitfalls, and when we added data from other strata, which are characteristic of forests (leaf-litter, vegetation). A shift on the complexity-richness trend is common in temperate latitudes or high altitudes, where ant richness decline in shaded forests and increase in open habitats (Seifert 2007, Longino et al. 2014). Ants are thermophilic animals (Pie 2016) and, below a certain threshold, less species are able to tolerate low insolation and temperature inside forests. Even considering only grasslands, a decrease in species richness might be observed with altitude (Dröse et al. 2017). Thus, although most species we found also occur in dense Atlantic Forest, the community is organized differently, and some species change their behavior accordingly. This might be the case for *Gnamptogenys striatula* and *Pachycondyla striata*, two species very common inside lowland forests (Lattke 1995, Rosumek et al. 2008, Medeiros & Oliveira 2009), which shifted their occurrence mainly to open areas in our study site.

A similar environmental effect reduces richness and abundance of arboreal-nesting ants in temperate habitats (Benson & Harada 1988, Blüthgen & Feldhaar 2010). Arboreal nests are more subject to temperature fluctuations along the year, particularly the harsh winter, which few species are able to withstand. In our site, arboreal pitfalls had low effect in total richness. In the mixed forest, only two species were found exclusively in arboreal pitfalls, all others also occurring on the ground. With pitfalls located at 1.5 m high, it is expected to find several ground species which climb up trees to forage. However, the low efficiency of the pitfalls and lack of exclusive species suggests a relatively small arboreal community. In grasslands, it was also observed that few species occur exclusively in vegetation (Dröse et al. 2017). Most plants in this habitat do not provide proper nesting sites above the ground, and these would be even more affected by cold temperatures.

While richness was similar, our results indicate that grassland and forest habitats maintain different ant assemblages. This division was confirmed by the GLM_{mv}, although it was not very strong (see axis values in Figure 3) and composition overlapped at many points. Two-thirds of the species were exclusive to one habitat, which accounted for about half of the total for each habitat. This finding agrees with the idea that ants have close relationships with vegetation structure, and, consequently, with microhabitat conditions and resources available, such as nesting sites and nutritional supplies (Rico-Gray & Oliveira 2007, Blüthgen & Feldhaar 2010, Gibb & Parr 2010). Indeed, most studies comparing ant species composition between different ecosystems found great dissimilarities (Marques & Del-Claro 2006, Vasconcelos & Vilhena 2006, Vasconcelos et al. 2008, Groc et al. 2014).

3. *Concluding remarks*

Here we assessed the ant community in highlands of the state of Santa Catarina, and showed that grasslands and forests harbor similar richness, yet distinct ant species composition. This work joins recent studies that assessed ant communities in the grassland/mixed forest mosaics of the southern Atlantic forest (Pinheiro et al. 2010, Dröse et al. 2017, Franco & Feitosa 2018). Our results provide insights on the drivers of community organization, and suggest that the combination of relatively high latitude and altitude gives to the ant community some features of temperate habitats, thus distinct from the dense Atlantic Forest and from most Brazilian ecosystems. Such patterns might be context-dependent, and more factors could be involved in the distribution of the biodiversity in this ecosystem, e.g. edge effects (Pinheiro et al. 2010). Nevertheless, we suggest that these grasslands and forests are equally relevant for biodiversity conservation. We call special attention to grasslands, because they are frequently neglected in conservation programs (Overbeck et al. 2015), a conclusion shared by the aforementioned recent studies in the same ecosystem. While grasslands occupy nearly 13.7 million ha in Brazilian territory, less than 0.5% of the ecosystem is within protected areas (Overbeck et al. 2007). As highlighted by some authors, managed grasslands lead to different plant and ant assemblages, and more research is necessary to effectively protect the biodiversity linked with those habitats (Azcárate & Peco 2012). Even though the issues in conservation status of plant species have been raised for over a decade (Overbeck et al. 2007), more recently attention has also been called for arthropod diversity in similar ecosystems around the world (Littlewood et al. 2012).

Supplementary material

The following online material is available for this article:

Figure S1 - Map of the sample grid established in "Parque Nacional de São Joaquim". The rectangular line shows the main trails of the 5x1 km grid. Red lines indicate 250 m plots, which follow the isocline of the ground. The thick yellow line shows the limits of the protected area.

Figure S2 - Schematic representation of the sample design.

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Author Contributions

Cristian Luan Klunk: Contribution to data collection; contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Eduardo Luís Hettwer Giehl: Contribution to data analysis and interpretation; contribution to manuscript preparation; contribution to critical revision, adding intellectual content.

Benedito Cortês Lopes: Substantial contribution in the concept and design of the study; contribution to data analysis and interpretation; contribution to critical revision, adding intellectual content.

Frederico Rottgers Marcineiro: Contribution to data collection; contribution to critical revision, adding intellectual content.

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

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

Size-related and seasonal changes in the diet of the non-native *Cichla kelberi* Kullander & Ferreira, 2006 in a lowland reservoir in the southeastern Brazil

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Abstract: We examined size-related and seasonal changes in the diet of the peacock bass *Cichla kelberi* in a tropical lowland reservoir in southeastern Brazil over three hydro-climatic seasons: summer (high rainfall and temperature), winter (low rainfall and temperature), and late spring (increasing rainfall and temperature) during two years (2006-2007). The tested hypothesis is that this non-native predator fish changes diet during the subadult and adult phases and among seasons to adapt in new colonized environment. Fishes of the families Clupeidae (*Platanichthys platana*), Characidae (*Astyanax* spp.) and Cichlidae (*Cichla kelberi*) were the most important food items, followed by insects of the order Odonata. Cannibalism was also recorded for the largest individuals. A significant size-related change in diet was found with the smallest individuals (Total Length, TL < 20 cm) preying mainly on fishes, whereas the larger individuals (TL > 30 cm) preyed mainly on Odonata. The niche breadth increased during growth, with the largest individuals having a diet more evenly distributed among the available resources. No significant seasonal differences in diet composition were found, but stomachs with higher degree of volume occupied by food were more frequent in late spring than in summer. Conversely, the highest niche breadth was found during the summer compared to the other seasons. Together, these observations suggest an efficient use of the available resources by this top predator in this new colonized system.

Keywords: *Feeding ecology, freshwater fishes, non-native species, piscivory, impoundment.*

Mudanças sazonais e relacionadas ao tamanho da dieta do não-nativo *Cichla kelberi* Kullander & Ferreira, 2006 em um reservatório de planície no sudeste do Brasil

Resumo: Foram examinadas as mudanças sazonais na dieta e relacionadas ao tamanho para o tucunaré *Cichla kelberi* em um reservatório tropical de várzeas no Sudeste do Brasil em três ciclos hidrológicos: verão (elevadas precipitações e temperaturas), inverno (baixas precipitações e temperaturas), e final da primavera (aumentos das precipitações e temperaturas) durante dois anos (2006-2007). A hipótese testada é que este peixe predador não-nativo muda a dieta durante as fases adulta e subadulta e entre as estações do ano para se adaptar neste novo ambiente colonizado. Peixes das famílias Clupeidae (*Platanichthys platana*), (Characidae (*Astyanax* spp.) e Cichlidae (*Cichla kelberi*) foram os itens alimentares mais importantes, seguidos por insetos da ordem Odonata. O canibalismo também foi registrado para os maiores indivíduos. Mudanças significativas na dieta foram relacionadas ao tamanho, com os indivíduos menores (Comprimento Total, CT <20 cm) utilizando principalmente peixes, enquanto os indivíduos maiores (CT >20 cm) se alimentaram principalmente de Odonata. A amplitude de nicho aumentou ao longo do crescimento, com indivíduos de maior porte tendo dieta mais uniformemente distribuída entre os recursos disponíveis. Nenhuma diferença significativa na composição da dieta foi encontrada entre as estações do ano, mas estômagos com maiores volumes ocupados pelo alimento foram mais frequentes no fim da primavera e menos frequentes no verão. Por outro lado, a maior amplitude de nicho foi encontrada no verão comparada com as outras estações, o que indica o uso mais uniforme dos diversos recursos disponíveis. Juntas, estas observações sugerem uma utilização eficiente dos recursos disponíveis por esta espécie predadora de topo neste novo sistema colonizado.

Palavras-chave: *Ecologia alimentar, peixes de água doce, espécies não nativas, piscívora, represamento.*

Introduction

Many rivers in southeastern Brazil have undergone considerable changes due to the construction of dams. The artificial lakes fragment the lotic system, blocking routes for fish migration and forming systems with new environmental conditions (Agostinho et al. 2008). As a result, the natural structure of the fish assemblages changes in the new environment favoring the establishment of non-native fish species. The non-native species are, in many cases, more tolerant to the lentic conditions than the previously native lotic fish fauna (Hoeinghaus et al. 2009, Araújo et al. 2013).

Knowing the ecology of invasive species is crucial to help environmental managers in policies aiming the biodiversity conservation (Gozlan 2008). Trophic plasticity is a fundamental condition for species to adapt to a newly created lentic environment such as the reservoirs. Introduced species can affect the functioning of ecosystems by changing trophic relationships, besides presenting other deleterious effects (Adams 1991, Meffe et al. 1997, Marchetti 1999, Kati et al. 2015). These changes may have important economic and ecological consequences (Gomiero & Braga 2003, Fugi et al. 2008, Pelicice & Agostinho 2009). Native species may change their abundance due to competition with non-native species (Zaret & Paine 1973, Lodge 1993, Khan & Panikkar 2009, Kati et al. 2015). On the other hand, non-native species can alter the functioning of local ecosystems by intensifying predation pressure on native species, or by searching for other prey that are not used by native predators (Zavaleta et al. 2001, Laxson et al. 2003).

Cichla species have successfully invaded Neotropical reservoirs and this may seriously threaten native fish diversity due to their high predatory capacity (e.g., Santos et al. 2001, Latini & Petrere, 2004, Novaes et al. 2004, Fugi et al. 2008. Pinto-Coelho et al. 2008, Kolavensko et al. 2010, Menezes et al. 2012, Pelicice et al. 2015). Species of *Cichla* are able to change their diets according to resources availability, usually related to seasonal and ontogenetic variations (Gomiero et al. 2010). Seasonal changes in the fish diet associated to changes in water temperature and photoperiod also influence food availability (Zavala-Camin 1996). Moreover, in environments where species of *Cichla* have been introduced, high cannibalism rates can occur (Santos et al. 1994, Durães et al. 2000, Gomiero & Braga 2004b).

The peacock bass *Cichla kelberi* Kullander and Ferreira 2006 is native and endemic to the Araguaia and Tocantins Rivers basins (Kullander & Ferreira 2006) but has been introduced in several aquatic systems across the country. This species has been introduced in Brazilian reservoirs since the 1950s by hydroelectric companies and by amateur anglers and has colonized large areas of river basins (Oliveira et al. 1986). The wide distribution and high abundance of *C. kelberi* in Brazilian reservoirs suggests a high impact on the native fish communities and other aquatic organisms (Magalhães et al. 1996, Câmara et al. 2002, Chellapa et al. 2003, Gomiero & Braga 2003, Fugi et al. 2008, Santos et al. 2011, Pelicice et al. 2015). It is a top predator with piscivorous habits (Lowe McConnel 1969, Braga 1990, Arcifa & Meschiatti 1993, Jepsen et al. 1997, Novaes et al. 2004, Pelicice & Agostinho 2009, Montaña 2011), favored by its accurate visual capacity, preying largely on small fishes but occasionally consuming shrimps and other aquatic invertebrates when adults (Winemiller 2001, Kullander & Ferreira 2006, Santos et al. 2011). As most successful invaders, this species must be able to adapt to novel environmental conditions that include shifting feeding habits to explore the available resources. It is widely accepted that species of *Cichla* change diet during growth, with the juveniles feeding mainly on crustaceans and insects whereas the adults fed mainly on fishes (Gomiero & Braga 2004a, Novaes et al. 2004).

Cichla kelberi was introduced in the Juturnaíba Reservoir, a tropical impoundment in the north of Rio de Janeiro State, shortly after the reservoir construction, about three decades ago. This study aimed to assess dietary preference of *Cichla kelberi* and eventual changes among seasons and size-classes in the Juturnaíba Reservoir. Knowing the diet of *C. kelberi* from gut analyses, although may reflect more on prey availability than preference towards a particular prey species, is one of the first step to assess their success in the new colonized system. We hypothesized that there are size-related and seasonal changes in the diet of *Cichla kelberi* in the Juturnaíba Reservoir, which may be a mechanism that favor the use of available resources.

Material and Methods

1. Studied area

The dam on the São João River that formed the Juturnaíba Reservoir (22º36'S, 42º 16'W) is located at 65 km from the river headwaters and 38.5 km from its mouth in the Atlantic Ocean (Fig. 1). The reservoir has approximately 30.6 km^2 of water surface, a maximum depth of 9 meters and water volume of 100 million m^3 (Afonso & Cunha 1989). The reservoir has an average depth of 4 meters and is located in lowland areas of northeast floodplains of Rio de Janeiro State. We defined three instead of four seasons because changes in the major factors of temperature and rainfall reflect better three and not four seasonal periods: (i) summer, with the highest temperatures and rainfalls; (ii) winter, when environmental conditions are stable, characterized by the lowest temperature and rainfall; (iii) late spring, when the area experiences changes in environmental variables, with strong southwestern winds coinciding with increases in rainfall and temperature. Such seasons were already established in other studies (e.g. Azevedo et al. 2016) and the database supporting this pattern is available from the Instituto Nacional de Meteorologia (INMET), available at http://www.inmet.gov.br/html/clima.php#.

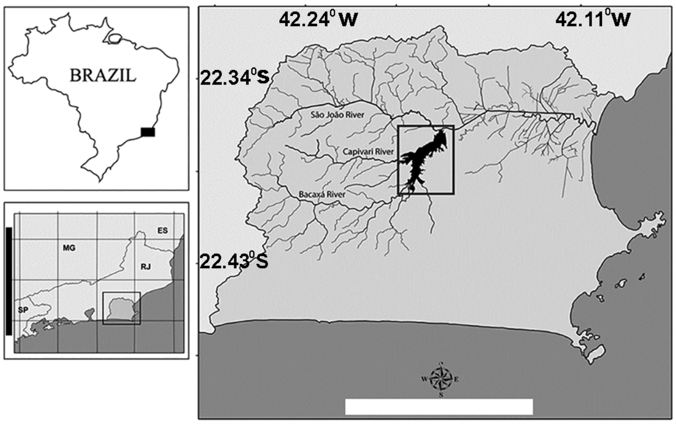

Figure 1. Map showing the study area, the Juturnaíba Reservoir and its drainage area, Rio de Janeiro, Brazil.

2. *Sampling and data handling*

Fishes were sampled in March (summer), August (winter) and December (late spring) during two years (2006 and 2007), covering two full hydro-climatic cycles. Information from each year were pooled by seasons, with years being used as replications of the seasons. At each season and each year, excursions were carried out at 7 sites randomly chosen covering most area of the reservoir, totaling 42 samples (7 sites × 3 seasons × 2 years). Seven gill nets (30 m long × 3 m high) of different mesh size (2-7 cm stretched mesh) at each sampling site were used. The nets were set up at sunrise and retrieved the following afternoon, remaining in operation for ca. 20 h. All fish were identified, measured for total length (TL, in centimeters) and weighed (in grams). The stomachs were removed and fixed in 10% formalin, and after 48 hours transferred to 70% ethanol.

For each individual, the degree of fullness in the stomach (SF) was measured and classified according to the following categories: SF-0 (empty stomach), SF-1 (< 30% of stomach volume occupied by food); SF-2 (between 30 and 70%); and SF-3 (above 70%).

The food items were identified under a stereomicroscope. Fish with empty stomachs or those with unidentifiable contents were excluded from the analyses. The volume of each identified food item was measured according to Albrecht & Caramaschi (2003). The volume and the frequency of occurrence of the food items were used to calculate the Alimentary Index (AI) proposed by Kawakami & Vazzoler (1980) according to the following equation: $IAi = \%Fi \times \%Vi / \Sigma (\%Fi \times \%Vi)$, where: IAi = Index Feeding; %Fi = Frequency of occurrence of the item i in the diet (%); V = Volume of the item i in the diet (%).

3. *Data analyses*

The stomach contents of individuals were analyzed covering the three periods (March, August and December 2006 and 2007). To assess possible changes in diet with respect to size, fish were divided into three size classes that were assigned according to observed size ranges: Total Length (TL) TL1< 20 cm; TL2 = 20-30 cm; TL3 > 30.0 cm. Although these size classes do not encompass the juveniles (TL < 10 cm, according to Novaes et al. 2004) at smaller lengths, they included the subadult and adult, which are the focus of the present study.

The volume of the food items for each sample was square root transformed and converted into a triangular matrix of similarities among all samples, using the Bray–Curtis similarity measure (Schafer et al. 2002). The diet composition was compared among seasons (three seasons) and size class (three size classes) that were the fixed factors. We used ANOSIM procedure at a significance level of $P < 0.05$ to compare the diet composition among seasons and size classes. Theses analyses were performed with the PRIMER software package version 6.0+ PERMANOVA (Anderson et al. 2008). The volumetric data obtained for the food items were used to calculate the standardized Levin's index as a measure of the niche breadth for each season and size class.

Results

A total of 125 specimens of *C. kelberi* were examined from which 98 had stomachs with identifiable food items and 27 were empty. A total of 34 individuals were examined in summer, 38 in winter and 26 in late spring. The specimens examined had sizes ranging from 9.7 to 48.1 cm Total Length (TL), with 36 individuals in size class TL1 (< 20 cm TL), 45 individuals in size class TL2 (20-30 cm TL) and 17 individuals in size class TL3 (> 30 cm TL).

The diet consisted mainly of fishes and insects (Table 1). During the summer, the most important food items was fish remains (IAi =36.15%) and juveniles of *Cichla kelberi* (IAi =22.39%). During the winter, the most important food item was Odonata (IAi =52.15%) followed by characid fishes *Astyanax* spp. (IAi = 34.63%), whereas in the late spring, Odonata (IAi=55.86%), followed by fish remains (IAi=24.19%) and the clupeid *Platanichthys platana* (Regan, 1917) (IAi=18.18%) were the main food items (Table 1). The Cichlidae family was represented by *C. kelberi,* indicating cannibalism habit. No significant seasonal differences were found in the diet composition (R global= 0.022; P = 0.132) according to the ANOSIM test.

The frequency of stomachs with lower than 30% of fullness (SF-1) and with 30-70% of fullness (SF-2) was higher in late spring and lower in summer (Fig. 2). Conversely, the frequency of empty stomachs (SF-0) was higher in summer and lower in late spring. No seasonal differences were found in the frequency of stomachs with higher than 70% of fullness (SF-3).

The diet composition changed significantly (R global= 0.286; P = 0.03) among the three size classes according to the ANOSIM (Fig. 3). The smallest individuals (TL1) fed mainly on fish remains and fishes from the family Clupeidae, whereas the medium-sized individuals (TL2) fed preferably on fish remains and *Platanichthys platana*. The largest individuals (TL3) fed on Odonata and fishes from the Characidae family. Cannibalism was recorded mainly in the largest individuals that fed mainly on juveniles of *C. kelberi*.

Niche breadth was low, with comparatively higher values in summer (0.35) and lower in winter (0.21) and late spring (0.14). There was a trend of increasing niche breadth as fish grew, with lower values (0.12) in the sizes smaller than 20 cm (TL1), then increases to 0.21 in the 20-30 cm TL size class (TL2), reaching the highest value (0.24) at sizes larger than 30 cm (TL3).

Discussion

The non-native *Cichla kelberi* seems to adapt its trophic niche to use a broad spectrum of the available resources in the Juturnaíba Reservoir. It is noticeable its opportunist carnivore strategy by using 16 different food item, usually in high volume and in low frequency. A generalist trait favors successful invasions (Ribeiro et al. 2007, Brandner et al. 2013) since it is less affected by shifting prey availability, whereas a specialist feeding strategy could result in fluctuating population abundance of the predator when preferred prey abundance varies (Volterra 1928).

Specialization in piscivory as the main feeding habit for species of *Cichla* is widely known (e.g., Santos et al. 2001, Rabelo et al. 2002, Gomiero & Braga 2004a, Novaes et al. 2004, Santos et al. 2004, Villares-Junior & Gomiero 2010, Pereira et al. 2015). However, some degree of trophic plasticity is also described for these species, which prey occasionally on shrimps and other aquatic invertebrates (Winemiller et al. 2001, Kullander & Ferreira 2006). Winemiller et al. (1997) found that Characiformes and Perciformes fishes are the major prey for species of *Cichla,* and food partitioning has been documented for *C. temensis*

Table 1. Diet composition (% of the Alimentary Index – IAi) of *Cichla kelberi* in the Juturnaíba Reservoir in the three seasons (summer, winter and late spring).

Resources	Summer	Winter	Late Spring	Total
ANGIOSPERMA				
Vegetal	0.67	0.66	0.00	0.14
MOLLUSCA				
Bivalvia	0.00	0.39	0.00	0.04
ARTHROPODA				
Crustacea				
Decapoda	0.00	0,85	0.28	0.28
Ostracoda	0.23	0.00	0.00	0.01
Hexapoda				
Ephemeroptera	5.25	0,21	0.00	0,22
Odonata	4.99	52.15	55.86	68.07
Diptera	0.00	0.00	0.01	0.01
Trichoptera	0.12	0.02	0.00	0,01
Hemynoptera	15.98	0.00	0.00	0.31
Hemiptera	0.01	0.04	0.00	0.01
Remains insecta	0.02	0.10	0.00	0.02
FISHES				
Clupeidae				
Platanichthys platana		1.55	18.18	6.72
Characidae				
Astyanax sp.	8,45	34.63	1.49	8.03
Auchnenipteridae				
Trachelyopterus striatulus	0.00	0.15	0.00	0.02
Cichlidae				
Cichla kelberi	22.39	0.06	0.00	0.53
Fish remains	36.15	9.17	24.19	15.61

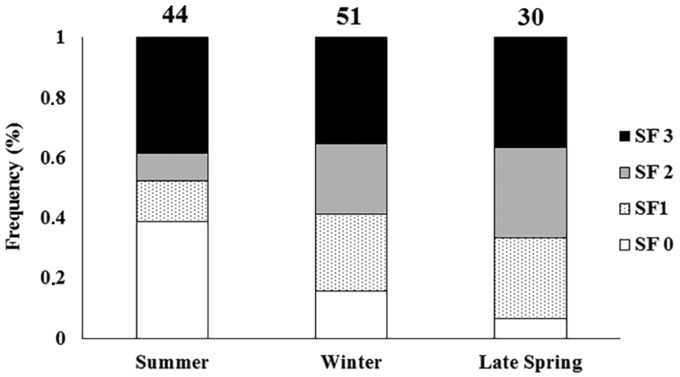

Figure 2. Frequency (%) of stomach of *Cichla kelberi* in the Juturnaiba Reservoir in different fulness degrees. SF-0, empty stomachs; SF-1, < 30% of the stomachs volume occupied by food; SF-2, 30-70%; SF-3, > 70%. Numbers of examined individuals also indicated.

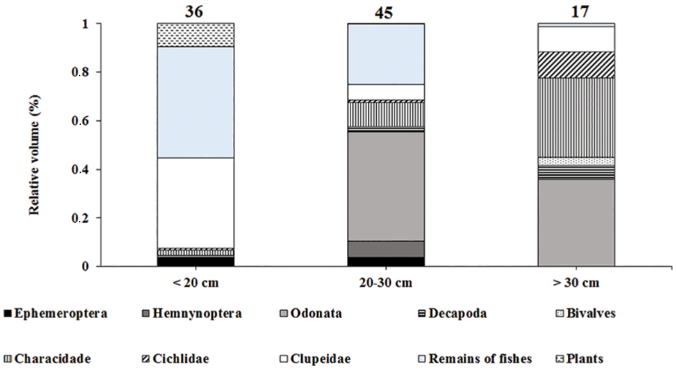

Figure 3. Main food items (% volume) by size classes of *Cichla kelberi* in the Juturnaíba Reservoir. Numbers of individuals examined also indicated.

and *C. intermedia* in the Cinaruco River, in Venezuela. In the Guri Reservoir, *C. temensis* feeds more on Characiforms and less on Cichlids than *C. orinocensis*. In the Tocantins River, the Tetragonopterinae subfamily was reported as the main prey for *Cichla kelberi* during the construction phase of a large reservoir (Serra da Mesa Reservoir), whereas the cichlids were the dominant prey during the operation phase (Novaes et al. 2004).

In its natural environment, the Tocantins-Araguaia watershed, *Cichla* kelberi preys mainly on small Tetragonopterinae, and to a lesser extent on shrimps and insects, besides practicing cannibalism (Novaes et al. 2004). When introduced in other reservoirs, this species prey on a variety of fish species that are available (Table 2), mainly small characiforms, siluriforms and cichlids, besides shrimps and insects (Santos et al. 2001, Gomieiro & Braga 2004a, Santos et al. 2004, Villares Junior & Gomiero 2010, Pereira et al. 2015). In the present study, this trend for using the available resources was confirmed, with *C. kelberi* preying mainly on characids, clupeids and cichlids. Additionally, a remarkable amount of insects of the order Odonata was also recorded in the stomachs examined.

Cannibalism is another aspect of the trophic behavior of *Cichla* species that is developed especially in invaded reservoirs. The high degree of cannibalism displayed by *C. kelberi* in the Corumbá (Fuji et al. 2008), Lajes (Santos et al. 2011) and Porto Primavera (Pereira et al. 2015) reservoirs highlights the aggressive behavior exhibited by this species. In the Lajes Reservoir, high cannibalism was recorded accounting for 50% of the diet (Santos et al. 2011). We found that cannibalism is higher in larger individuals of *C. kelberi*, as also reported in other reservoirs (Gomiero & Braga 2004, Pereira et al. 2015). Cannibalism, although being recorded in the Juturnaíba Reservoir, does not seem to be the most important source of feeding for *C. kelberi*. During the summer, the spawning season increases the number of early juveniles, which are easily preyed by the adult individuals (Gomiero et al. 2009). In the present study, cannibalism was also more frequent in summer, when the fullness indexes were at the lowest levels.

A conspicuous change in the diet was found during the subadult and adult phase, with the smallest individuals preying on a limited number of resources, mainly fishes of the family Clupeidae, whereas the largest individuals increased their trophic breadth preying mainly on insect of the order Odonata and fishes of the family Characidae. During and after the parental care period, juveniles (< 10 cm TL) of *Cichla* spp feed themselves on zooplankton, such as cladocerans, copepods, and microcrustacea; above this length, the diet is based on crustaceans, insects, and small fishes, with fish being the preferential item for adults (Lowe-McConnell 1969, Santos et al. 1994, Jepsen et al. 1997, Winemiller 2001). We also found that the niche breadth increased with growth, because the largest adults (>20 cm TL) consumed in an even proportion more items compared with the subadults (<20 cm TL). This is the opposite to the pattern detected by Gomiero & Braga (2004a) for species of *Cichla* in the Volta Grande Reservoir, where the smallest individuals had the largest niche breadth, feeding mainly on

Table 2. Main food items in decreasing importance used by *C. kelberi* in different aquatic systems.

Aquatic system	Origin	Main food items	References
Porto Primavera Reservoir (Southern Brazil), Paraná river basin	Non-native	Fishes (Anostomidae, Cheirodontidae, Curimatidae, Sternopygidae e Cichlidae) and Crustacea (Decapoda)	Pereira et al. 2015
Lajes Reservoir (Southeastern Brazil)	Non-native	Fishes (Cichlidae - *Cichla kelberi*, *Crenicichla lacustris* and *Tilapia rendalli*, Characidae - *Astyanax* spp.; *Oligosarcus hepsetus*; Pimelodidae - *Pimelodella eigenmani* and *Rhamdia parahybae*) Insects (Odonata - nymphs and adults; Hemiptera); fish eggs; Crustacea (*Macrobrachium* sp.) and unidentifield fishes.	Santos et al. 2011
Leme Lake (Southeastern Brazil)	Non-native	Fishes (Cichlidae - *Tilapia* sp., *C. kelberi* and *Geophagus brasiliensis*; Callichthyidae - *Hoplosternum litoralle*), Ophidia and unidentifield fishes	Villares Junior & Gomiero 2010
Corumbá Reservoir, Rio Grande, Paraná river basin	Non-native	Fishes (*Galeocharax knerii*, *Cichlasoma paranaense*, *Astyanax altiparanae*, *Cichla kelberi* and *P. maculatus*	Fugi et al. 2008
Lajes Reservoir (Southeastern Brazil)	Non-native	Fishes (Cichlidae - *Cichla monoculus*, *Crenicichla lacustris* and *Tilapia rendalli*, Characidae - *Astyanax* spp.; *Oligosarcus hepsetus*; Pimelodidae - *Pimelodella eigenmani* and *Rhamdia parahybae*) Insects (Odonata - nymphs and adults; Hemiptera); fish eggs; Crustacea (*Macrobrachium* sp.) and plants.	Santos et al. 2004
Volta Grande Reservoir, (Southeastern Brazil), Paraná river basin	Non-native	Fishes (Cichlidae - *Cichla* sp., *Tilapia rendali* and Scianidae – *Plagioscion squamosissimus*, fishes remains and others species), Insects (Culicidae, Odonata) and Shrimp (Macrobrachium sp.)	Gomiero & Braga 2004a
Serra da Mesa Reservoir (Western Brazil), Tocantins river basin	Native	Fishes (Tetragonopterinae, unidentifield fishes and Cichlidae), Insects.	Novaes et al. 2004
Lajes Reservoir (Southeastern Brazil)	Non-native	Fishes (Cichlidae, Characidae and Pimelodidae), Shrips (Macrobrachium sp.) & Insects Odonata.	Santos et al. 2001
Juturnaíba Reservoir (Southeastern Brazil)	Non-native	Fishes (Characidae, Clupeidae and Cichlidae, unidentifield fishes), Insects (Odonata and Ephemeroptera)	This study

juvenile fishes and insects whereas the largest individuals feed mainly on fishes. It seems that the opportunism is a more favorable strategy, rather than the specialism for *C. kelberi* in the Juturnaíba Reservoir, as this fish reaches larger size.

Seasonally, there was an inverse relationship between stomach fullness and the niche breadth for *C. kelberi* in the Juturnaíba Reservoir. In summer, it was detected the lowest stomach fullness and the highest niche breadth, whereas the opposite was recorded in late spring. The greater contribution of Odonata during the winter and late spring may have contributed for the high stomach fullness and low niche breadth in this period. Seasonal changes in feeding activity have been reported for this species in other reservoirs, as the case of the Volta Grande Reservoir (Gomiero & Braga 2004a) and the Leme Lake (Villares Junior & Gomiero 2010), which are likely to be associated to the dynamic in environmental conditions that affect seasonal resources availability. Villares-Junior & Gomiero (2010) reported that *C. kelberi* changes diet according to season, with low feeding activity during the winter when it feed on several fish species, increasing feeding activity during the spring, by preying mainly on *Tilapia* spp. and practicing cannibalism in summer/early autumn. In the present study, no significant seasonal differences were found in the diet composition of *C. kelberi.*

Piscivorous invaders change the diet composition in the new environment more than other trophic levels (Pereira et al. 2015), with direct effects on prey populations. In the case of *C. kelberi*, piscivory is the main feeding habit that have been recorded in several other studies (e.g., Santos et al. 2001, Rabelo et al. 2002, Gomiero & Braga 2004a, Novaes et al. 2004, Santos et al. 2004, Villares-Junior & Gomiero 2010, Pereira et al. 2015). It is expected that non-native species exploit the available resources in the new environment, and it was confirmed in this study, especially by the high consumption of Odonata and others invertebrates. We found that feeding plasticity is probably important to explain persistence of *C. kelberi* in reservoirs. Moreover, invasive species face new ecological interactions in the invaded environment and the success of an invasion might be affected by the biotic resistance presented by competitors and predators (Thompson et al. 2012).

The colonization and adaptation of *Cichla* spp. in altered environments such as reservoirs, is likely to result in decline of the original fish community. The presence of these highly adapted and quickly proliferating predators can cause serious losses to these communities by predation, competition, and cascade effects throughout the whole trophic chain. However, *Cichla* species attract recreational anglers, involving in this way the whole riverine community that depends on native fish species. The impact on the native ichthyofauna have been reported by several studies where there were introductions of species of *Cichla* (Pelicice & Agostinho 2009, Menezes et al. 2012, Orsi & Britton 2014). In the Juturnaíba Reservoir, *C. kelberi* seems to benefit from some native species (e.g., *Astyanax* spp. and *P. platana*). The opportunist feeding habit of *C. kelberi* enables to explore efficiently native species, thus facilitating its success to adapt and disperse in new colonized areas. A generalist feeding strategy, coupled with exceptionally high predatory ability may result in the proliferation of *C. kelberi* population.

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Author Contributions

Helaine da Silva Mendonça and Alexandre Clistenes de A. Santos: Substantial contribution in the concept and design of the study.

Helaine da Silva Mendonça and Alexandre Clistenes de A. Santos: Contribution to data collection.

Helaine da Silva Mendonça; Alexandre Clistenes A. Santos, Mariana Marques Martins e Francisco Gerson Araújo: Contribution to data analysis and interpretation.

Helaine da Silva Mendonça; Mariana Marques Martins and Francisco Gerson Araújo: Contribution to manuscript preparation.

Helaine da Silva Mendonça; Alexandre Clistenes de A. Santos and Francisco Gerson Araújo: 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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Editorial

Biodiversity at risk in the Americas

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At the end of March 2018, the Intergovernmental Platform for Biodiversity and Ecosystem Services (IPBES) launched the First Assessment on Biodiversity and Ecosystem Services for the Americas region. The conclusions of this colossal work, developed by about 100 experts over three years, are at least alarming.

The mainland of the Americas is the world's longest north-to-south landmass, and the distance between the northernmost point to the southernmost point is about 14,000 km. Including the Caribbean the area is divided into 36 countries (Canada, United States, Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica, Panama, Antigua and Barbuda, Bahamas, Barbados, Cuba, Dominica, Dominican Republic, Grenada, Haiti, Jamaica, Saint Lucia, Saint Kitts and Nevis, Saint Vincent and the Grenadines, Trinidad and Tobago, French Guyana, Suriname, Guyana, Brazil, Venezuela, Colombia, Ecuador, Peru, Bolivia, Chile, Paraguay, Uruguay, Argentina).

The American continent, including the Caribbean, is one of the richest regions in the world in terms of biodiversity and ecosystem services, housing seven of the 17 megadiverse countries, the largest rainforest, and an unparalleled array of freshwater environments - including rivers, lakes, dams and wetlands. Around 29 per cent of the world´s seed plants, 35 per cent of mammals, 35 per cent of reptiles, 41 per cent of birds and 51 per cent of amphibians are found in the Americas, totaling over 122,000 species for those species groups alone. In addition it hosts over one third of the world´s freshwater fish fauna, consisting of over 5,000 species. Conservatively, 33 per cent of the plants used by humans are found in the Americas.

The Americas is also a highly culturally and socioeconomically diverse region, home to 15 per cent of global languages. It is populated by over 66 million indigenous people whose cultures have persisted in all subregions and, in addition, by an exceptionally large proportion of new immigrants and descendants of immigrants, mainly from Europe, Asia and Africa. The cultural diversity of indigenous peoples and local communities in the Americas provides a plethora of knowledge and world views for managing biodiversity and nature's contributions to people in a manner consistent with cultural values promoting the respectful interaction of people with nature. This collective diversity provides many opportunities to develop world views compatible with sustainable uses of and respect for nature in a globalized world.

Socioeconomically, the region contains 2, US and Canada, of the 10 countries with the highest Human Development Index, as well as 1, Haiti, of the 30 countries with the lowest Human Development Index. Such heterogeneity makes it difficult to develop general conclusions that apply uniformly across all subregions.

We also have about 40% of the world's capacity to produce nature-based products, which are essential to guarantee food, water and energy security, pollinating services, climate regulation, as well as non-material services such as cultural continuity, spiritual and aesthetic. Considering only the terrestrial part of the continent, the value of these ecosystem services is estimated at US $ 24.3 trillion, the equivalent of the GDP of all countries of the region.

However, both biodiversity and the ecosystem services that depend on it, are under tremendous pressure. At least 65% of services are declining, while 21% are declining very fast. About ¼ of the 14,000 species of well-known taxonomic groups are at risk of extinction, to a greater or lesser degree, in terrestrial, fresh water and marine environments.

Among the endemic species the threat of extinction can reach 40%, in the case of the Caribbean species. On the continent as a whole, these losses are already affecting the ecosystem services associated with the provision and protection of water resources, as well as those associated with food production, including subsistence crops.

By 2005, the Millennium Ecosystem Assessment had already ignited the yellow light on the health of the planet's ecosystems. Unfortunately this warning has not been taken into account, and in the last two decades, since the end of the 20th century, the problems only worsened.

Population growth, migration, climate change, economic growth model and the fragility of environmental governance are the main indirect anthropogenic factors that impact biodiversity and ecosystem services on the continent. The region's GDP has grown six times since 1960, which has meant an improvement in the quality of life of millions of inhabitants in the region, but unfortunately, due to the predatory model of economic growth, it also represented a disproportionate increase in the conversion and fragmentation of habitats, generally for unsustainable production of export commodities such as soybeans and meat.

In the Americas we find a diversity of governance models of biodiversity and ecosystem services, embedded in a myriad of socioeconomic and cultural realities. Many of them aim to reduce the pressures of environmental degradation factors. But for the most part environmental policy is subordinated to short-term economic policies, lacking the necessary integration for the implementation of a model of sustainable development that guarantees an improvement in life quality without the predatory exploitation of biodiversity and ecosystem services. Short term plans to reduce inequity, for example, often compromise resources from the next generations.

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The report also identifies policy decisions that, if taken immediately, can reverse this alarming picture. For example, large-scale restoration of degraded areas, such as that proposed by Brazil in the Paris Agreement, as well as changing production/consumption patterns, adopting less caloric diets and avoiding food wastage.

But the most effective solution would be to put biodiversity and ecosystem services associated with life quality at the center of the economic development policies of Americas' countries.

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Anuran amphibians in state of Paraná, southern Brazil

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Abstract: The state of Paraná, located in southern Brazil, was originally covered almost entirely by the Atlantic Forest biome, with some areas of Cerrado savanna. In the present day, little of this natural vegetation remains, mostly remnants of Atlantic Forest located in the coastal zone. While some data are available on the anurans of the state of Paraná, no complete list has yet been published, which may hamper the understanding of its potential anuran diversity and limit the development of adequate conservation measures. To rectify this situation, we elaborated a list of the anuran species that occur in state of Paraná, based on records obtained from published sources. We recorded a total of 137 anuran species, distributed in 13 families. Nineteen of these species are endemic to the state of Paraná and five are included in the red lists of the state of Paraná, Brazil and/or the IUCN. Two anuran species were categorized as Near Threatened by the IUCN and 27 species were listed as Data Deficient in one or more lists. According to IUCN 49.6% of the anuran species recorded had their population trends stable, 19% in declined, only 1.4% is increased and 20.4% had your population trends unknown. We also recorded the occurrence in Paraná of the exotic invader anuran *Lithobates catesbeianus*. We consider our list of species to be a relatively reliable estimate of the anuran diversity of the Brazilian state of Paraná, although new species records are expected, mainly because there are many regions that have not yet been sampled.

Keywords: *Atlantic Forest, checklist, Cerrado, inventory, species richness.*

Anfíbios anuros do estado do Paraná, sul do Brasil

Resumo: O estado do Paraná, localizado no sul do Brasil, originalmente possuía praticamente toda sua área coberta pelo bioma Mata Atlântica com algumas porções do bioma Cerrado. Atualmente, pouco resta das formações destes biomas, sendo a maior parte remanescentes florestais de Mata Atlântica, localizados na região costeira do Estado. Apesar de haver estudos sobre anuros no estado do Paraná, até então nenhum deles proveu uma lista dos anuros com ocorrência no Estado, o que pode dificultar o conhecimento sobre a potencial diversidade de anuros e restringir medidas de conservação. A partir dessa lacuna, elaboramos uma lista das espécies de anuros com registro para o Paraná, a partir de dados de estudos publicados. Registramos um total de 137 espécies de anuros, pertencentes a 13 famílias. Dentre estas, 19 espécies de anuros são endêmicas do estado do Paraná e cinco encontram-se relacionadas sob algum grau de ameaça de extinção na lista vermelha do estado do Paraná, do Brasil e/ou da lista global da IUCN. Duas espécies de anuros foram categorizadas como Quase Ameaçada pela IUCN e 27 espécies foram listadas como Dados Insuficientes nestas listas. De acordo com a IUCN, 49,6% das espécies de anuros registradas apresentaram tendências populacionais estáveis, 19% diminuíram, apenas 1,4% aumentaram e 20,4% apresentaram tendências de sua populações desconhecidas. Registramos também a ocorrência do anuro exótico e invasor *Lithobates catesbeianus* para diferentes áreas do estado do Paraná. Consideramos nossa lista de espécies uma estimativa relativamente confiável da diversidade de anuros do estado brasileiro do Paraná, embora sejam esperados novos registros de espécies, principalmente porque existem muitas regiões que ainda não foram amostradas.

Palavras-chave: *Mata Atlântica, lista de espécies, Cerrado, inventário, riqueza de espécies.*

Introduction

Currently 6836 species of anuran amphibians are known to exist worldwide (Frost 2017). Just under a sixth (15.2%; 1039 species) of this total is found in Brazil (Segalla et al. 2016). This high species richness is partly related to the continental dimensions of the country and its considerable variation in altitude, as well as the enormous variety of tropical and subtropical habitats and ecosystems found in the different Brazilian biomes (Araújo et al. 2009), which provide a diversity of environments appropriate for anuran populations. In the Brazilian state of Paraná, most of these ecosystems are associated with the Atlantic Forest biome, although some areas of the Cerrado biome are also found in the state. Both these biomes have been classified as world biodiversity hotspots (Myers et al. 2000). Based on the modeling of the distribution data available for anuran species, Toledo & Batista (2012) estimated that as many as 147 anuran species may potentially occur in the state. Crivellari et al. (2014) recently listed the anurans that occur in the southern grasslands of Paraná, citing a total of 61 species. Up until now, however, no complete list of the anurans known to occur in the state of Paraná has been published.

While species lists for the country as a whole or for its different political divisions are important for the understanding of their biodiversity and provide an additional tool for the development of conservation measures, knowledge on the diversity of most groups of animals, through studies that provide species lists, is still incipient for most Brazilian states. Currently, lists of anuran amphibians are only available for the Brazilian states of Alagoas (Almeida et al. 2016), Espírito Santo (Almeida et al. 2011), Piauí (Roberto et al. 2013), Rio de Janeiro (Rocha et al. 2004), Rio Grande do Sul (Machado & Maltchik 2007), and São Paulo (Rossa-Feres et al. 2011), less than a quarter (23.1%) of the total number of Brazilian states.

In the present study, we estimated the number of anuran taxa that occur in the Brazilian state of Paraná based on a compilation of published records. We identified the endemic species, the taxa listed as threatened, near threatened and data deficient and the population trend of each species (*sensu* IUCN 2017). We also mapped the localities in Paraná where anuran inventories have been conducted.

Material and Methods

To identify the anuran species that occur in the Brazilian state of Paraná, we considered data obtained from papers published in journals based on a search of the Web of Science, Scielo, Scopus, and Google Scholar databases. The search terms used to identify the papers were amphib* AND Paraná, anur* AND Paraná and frog* AND Paraná. We consulted the papers cited as references in these articles. We terminated the search in November 06, 2017. We also did a supplementary search on the website SpeciesLink (2017) to know if there was still some anuran species collected in the state of Paraná that had not been registered by us through the publications accessed. To identify the endemic species of the Paraná state and the Atlantic Forest, we analyzed the known geographic distribution of each recorded species by Frost (2017). The current conservation status of each species was obtained from the Red List of Threatened Fauna of the state of Paraná (Segalla & Langone 2004), the Red List of Threatened Brazilian Fauna (MMA 2014), and the online version of the IUCN Red List of Threatened Species (IUCN 2017). The IUCN website was accessed to know the population trends of the anurans recorded in the present study (IUCN 2017). We followed the taxonomic nomenclature of Frost (2017).

Results

Based on the combined database (Table 1), we recorded 137 anuran species for the state of Paraná, belonging to 13 families: Hylidae (n = 58 species), Leptodactylidae (26), Brachycephalidae (15), Bufonidae (12), Cycloramphidae (7), Hylodidae (5), Odontophrynidae (5), Mycrohylidae (3), Hemiphractidae (2), Alsodidae (1), Centrolenidae (1), Craugastoridae (1), and Ranidae (1).

The Hylidae family was the richest in species (58 species, 42.3% of the total), while Alsodidae, Centrolenidae, Craugastoridae, Hemiphractidae and Ranidae were the least diverse, each one represented by a single species (0.7% of the total) (Table 1). The most diverse genus was *Boana*, with 13 species, 9.5% of the total recorded for the state. The most frequently recorded species in published studies were *Boana faber* (Wied-Neuwied, 1821) and *Scinax fuscovarius* (Lutz, 1925), each one recorded in 18 studies, and *Dendropsophus minutus* (Peters, 1872) and *Ischnocnema henselii* (Peters, 1870), both recorded in 17 studies. By contrast, out of 137 recorded species, 32 (23.3%) were cited in only a single study, nine of them (28.1%) are endemic to Paraná, and a further eight (25%) are also found only in the neighboring states of Santa Catarina or São Paulo.

Out of 137 species recorded in this study (Table 1), 19 (13.9%) are endemic to the state of Paraná: Brachycephalidae: *Brachycephalus brunneus* Ribeiro, Alves, Haddad & Reis, 2005 (Pico Caratuva, Campina Grande do Sul municipality), *B. coloratus* Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017 (Serra da Baitaca, Piraquara municipality), *B. curupira* Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017 (Serra do Salto, São José dos Pinhais municipality), *B. ferruginus* Alves, Ribeiro, Haddad & Reis, 2006 (Pico do Marumbi, Morretes municipality), *B. izecksohni* Ribeiro, Alves, Haddad & Reis, 2005 (Pico Caratuva, Campina Grande do Sul municipality), *B. leopardus* Ribeiro, Firkowski & Pie, 2015 (Serra do Araçatuda, Tijucas do Sul municipality; Morro dos Perdidos, Guaratuba municipality), *B. pernix* Pombal, Wistuba & Bornschein, 1998 (Quatro Barras, Morretes and São José dos Pinhais municipality), *B. pombali* Alves, Ribeiro, Haddad & Reis, 2006 (Pico da Igreja, Guaratuba municipality), *B. tridactylus* Garey, Lima, Hartmann & Haddad, 2012 (Pico do Morato, Guaraqueçaba municipality), *Ischnocnema paranaensis* (Langone & Segalla, 1996) (next to Pico do Paraná, Antonina municipality) and *I. sambaqui* (Castanho & Haddad, 2000) (Guaraqueçaba and Morretes municipality); Bufonidae: *Dendrophryniscus stawiarskyi* Izecksohn, 1994 (Bituruna municipality), *Melanophryniscus alipioi* Langone, Segalla, Bornschein & de Sá, 2008 (Campina Grande do Sul municipality) and *M. vilavelhensis* Steinbach-Padilha, 2008 (Ponta Grossa municipality); Cycloramphidae: *Cycloramphus duseni* (Anderson, 1914) (Ipiranga municipality) and *C. mirandaribeiroi* Heyer, 1983 (São João da Graciosa, Morretes municipality); Hylidae: *Bokermannohyla langei* (Bokermann, 1965) (Morretes municipality) and *Boana jaguariaivensis* (Caramaschi, Cruz & Segalla, 2010) (Jaguariaíva municipality); Leptodactylidae: *Physalaemus insperatus* Cruz, Cassini & Caramaschi, 2008 (Guaratuba municipality).

Table 1. Anuran amphibians in the state of Paraná, southern Brazil, including geographical distribution in Brazil, endemism for the state of Paraná (both sensu Frost 2017), and conservation status [according to Red List of Threatened Fauna of the state of Paraná (Segalla & Langone 2004), Red List of Threatened Brazilian Fauna (MMA 2014), and the IUCN Red List of Threatened Species (2017)]. Population trend of anurans follows IUCN (2017): S = stable, D = decreasing, I = increasing and U = unknown. Source of records: 1, Affonso & Delariva (2012); 2-3, Affonso et al. (2011, 2014); 4, Alves et al. (2006); 5, Antonucci et al. (2011); 6, Armstrong & Conte (2010); 7, Baldo et al. (2008); 8, Benarde & Anjos (1999); 9, Bernarde & Machado (2001); 10, Bokermann (1965); 11, Bornschein et al. (2015a); 12, Bornschein et al. (2015b); 13, Bornschein et al. (2016b); 14, Caramaschi & Cruz (2002); 15, Caramaschi & Rodrigues (2007); 16, Caramaschi et al. (2010); 17, Castanho & Haddad (2000); 18-19, Clemente-Carvalho et al. (2009, 2011); 20, Condez et al. (2016); 21, Conte & Machado (2005); 22-23, Conte & Rossa-Feres (2006, 2007); 24-25, Conte et al. (2005, 2010); 26, Costa et al. (2009); 27, Crivellari et al. (2014); 28, Cruz (1990); 29, Cruz et al. (2008); 30, Cunha et al. (2010); 31, Figueiredo et al. (2014); 32, Fontoura et al. (2011); 33, Gambale et al. (2014); 34, Garey & Hartmann (2012); 35-36, Garey et al. (2012a, 2012b); 37-38, Heyer (1978, 1983); 39, Heyer & Heyer (2004); 40, Hiert & Moura (2010); 41, Hiert et al. (2012); 42, Izecksohn (1993); 43, Langone & Segalla (1996); 44, Langone et al. (2008); 45, Leivas & Hiert (2016); 46, Lima et al. (2010); 47, Lingnau & Bastos (2007); 48, Lingnau et al. (2008); 49, Machado & Bernarde (2002); 50, Machado et al. (1999); 51, Marcelino et al. (2009); 52, Miranda et al. (2008); 53-55, Moresco et al. (2009, 2013, 2014); 56, Nazaretti & Conte (2015); 57, Nunes et al. (2012); 58, Oda & Landgraf (2012); 59, Oda et al. (2015); 60, Oliveira et al. (2010); 61, Pederassi et al. (2015); 62, Pie et al. (2013); 63, Pimenta et al. (2014); 64, Pombal et al. (1998); 65, Prado & Pombal (2008); 66, Ribeiro et al. (2015); 67, Ribeiro et al. (2017); 68, Sá & Langone (2002); 69-70, Sá et al. (2007, 2014); 71, Santos-Pereira & Rocha (2015); 72-74, Santos-Pereira et al. (2011, 2015, 2016); 75, Steinbach-Padilha (2008); 76-77, Toledo et al. (2007, 2012); 78, Thomé et al. (2012); 79, Trein et al. (2014); 80, Vieira et al. (2012); 81, Winkelmann & Noleto (2015). *considered the anuran species as "aff.". **considered the anuran species as "cf.".

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
ANURA							
Alsodidae							
Limnomedusa macroglossa (Duméril & Bibron, 1841)	Southern		CR		LC	S	9, 22
Brachycephalidae							
Brachycephalus brunneus Ribeiro, Alves, Haddad & Reis, 2005	PR (Pico Caratuva, Campina Grande do Sul)	X			DD	U	13, 18, 19, 32, 62
Brachycephalus coloratus Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017	PR (Serra da Baitaca, Piraquara)	X					67
Brachycephalus curupira Ribeiro, Blackburn, Stanley, Pie & Bornschein, 2017	PR (Serra do Salto, Malhada District, São José dos Pinhais)	X					67
Brachycephalus ferruginus Alves, Ribeiro, Haddad & Reis, 2006	PR (Pico Marumbi, Morretes)	X			DD	U	4, 13, 18
Brachycephalus hermogenesi (Giaretta & Sawaya, 1998)	RJ, SP and PR				LC	S	30*, 72, 74
Brachycephalus izecksohni Ribeiro, Alves, Haddad & Reis, 2005	PR (Pico Caratuva, Campina Grande do Sul)	X			DD	U	13, 18, 19, 62
Brachycephalus leopardus Ribeiro, Firkowski & Pie, 2015	PR (Serra do Araçatuba, Tijucas do Sul and Morro dos Perdidos, Guaratuba)	X					13, 66
Brachycephalus pernix Pombal, Wistuba & Bornschein, 1998	PR (Quatro Barras, Morretes and São José dos Pinhais)	X	CR	CR	DD	U	18, 19, 22, 62, 64
Brachycephalus pombali Alves, Ribeiro, Haddad & Reis, 2006	PR (Pico da Igreja, Guaratuba)	X			DD	U	4, 13, 18, 19, 62
Brachycephalus sulfuratus Condez, Monteiro, Comitti, Garcia, Amaral & Haddad, 2016	SP, PR and SC						20
Brachycephalus tridactylus Garey, Lima, Hartmann & Haddad, 2012	PR (Pico do Morato, Guaraqueçaba)	X					11, 13, 34, 36, 74
Ischnocnema henselii (Peters, 1870)	Southern				LC	U	6*, 8, 9, 21, 22, 23, 25, 27, 30*, 34, 45, 49, 50, 72, 73, 74
Ischnocnema paranaensis (Langone & Segalla, 1996)	PR (Next to Pico do Paraná)	X	EN		DD	U	12, 43

Continued Table 1.

TAXON	**Geografic Distribution in Brazil**	**Endemism**	**Red lists**			**Population trend (IUCN)**	**Source of record**
			Paraná	**Brazil**	**Global (IUCN)**		
Ischnocnema sambaqui (Castanho & Haddad, 2000)	PR (Guaraqueçaba and Morretes)	X	DD		DD	U	6, 17, 22, 74
Ischnocnema cf. *spanios*					DD	U	74
Bufonidae							
Dendrophryniscus berthalutzae Izecksohn, 1994	PR and SC				LC	D	34, 74
Dendrophryniscus leucomystax Izecksohn, 1968	RJ, SP, PR and SC				LC	D	34, 74
Dendrophryniscus stawiarskyi Izecksohn, 1994	PR (Bituruna)	X	DD		DD	U	42
Melanophryniscus alipioi Langone, Segalla, Bornschein & de Sá, 2008	PR (Campina Grande do Sul)	X			DD	U	27, 44
Melanophryniscus tumifrons (Boulenger, 1905)	PR and RS				LC	U	14
Melanophryniscus vilavelhensis Steinback-Padilha, 2008	PR (Ponta Grossa)	X					27, 75
Rhinella abei (Baldissera, Caramaschi & Haddad, 2004)	Southern				LC	U	6, 21, 22, 23, 27, 30, 34, 45, 72, 74, 78
Rhinella henseli (Lutz, 1934)	Southern				LC	U	27, 78
Rhinella hoogmoedi Caramaschi & Pombal, 2006	CE to PR				LC	U	34, 74
Rhinella icterica (Spix, 1824)	RS to BA, MG and GO				LC	S	6, 21, 22, 23, 27, 30, 34, 45, 49, 74
Rhinella ornata (Spix, 1824)	ES, RJ, SP and north PR				LC	U	56, 78
Rhinella schneideri (Werner, 1894)	CE to RS				LC	I	1, 3, 5, 8, 22, 56, 58
Centrolenidae							
Vitreorana uranoscopa (Müller, 1924)	Southeastern and Southern		DD		LC	D	6, 8, 9, 22, 27, 30, 34, 45, 49, 50, 74
Craugastoridae							
Haddadus binotatus (Spix, 1824)	BA to RS, MS				LC	S	8, 9, 22, 27, 34, 49, 50, 72, 74
Cycloramphidae							
Cycloramphus cf. *asper*			DD		DD	D	74
Cycloramphus bolitoglossus (Werner, 1897)	PR and SC		DD		DD	D	21, 22, 23, 38
Cycloramphus duseni (Andersson, 1914)	PR (Ipiranga)	X	DD		DD	U	38
Cycloramphus eleutherodactylus (Miranda-Ribeiro, 1920)	RJ, SP and PR		DD		DD	U	38
Cycloramphus lutzorum Heyer, 1983	SP and PR		DD		DD	D	38, 46
Cycloramphus mirandaribeiroi Heyer, 1983	PR (São João da Graciosa)	X	DD		DD	U	38, 74

Continued Table 1.

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
Cycloramphus rhyakonastes Heyer, 1983	PR and SC		DD		LC	U	38
Hemiphractidae							
Gastrotheca microdiscus (Andersson, 1910)	SP, PR and SC		DD		LC	D	15, 74
Fritziana gr. *fissilis*					LC	S	34, 74
Hylidae							
Aplastodiscus albosignatus (Lutz & Lutz, 1938)	GO, MG, RJ, SP and PR				LC	D	21, 22, 23, 27, 30, 45, 74
Aplastodiscus ehrhardti (Müller, 1924)	SP, PR and SC				LC	D	6, 22, 24
Aplastodiscus perviridis Lutz, 1950	Central, Southeastern and Southern				LC	S	8, 9, 21, 22, 23, 26, 45, 49, 50
Boana albomarginata (Spix, 1824)	PE to SC				LC	S	6, 30, 34, 74
Boana albopunctata (Spix, 1824)	Central, Southeastern and Southern				LC	S	1, 3, 8, 21, 22, 27, 45, 49, 50, 56, 75
Boana bischoffi (Boulenger, 1887)	RJ to RS				LC	S	6, 21, 22, 23, 27, 30, 45, 51
Boana caingua (Carrizo, 1991)	MS, SP and Southern				LC	S	27
Boana faber (Wied-Neuwied, 1821)	PE to RS				LC	S	1, 3, 6, 8, 9, 21, 22, 23, 27, 28, 34, 45, 49, 50, 55, 74, 80
Boana jaguariaivensis (Caramaschi, Cruz & Segalla, 2010)	PR (Jaguariaíva)	X					16
Boana leptolineata (Braun & Braun, 1977)	PR and RS				LC	S	27, 40, 41
Boana prasina (Burmeister, 1856)	MG, RJ, SP and PR				LC	S	8, 9, 21, 22, 23, 27, 45, 49, 50, 56
Boana pulchella (Duméril & Bibron, 1841)	Southern				LC	S	27
Boana raniceps (Cope, 1862)	Southeastern (except ES) and PR				LC	S	1, 3, 22, 33, 49, 50, 56
Boana semiguttata (Lutz, 1925)	PR and SC			EN	LC	S	22, 49
Boana semilineata (Spix, 1824)	AL to SC				LC	S	34, 74
Boana cf. *stellae*					LC	S	27
Bokermannohyla circumdata (Cope, 1871)	BA, Southeastern, PR and SC				LC	D	21, 22, 23, 27
Bokermannohyla hylax (Heyer, 1985)	SP and PR				LC	D	6, 30, 34, 74
Bokermannohyla langei (Bokermann, 1965)	PR (Morretes)	X	DD		DD	U	10
Dendropsophus anceps (Lutz, 1929)	BA to PR		CR		LC	S	22, 25, 56

Continued Table 1.

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
Dendropsophus berthalutzae (Bokermann, 1962)	ES, SP and PR				LC	S	6, 30, 34, 74
Dendropsophus elegans (Wied-Neuwied, 1824)	BA to SC				LC	S	6, 30, 34, 74
Dendropsophus microps (Peters, 1872)	BA to RS				LC	S	6, 22, 23, 27, 30, 34, 49, 74
Dendropsophus minutus (Peters, 1872)	Brazil				LC	S	1, 3, 6, 8, 9, 21, 22, 23, 27, 30, 34, 45, 49, 50, 56, 74, 75
Dendropsophus nahdereri (Lutz & Bokermann, 1963)	Southern				LC	S	22, 25, 30
Dendropsophus nanus (Boulenger, 1889)	Brazil				LC	S	1, 3, 8, 22, 49, 50, 56
Dendropsophus sanborni (Schmidt, 1944)	Southern				LC	S	21, 22, 23, 27, 49, 56
Dendropsophus seniculus (Cope, 1868)	MG, ES, RJ and PR				LC	S	34, 74
Dendropsophus werneri (Cochran, 1952)	Southern				LC	S	30, 34, 52, 60, 74
Itapothihyla langsdorffii (Duméril & Bibron, 1841)	SE, BA, Southeastern, PR and RS						74
Julianus uruguayus (Schmidt, 1944)	PR and SC				LC	S	22, 27, 49
Lysapsus limellum Cope, 1862	MT and PR				LC	S	3
Ololygon argyreornata (Miranda-Ribeiro, 1926)	ES, RJ, SP and PR				LC	S	6*, 34, 74**
Ololygon aromothyella Faivovich, 2005	PR and RS				DD	U	27
Ololygon berthae (Barrio, 1962)	Southern				LC	S	21, 22, 23, 34*, 49, 56, 74*
Ololygon catharinae (Boulenger, 1888)	Southeastern and Southern				LC	S	22, 23, 27**
Ololygon littoralis (Pombal & Gordo, 1991)	SP, PR and SC				LC	D	6, 34, 35, 74, 77
Ololygon aff. *perpusilla*					LC	S	6, 34, 74
Ololygon rizibilis (Bokermann, 1964)	SP and PR				LC	D	6, 22, 23, 27, 30, 31
Phasmahyla guttata (Lutz, 1924)	RJ, SP, ES and PR		DD		LC	D	28
Phyllomedusa distincta Lutz, 1950	SP, PR and SC				LC	D	6, 22, 23, 27, 30, 34, 45, 74
Phyllomedusa tetraploidea Pombal & Haddad, 1992	SP and PR				LC	S	1, 3, 8, 9, 22, 27, 49, 50, 56, 81
Pithecopus rusticus (Bruschi, Lucas, Garcia & Recco-Pimentel, 2015)	PR and SC						27
Pseudis cardosoi Kwet, 2000	Southern				LC	S	25, 27

Continued Table 1.

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
Scinax alter (Lutz, 1973)	BA, MG, ES, RJ and PR				LC	S	6*, 30
Scinax fuscomarginatus (Lutz, 1925)	Southern, Central, Southeastern, Northeast, Southern AM				LC	S	56
Scinax fuscovarius (Lutz, 1925)	Southeastern and PR				LC	S	1, 3, 6, 8, 9, 22, 23, 27, 30, 33, 34, 45, 49, 50, 56, 59, 74
Scinax granulatus (Peters, 1871)	Southern				LC	S	25, 27, 53
Scinax imbegue Nunes, Kwet & Pombal, 2012	SP, PR and SC						57
Scinax nasicus (Cope, 1862)	MT, MS, GO, MG, SP, PR, SC and RS				LC	S	61
Scinax perereca Pombal, Haddad & Kasahara, 1995	SP, PR and RS				LC	U	6, 8, 9, 21, 22, 23, 27, 30, 34, 45, 49, 50, 56*, 74
Scinax squalirostris (Lutz, 1925)	MS, Southeastern and PR				LC	S	21, 22, 27, 49
Scinax tymbamirim Nunes, Kwet & Pombal, 2012	RJ to RS						57, 74**
Sphaenorhynchus caramaschii Toledo, Garcia, Lingnau & Haddad, 2007	Southern, Southeastern and BA				LC	S	27, 76
Sphaenorhynchus surdus (Cochran, 1953)	Southern				LC	S	21, 22, 23, 27
Trachycephalus dibernardoi Kwet & Solé, 2008	Southern				LC	U	22, 23, 25, 27
Trachycephalus mesophaeus (Hensel, 1867)	BA to RS				LC	D	6, 30, 34, 74
Trachycephalus typhonius (Linnaeus, 1758)	Southern				LC	S	1, 3, 22, 49
Hylodidae							
Crossodactylus caramaschii Bastos & Pombal, 1995	SP, PR and SC				LC	S	27**, 63, 74
Crossodactylus schmidti Gallardo, 1961	Southern				NT	D	3
Hylodes cardosoi Lingnau, Canedo & Pombal, 2008	SP and PR				LC	U	48, 74, 80
Hylodes aff. *asper*					LC	S	34, 74
Hylodes heyeri Haddad, Pombal & Bastos, 1996	SP and PR				DD	D	6, 22, 26, 30, 34, 47, 74**
Leptodactylidae							
Adenomera araucaria Kwet & Angulo, 2002	Southern				LC	S	25
Adenomera cf. *bokermanni*					LC	S	6*, 34*, 74**
Adenomera marmorata Steindachner, 1867	RJ to SC				LC	S	21, 22, 23*, 27*, 30**, 34, 72, 73, 74

Continued Table 1.

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
Adenomera nana (Müller, 1922)	PR and SC				LC	U	25, 27
Leptodactylus flavopictus Lutz, 1926	ES to SC				LC	D	70
Leptodactylus furnarius Sazima & Bokermann, 1978	TO, BA, Central, Southeastern and PR				LC	S	7, 37, 39, 70
Leptodactylus fuscus (Schneider,1799)	Brazil				LC	S	1, 3, 8, 22, 27, 33, 49, 50, 56, 70
Leptodactylus gracilis (Duméril & Bibron, 1840)	Southern				LC	S	22, 27, 49, 70
Leptodactylus labyrinthicus (Spix, 1824)	Central, Southeastern and PR				LC	S	22, 27, 49, 50, 56, 70
Leptodactylus latrans (Steffen, 1815)	MG, BA, SP and PR				LC	S	1, 3, 27**, 30**, 34, 45, 56, 70, 74
Leptodactylus mystaceus (Spix, 1824)	Norte, Northeast, Central, MG, SP and PR				LC	S	2, 3, 70
Leptodactylus mystacinus (Burmeister, 1861)	Central, Southeastern and Southern				LC	S	1, 3, 9, 22, 27, 49, 50, 55, 70
Leptodactylus notoaktites Heyer, 1978	SP, PR and SC				LC	S	6, 22, 23, 27, 30, 34, 45, 69, 70, 74
Leptodactylus plaumanni Ahl, 1936	Southern				LC	S	27, 70
Leptodactylus podicipinus (Cope, 1862)	Northern, Central and PR		DD		LC	S	3, 22, 49, 56, 70
Physalaemus cuvieri Fitzinger, 1826	Northeast, Central and Southern				LC	S	1, 3, 6, 8, 9, 21, 22, 23, 27, 33, 45, 49, 50, 54, 55
Physalaemus gracilis (Boulenger, 1883)	Southern				LC	S	9, 21, 22, 23, 27*, 45*, 49, 75
Physalaemus insperatus Cruz, Cassini & Caramaschi, 2008	PR (Guaratuba)	X			DD	U	29
Physalaemus lateristriga (Steindachner, 1864)	SP, PR and SC						27, 45
Physalaemus maculiventris (Lutz, 1925)	RJ, SP, PR and SC		DD		LC	D	30
Physalaemus nanus (Boulenger, 1888)	SP and Southern				LC	S	27
Physalaemus nattereri (Steindachner, 1863)	Central, Southeastern and SC				LC	D	3, 56
Physalaemus olfersii (Lichtenstein & Martens, 1856)	MG, ES, SP and PR				LC	S	6**, 22, 23, 30, 34*, 74*
Physalaemus spiniger (Miranda-Ribeiro, 1926)	SP and PR				LC	D	34, 72, 74, 77
Pleurodema bibroni Tschudi, 1838	Southern				NT	D	27**, 79
Scythrophrys sawayae (Cochran, 1953)	PR and SC		DD		LC	D	22

Continued Table 1.

TAXON	Geografic Distribution in Brazil	Endemism	Red lists			Population trend (IUCN)	Source of record
			Paraná	Brazil	Global (IUCN)		
Microhylidae							
Chiasmocleis leucosticta (Boulenger, 1888)	SP, PR and SC		DD		LC	S	22, 30
Elachistocleis bicolor (Guérin-Méneville, 1838)	Southern				LC	S	3**, 6, 22, 23, 50, 56
Elachistocleis cf. *cesarii*							1
Odontophrynidae							
Odontophrynus americanus (Duméril & Bibron, 1841)	Southern				LC	S	3, 21, 22, 23, 27, 49, 50, 56
Proceratophrys avelinoi Mercadal de Barrio & Barrio, 1993	PR and RS				LC	U	8, 9, 22, 23*, 49, 50, 68
Proceratophrys brauni Kwet & Faivovich, 2001	Southern				LC	D	27
Proceratophrys boiei (Wied-Neuwied, 1824)	Southeastern, PR and SC				LC	S	6, 21, 22, 23, 27, 30, 34, 45, 72, 74
Proceratophrys subguttata Izecksohn, Cruz & Peixoto, 1999	PR and SC				LC	D	65
Ranidae							
Lithobates catesbeianus (Shaw, 1802)	Brazil				LC	I	1, 3, 6, 9, 22, 27, 45, 49, 71

We recorded a total of 24 anuran species whose type locality is in the state of Paraná (Figure 1). Nineteen of these species are endemic to the state, and 15 (except *B. leopardus*, *B. pernix*, *I. sambaqui*, and *B. jaguariavensis*) are known only from the type locality (Figure 1).

We also reported the occurrence of the exotic invader anuran *Lithobates catesbeianus* (Shaw 1802) (Ranidae), known as the bullfrog. We found five studies that recorded the occurrence of this anuran species in the state of Paraná (Table 1).

Five (3.6%) anuran species are listed as threatened in the red lists of Paraná, Brazil and/or IUCN (Table 1): *Brachycephalus pernix*, *Dendropsophus anceps* (Lutz, 1929), *Limnomedusa macroglossa* (Duméril & Bibron, 1841), *Boana semiguttata* (Lutz, 1925) and *Ischnocnema paranaensis*. *Brachycephalus pernix* is classified as Critically Endangered (CR) in Paraná and Brazil and *L. macroglossa* only in Paraná, while *B. semiguttata* and *I. paranaensis* are listed as Endangered (EN) in Brazil and in Paraná, respectively. Moreover, *Crossodactylus schmidti* Gallardo, 1961 and *Pleurodema bibroni* Tschudi, 1838 are listed as Near Threatened (NT) by the IUCN. A further 27 species (19.2% of the total) are listed as Data Deficient (DD) in Paraná and/or by the IUCN. Two of the species endemic to Paraná (*B. pernix* and *I. paranaensis*) are listed as Critically Endangered, corresponding to 10.5% of the anuran species endemic to the state.

Based on IUCN (2017), the population trends of 68 (49.6%) anuran species recorded in the present study are stable (Table 1). Most of these species belongs to the family Hylidae (40 species, 58.8% of the total number of registered species), representing 69% of the recorded hylids, and Leptodactylidae (17 species, 25.0% of the total number of registered species), representing 65.4% of the recorded leptodactylids. By contrast, 26 species (19% of the total number of registered species) have declining populations, including nine hylids (15.5% of hylid species) and five leptodactylids (19.2% of leptodactylids species), and two species have increasing populations (1.4% of the total number of registered species), *Rhinella schneideri* (Werner, 1894) and *Lithobates catesbeianus.* A further 28 (20.4%) species recorded for Paraná have unknown population trends, nine species from the family Brachycephalidae (6.6% of the total number of registered species, 60% of the brachycephalids) and seven species from the family Bufonidae (5.1% of the total number of registered species, 58.3% of the bufonids). The remaining 11 anuran species (8.1% of the total number of registered species) have yet to be assessed by the IUCN. Two recently described anuran species (*Brachycephalus coloratus* and *B. curupira*) have not yet been assessed by the IUCN and therefore, we have not been able to access their population trends.

In our supplementary search of the SpeciesLink database (Table 2), we recorded 24 anuran species belonging to seven families: Hylidae (n = 9 species), Leptodactylidae (5), Hylodidae (4), Bufonidae (2), Centrolenidae (2), Allobatidae (1) and Mycrohylidae (1). These species included 10 anurans not identified in our literature search: *Allobates brunneus* (Cope, 1887), *Vitreorana eurygnata* (Lutz, 1925), *Aplastodiscus cochranae* (Mertens, 1952), *A. leucopygius* (Cruz & Peixoto, 1985), *Boana polytaenia* (Cope, 1870), *Ololygon brieni* (De Witte, 1930), *Crossodactylus gaudichaudii* Duméril & Bibron, 1841, *Leptodactylus chaquensis* Cei, 1950, *Physalaemus biligonigerus* (Cope, 1861) and *Pseudopaludicola falcipes* (Hensel, 1867).

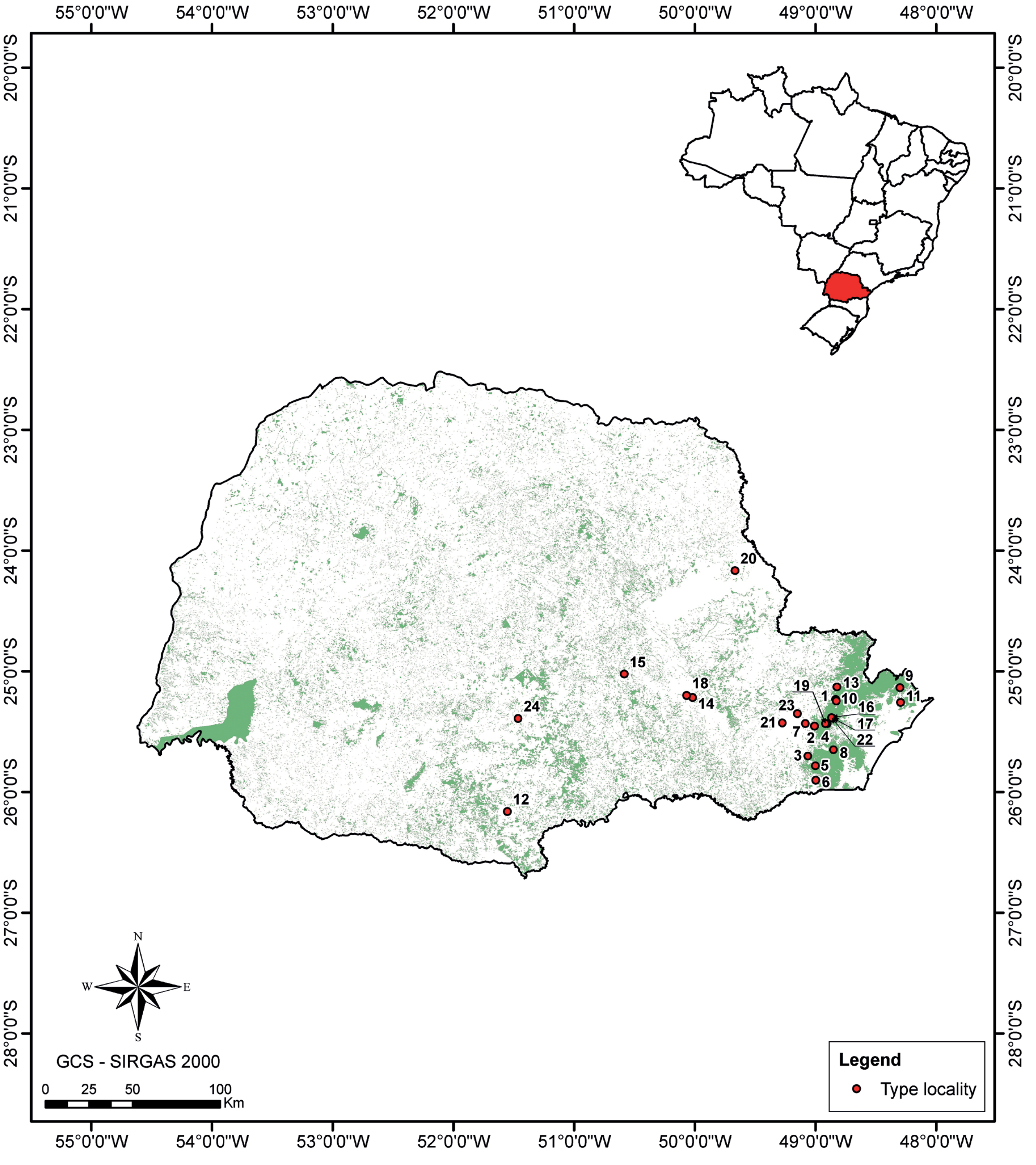

Figure 1. Type localities of the anuran species in the state of Paraná, southern Brazil. Abbreviations: mun., municipality; P.E., Parque Estadual (State Park). *Brachycephalus brunneus* (1 = Pico Caratuva, mun. Campina Grande do Sul), *B. ferruginus* (2 = Pico Marumbi, mun. Morretes), *B. izecksohni* (3 = Pico Torre da Prata, from mun. Guaratuba to mun. Paranaguá), *B. leopardus* (4 = Serra do Araçatuba, mun. Tijucas do Sul), *B. pernix* (5 = Morro Anhangava, in Serra da Baitaca, Conjunto Marumbi, mun. Quatro Barras), *B. pombali* (6 = Morro dos Padres, Pico da Igreja, mun. Guaratuba), *B. tridactylus* (7 = Pico do Morato, mun. Guaraqueçaba), *Ischnocnema paranaensis* (8 = Pico Paraná, mun. Antonina), *I. sambaqui* (9 = mun. Guaraqueçaba), *Dendrophryniscus stawiarskyi* (10 = mun. Bituruna), *Melanophryniscus alipioi* (11 = Serra do Capivarí, mun. Campina Grande do Sul), *M. vilavelhensis* (12 = P. E. de Vila Velha, mun. Ponta Grossa), *Cycloramphus duseni* (13 = mun. Ipiranga), *C. mirandaribeiroi* (14 = São João da Graciosa, mun. Morretes), *C. rhyakonastes* (15 = São João da Graciosa, mun. Morretes), *Gastrotheca microdiscus* (16 = mun. Ponta Grossa), *Bokermannohyla langei* (17 = Pico do Marumbi, mun. Morretes), *Boana jaguariaivensis* (18 = P. E. do Cerrado, mun. Jaguariaíva), *Sphaenorhynchus surdus* (19 = mun. Curitiba), *Hylodes cardosoi* (20 = Porto de Cima, mun. Morretes), *Physalaemus insperatus* (21 = Área de Proteção Ambiental Guaratuba, Serra da Pedra Branca do Araraquara, mun. Guaratuba) and *Scythrophrys sawayae* (22 = Banhado, mun. Guarapuava).

Table 2. Putative determinations of the anuran amphibians of the state of Paraná, southern Brazil, that have been deposited in scientific collections (search in the website SpeciesLink 2017). Scientific collections: DZSJRP = Departamento de Zoologia São José do Rio Preto, NMNH = National Museum of Natural History, MZUEL = Museu de Zoologia da Universidade Estadual de Londrina, ZUEC = Museu de Zoologia da Universidade Estadual de Campinas, FNJV = Fonoteca Neotropical Jacques Vielliard, CFBH = Coleção Célio F. B. Haddad. Source: [1] = Berneck et al. (2016), [2] = Garcia et al. (2007), [3] = Lingnau & Bastos (2007) and [4] = Sá et al. (2014).

Taxon	Tentatively updated taxon	Voucher number
Colostethus brunneus (Cope, 1887)	*Allobates brunneus* (Cope, 1887)	NMNH-Animalia_BR 148487
Dendrophryniscus brevipollicatus (Jiménez de la Espada, 1870)	*Dendrophryniscus stawiarskyi* Izecksohn, 1994	NMNH-Animalia_BR 217659
Bufo crucifer Wied-Neuwied, 1821	*Rhinella abei* (Baldissera, Caramaschi & Haddad, 2004)	MZUEL-Herpeto 1134
Vitreorana eurygnatha (Lutz, 1925)	*Vitreorana eurygnatha* (Lutz, 1925)	NMNH-Animalia_BR 284502
Vitreorana parvula (Boulenger, 1895)	*Vitreorana uranoscopa* (Müller, 1924)	ZUEC-AMP 284502
Aplastodiscus albofrenatus (Lutz, 1924)	*Aplastodiscus ehrhardti* (Müller, 1924) [1]	DZSJRP 5252
Aplastodiscus cochranae (Mertens, 1952)	*Aplastodiscus cochranae* (Mertens, 1952)	FNJV 0033953
Aplastodiscus leucopygius (Cruz & Peixoto, 1985)	*Aplastodiscus leucopygius* (Cruz & Peixoto, 1985)	FNJV 0033943
Boana joaquini (Lutz, 1968)	*Boana semiguttata* (A. Lutz, 1925) [2]	FNJV 0033075
Boana polytaenia (Cope, 1870)	*Boana polytaenia* (Cope, 1870)	ZUEC-AMP 10502
Bokermannohyla astartea (Bokermann, 1967)	*Bokermannohyla hylax* (Heyer, 1985)	NMNH 125516
Ololygon angrensis (Lutz, 1973)	*Ololygon littoralis* (Pombal & Gordo, 1991)	ZUEC-AMP 4724
Phrynohyas venulosa (Fitzinger, 1843)	*Trachycephalus typhonius* (Linnaeus, 1758)	MZUEL-Herpeto 362
Scinax brieni Faivovich, Haddad, Garcia, Frost & Wheeler, 2005	*Ololygon brieni* (De Witte, 1930)	NMNH-Animalia_BR 125515
Crossodactylus bresslaui Müller, 1924	*Crossodactylus caramaschii* Bastos & Pombal, 1995	MZUEL-Herpeto 748
Crossodactylus gaudichaudii Duméril & Bibron, 1841	*Crossodactylus gaudichaudii* Duméril & Bibron, 1841	DZSJRP-Amphibia-adults 6296
Hylodes asper (Müller, 1924)	*Hylodes cardosoi* Lingnau, Canedo & Pombal, 2008 or *Hylodes heyeri* Haddad, Pombal & Bastos, 1996 [3]	NMNH-Animalia_BR 149648
Hylodes perplicatus (Miranda-Ribeiroi, 1926)	*Hylodes cardosoi* Lingnau, Canedo & Pombal, 2008 or *Hylodes heyeri* Haddad, Pombal & Bastos, 1996 [3]	NMNH-Animalia_BR 125509
Leptodactylus bolivianus Boulenger, 1898	*Leptodactylus chaquensis* Cei, 1950 [4]	DZSJRP-Amphibia-adults 8625
Leptodactylus ocellatus Girard, 1853	*Leptodactylus latrans* (Steffen, 1815)	CFBH 21025
Physalaemus biligonigerus (Cope, 1861)	*Physalaemus biligonigerus* (Cope, 1861)	ZUEC-AMP 10371
Physalaemus marmoratus (Reinhardt & Lütken, 1862)	*Physalaemus marmoratus* (Reinhardt & Lütken, 1862)	DZSJRP-Amphibia-tadpoles 0832.03
Pseudopaludicola falcipes (Hensel, 1867)	*Pseudopaludicola falcipes* (Hensel, 1867)	NMNH-Animalia_BR 149646
Elachistocleis ovalis (Schneider, 1799)	*Elachistoclei bicolor* (Guérin-Méneville, 1838)	MZUEL-Herpeto 671

We identified 29 localities at which anurans have been inventoried in the state of Paraná, of which, just under half (48.3%) are located with the metropolitan region of Curitiba city, while a further 34.5% are found within the central-northern region of the state (Figure 2), creating a highly disproportionate distribution of sampling sites within the state.

Discussion

The 137 anuran species recorded in the present study for the Brazilian state of Paraná is close to the 147 species predicted for the state by Toledo & Batista (2012), and thus appears to be a relatively reliable estimate. In that study, the authors constructed a Brazilian list of species based on range distribution shapes (shadow maps analysis), including species occurrences by Brazilian states, and therefore, we assume that species recorded by them to Paraná but not registered in our study do not occur in the state; these anuran species are: *Ischnocnema manezinho* (Garcia, 1996), *Melanophryniscus spectabilis* Caramaschi & Cruz, 2002, *Rhinella granulosa* (Spix, 1824), *Cycloramphus diringshofeni* Bokermann, 1957, *Proceratophrys appendiculata* (Günther, 1873), *Fritziana goeldii* (Boulenger, 1895), *Dendropsophus elianeae* (Napoli & Caramaschi, 2000), *D. rubicundulus* (Reinhardt & Lütken, 1862), *Boana geographica* (Spix, 1824), *B. guentheri* (Boulenger, 1886), *Phyllomedusa burmeisteri* Boulenger, 1882, and *Pithecopus hypochondrialis* (Daudin, 1800). However, some of the taxa

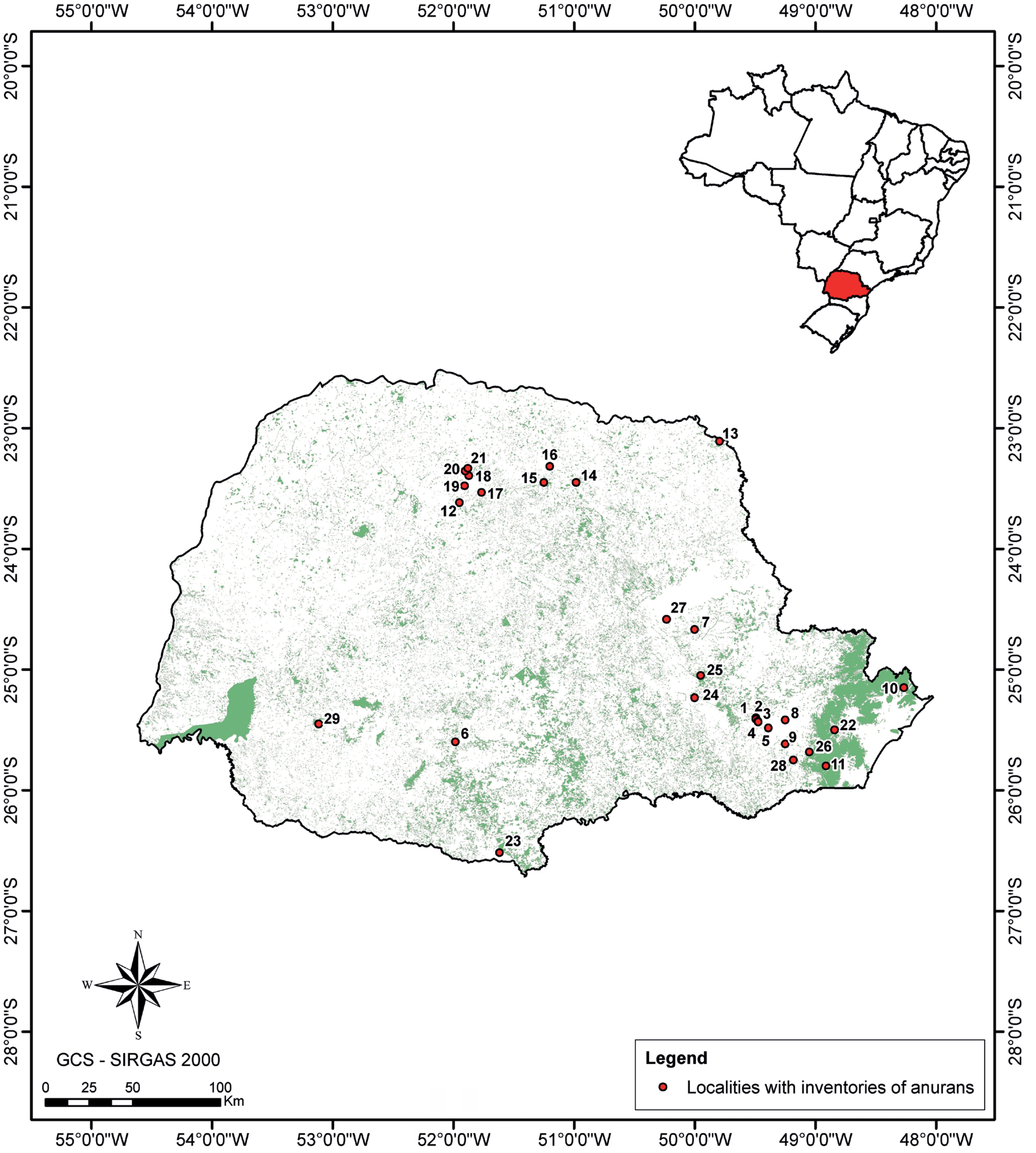

Figure 2. Municipalities with inventories of anurans in the state of Paraná, southern Brazil. 1-5 = Campo Largo (Leivas & Hiert 2016); 6 = Candói, Pinhão and Foz do Jordão (Crivellari et al. 2014); 7 = Castro (Crivellari et al. 2014); 8 = Curitiba (Crivellari et al. 2014); 9 = Fazenda Rio Grande (Conte & Rossa-Feres 2007); 10 = Guaraqueçaba (Garey & Hartmann 2012, Santos-Pereira et al. 2011, 2016); 11 = Guaratuba (Cunha et al. 2010); 12 = Itambé (Affonso & Delariva 2012); 13 = Jacarezinho (Nazaretti & Conte 2015); 14-16 = Londrina (Affonso & Delariva 2012, Benarde & Anjos 1999, Machado et al. 1999); 17 = Marialva (Affonso & Delariva 2012); 18-21 = Maringá (Affonso et al. 2014); 22 = Morretes (Armstrong & Conte 2010); 23 = Palmas (Crivellari et al. 2014); 24 = Ponta Grossa (Crivellari et al. 2014); 25 = Ponta Grossa, Castro and Carambeí (Crivellari et al. 2014); 26 = São José dos Pinhais (Conte & Rossa-Feres 2006); 27 = Tibagi (Crivellari et al. 2014); 28 = Tijucas do Sul (Conte & Machado 2005); and 29 = Três Barras do Paraná (Bernarde & Machado 2001).

recorded in Paraná require revision. For example, eight species were identified “aff.”, “cf.” or “gr.” in the articles consulted. One of them is *Adenomera* cf. *bokermanni*, cited by Santos-Pereira et al. (2016), and *A.* aff. *bokermanni* by Armstrong & Conte (2010) and Garey & Hartmann (2012). This species was originally described as *Adenomera bokermanni* by Heyer (1973), type locality in the municipality of Paranaguá, state of Paraná, and it seems very likely that the taxon cited in more recent studies corresponds to this species, given that these records refer to localities extremely close to the type locality of *A. bokermanni*, that is, municipalities of Morretes (Armstrong & Conte 2010) and Guaraqueçaba (Garey & Hartmann 2012, Santos-Pereira et al. 2016). *Ischnocnema spanios* (cited as “cf.” in Santos-Pereira et al. 2016) is known to occur only from the minicipalyti of Boracéia (type locality of this species, Heyer, 1985) to the municipality of São Paulo (L. Malagoli, pers. comm.). Santos-Pereira et al. (2016) collected an anuran of the genus *Cycloramphus*, which they identified as *C.* cf. *asper*, although vouchers were juveniles (MNRJ 87917-87918), which hampered the identification of the species. The occurrence of *Hylodes* aff. *asper* by the first time by Garey & Hartmann (2012) in the municipality of Guaraqueçaba may have been a mistake. Some years previously, Lingnau et al. (2008) described this species from a nearby municipality in the state of Paraná, Morretes, which is less than 90 km from Guaraqueçaba, and discussed the geographic distribution of *Hylodes asper* (Müller*,* 1924). For many years, herpetologists believed that the geographic range of *H. asper* extends from the state of Santa Catarina to the state of Rio de Janeiro, although this species has often been confused with *H. perplicatus* (Miranda-Ribeiro, 1926) or *H. cardosoi* Lingnau, Canedo & Pombal, 2008. The geographic range of *H. asper* is now restricted to an area from the state of Rio de Janeiro to Paranapiacaba, municipality of Santo André in the state of São Paulo, while *H. cardosoi*, but not *H. asper*, is found in the south of state of *São Paulo* (Lingnau et al. 2008). Therefore, we believe that the record of *Hylodes* aff. *asper* from Salto Morato, municipality of Guaraqueçaba, does in fact refers to *H. cardosoi*, but unfortunately, there are no voucher specimens or vocal records that might help confirm this hypothesis. *Boana stellae* (Kwet, 2008) is known only from the southern slope of the Araucaria Plateau in the center of the state of Rio Grande do Sul (Kwet 2008). *Ololygon perpusillus* is apparently restricted to the municipality of Rio de Janeiro (Peixoto 1987). *Elachistocleis cesarii* is known from northeastern Brazil, in the states of Ceará, Sergipe, and Bahia, central Brazil, in the states of Mato Grosso, Goiás, and Federal District, and southeastern Brazil, in the states of Minas Gerais, Espírito Santo, Rio de Janeiro and São Paulo (Caramaschi 2010). It is important to note that *Fritziana* gr. *fissilis* was recorded in two separate studies at the same locality, municipality of Guaraqueçaba, on the northern coast of the state of Paraná (Garey & Hartmann 2012, Santos-Pereira et al. 2016). In this case, further fieldwork will be required to confirm the occurrence of the species in the state of Paranábecause the genus *Fritziana* requires a taxonomic review and the specimens cannot be assigned reliably to a specific species at the present time.

In addition to these taxonomically problematic anurans, several other species assigned to Paraná by Frost (2017) were not included in our list. As we found no other reliable evidence or records of the occurrence of these species in Paraná, we did not include them in our inventory. These species are *Boana curupi* (Garcia, Faivovich & Haddad, 2007), *Bokermannohyla claresignata* (Lutz & Lutz, 1939), *Trachycephalus imitatrix* (Miranda-Ribeiro, 1926), *Cycloramphus izecksohni* Heyer, 1983 and *Boana crepitans* (Wied-Neuwied, 1824). In the case of *B. claresignata*, the only evidence is the record of Lutz (1973), who found tadpoles similar to those described for the species, but don’t recorded any adult individual. Garcia et al. (2007) assumed that *B. curupi* occurs in Paraná, due to its association with the Paraná and Uruguay rivers, but we don’t find any record of this anuran in the state of Paraná. Lutz (1973) found that the *T. imitatrix* specimens from southern Brazil (the “southern” form) were relatively large in size, and had a distinct type of iris, which is consistent with the description of *T. dibernardoi* Kwet & Solé, 2008. The analysis of the specimens from the municipality of Telêmaco Borba revealed the larger body size and dorsal markings typical of *T. dibernardoi* (Conte et al. 2010). In addition, the description of *T. dibernardoi* for the region near Curitiba (Kwet & Solé 2008) refers to a voucher specimen from a survey of the Fazenda Rio Grande (DZSJRP 8810; Conte & Rossa-Feres 2007), where this anuran was confused with *T. imitatrix* (Conte et al. 2010). Given this evidence, we concluded that *T. dibernardoi*, rather than *T. imitatrix*, occurs in Paraná, as recorded by Lutz (1973), and that the occurrence of *T. imitatrix* recorded *by* Conte & Rossa-Feres (2006, 2007) at São José dos Pinhais and Fazenda Rio Grande does in fact refer to *T. dibernardoi*. In the case of *C. izecksohni*, Frost (2017) only mentioned that the species occurs in the Serra do Mar coastal range, in the states of São Paulo, Paraná and Santa Catarina. Frost (2017) considered *B. crepitans* to occur in northern Brazil, with an allopatric population in the northeast, ranging as far south as Paraná and Santa Catarina. On these last two localities, we found available information about this anuran species only from the referred website.

Boana faber, *Scinax fuscovarius*, *Dendropsophus minutus* and *Ischnocnema henselii* were the anurans recorded most frequently in the studies analyzed. This reflects, in part, the wide geographic distribution of these species in Brazil (Frost 2017), as well as their life history strategies. In the case of the hylids, for example, populations are often associated with open areas or even disturbed habitats (Lucas & Fortes 2008; Almeida-Gomes et al. 2010, 2014), resulting in a greater tolerance of these species to anthropogenic impacts in the environment. In particular, *I. henseli*, which is found in the leaf litter, was considered to be one of the dominant species of the anuran community at Guaraqueçaba (Santos-Pereira et al. 2011), given the considerable variety of microhabitats found in the leaf litter of the forest floor. This suggests that *I. henseli* besides frequent, is also an abundant leaf-litter frog found in the forest floor, and this possibly also indicates a greater environmental tolerance by this frog.

By contrast, 32 (23.3%) of the 137 anuran species identified in the literature search were recorded in only one study, and most of these species are endemic to Paraná or occurs only also in the region that includes the neighboring states of Santa Catarina and São Paulo. While these records may reflect a relatively restricted distribution for these species, they may also be the result of subsampling in Paraná, given that research on anurans in this state is still incipient (Santos-Pereira 2016) with inventories of anuran species concentrated basically in two regions of state (see discussion below). The restricted occurrence of many species may also be related to the devastation of the state’s forests, which has often impacted their structure, resulting in direct effects on their anuran species richness, including the possible eradication of the most sensitive forms. Only 11.7% of the original Atlantic Forest cover of

Paraná now remains, and it has the highest historic rate of deforestation of any state of the Brazil (Fundação SOS Mata Atlântica & Instituto Nacional de Pesquisas Espaciais 2015).

Eleven (57.9%) of the 19 endemic species recorded in the present study are brachycephalids, and nine of these are *Brachycephalus* species, which is 26.5% of the 34 species currently recognized for this genus (Frost 2017). The species of this genus are found on the eastern coast of Brazil between the states of Bahia, in the northeast, and Santa Catarina, in the south (Frost 2017), with most occurring at altitudes of over 600 m in the Serra do Mar (Ribeiro et al. 2005, Alves et al. 2006). Many of these species have been described recently (Garey et al. 2012b, Ribeiro et al. 2015, 2017, Bornschein et al. 2016a), which emphasizes the importance of surveys in the least well-known remnants of Atlantic Forest, which are often found in relatively inaccessible areas of high altitude. The biodiversity corridors of the Atlantic Forest – the Central Atlantic Forest corridor, the Serra do Mar corridor, and the Northeast corridor (Rocha et al. 2003) – contain a considerable number of endemic vertebrates, including anuran amphibians, and the coast of Paraná is located within the largest continuous remnant of Atlantic Forest, which is part of the Serra do Mar corridor (Câmara 2005), which implies that the state may have more endemic anurans than currently known. This is consistent with the position of Pimm et al. (2010), who predicted that many endemic species of amphibians, birds, and mammals are yet to be discovered in the Brazil.

The type localities of the anuran species described from specimens collected in Paraná are concentrated in the east of the state. This probably reflects the greater research efforts in this region, where we recorded the highest number of localities with anuran inventories, and also to the principal remnants of native forest (Câmara 2005). Additionally, this can be reflected the flora inventories and the proximity of large urban centers or the presence of specialists in the taxonomic groups in these urban areas. Another factor is the presence (and density) of roads, which implies accessibility to natural areas (Pautasso 2007, Ficetola et al. 2013).

In the state of Paraná, the exotic invader anuran *Lithobates catesbeianus* has been recorded in 15 municipalities up until now (Santos-Pereira & Rocha 2015). In general, these municipalities are located within a 60 km radius of commercial frog farms, indicating that, in most cases, the invasion of natural areas by *L. catesbeianus* has resulted from the escape of captive individuals into the natural environment (Santos-Pereira & Rocha 2015). As the records show that *L. catesbeianus* is widely distributed in different regions of the state, it is possible that the actual area occupied by the bullfrog in the state of Paraná is much larger than currently thought. Further surveys in the state may provide new evidence on the extent of the area invaded by *L. catesbeianus*.

The red lists consulted during the present study revealed several inconsistencies in the conservation status of the anuran species identified in the state of Paraná. Some of these discrepancies may be the result of a certain asynchrony between the regional, national and global assessments (Rodríguez et al. 2000). The conservation status of non-endemic species will also vary according to the scale of analysis, even though it should be consistent among the state, national and global red lists (Brito et al. 2010). This indicates the existence of discrepancies between the national and global lists, given that two species endemic to the state of Paraná (*B. pernix* and *I. paranaensis*) are listed as DD by the IUCN, although *I. paranaensis* is not even listed by the Brazilian government. Similarly, *Pseudis cardosoi* is listed by the IUCN, but does not appear on the lists for Paraná or Brazil. In the specific case of the State list, the publication is more than 10 years old (Segalla & Langone 2004), and requires revision, whereas on a broader scale, species not endangered in Paraná may be under threat in other states. As the regional lists are not updated regularly, they tend to provide outdated assessments of conservation status. This situation may reduce the effectiveness of these lists as guidelines for conservation practices, given that most measures are currently based on the evaluation of the extinction risk of the species (Miller et al. 2006). It is important to note that the regional lists are important conservation tools, given that most measures are implemented on a local scale (Possingham et al. 2002). Half of the anuran species recorded by us in this study that were categorized as data deficient in Paraná list or by the IUCN are endemic of this state. This reinforces the need for further research into the viability of their populations and the habitats they occupy, and their potential risk of extinction.

Based on the assessment of the IUCN (2017), most of the anuran species recorded in the present study – predominantly hylids – have stable populations. This is probably at least partly due to the fact that these are among the most abundant species in anuran communities, in particular in the Atlantic Forest (e.g., Conte & Rossa-Feres 2006, Almeida-Gomes et al. 2010, Santos-Pereira et al. 2016). For example, the three species recorded most frequently in the publications analyzed in the present study (*Boana faber*, *Dendropsophus anceps* and *Scinax fuscovarius*) are known to be very common species (IUCN 2017), and *S. fuscovarius* is common even in deforested areas. The majority of leptodactylids have stable populations, and many of them [e.g., *Adenomera marmorata* Steindachner, 1867, *Physalaemus cuvieri* Fitzinger, 1826 and *Leptodactylus mystaceus* (Spix, 1824)], are common throughout their geographic ranges (IUCN 2017). Populations are declining in 26 species, which may reflect impacts on the ecosystems in which these anurans are found, in particular the high deforestation rates recorded throughout the state of Paraná. For example, *Aplastodiscus ehrhardti* (Müller, 1924), *Proceratophrys brauni* Kwet & Faivovich, 2001 and *Scythrophrys sawayae* (Cochran, 1953) are relatively common species and their populations, while classified as declining by the IUCN, are stable in suitable habitats. Other example is *Pleurodema bibroni*, a rare species that occurs in widely scattered populations, being probably extinct in Montevideo Department, Uruguay, because of habitat destruction (IUCN 2017). The populations of only two anuran species listed here (*Rhinella schneideri* and *Lithobates catesbeianus*) are thought to be increasing (IUCN 2017). *Lithobates catesbeianus* is widespread in Paraná (Santos-Pereira & Rocha 2015). This species is known worldwide from thousands of localities and is usually very abundant with increasing populations (IUCN 2017). In 28 cases, mostly brachycephalids and bufonids, population trends are unclear, although some brachycephalids, such as *Brachycephalus brunneus*, *B. ferruginus*, *B. izecksohni*, *Ischnocnema henselii* and *I. sambaqui*, are locally abundant (IUCN 2017). No data are available on the population trends of other brachycephalids, i.e., *B. pombali* and *I. spanios* (IUCN 2017). The populations of the *Brachycephalus* species are typically restricted to fragments of hill forests (Pombal et al. 1998, Ribeiro et al. 2005, Alves et al. 2006), separated by valleys, which isolates the populations (Pie et al. 2013) and makes them difficult to survey effectively. In the case of the bufonids, *Dendrophryniscus stawiarskyi*, for example, is known only from three specimens collected in the early 1980s, and there is currently no information on its population status, while the species of the genus *Melanophryniscus*, *M. alipioi* and *M. tumifrons* (Boulenger, 1905), are extremely difficult to survey, although the former species is commonly encountered during the breeding season (IUCN 2017). This possibly is due to the fact that the anuran species of this genus show explosive breeding, reproducing in small and ephemeral water bodies (e.g. Cairo et al. 2008, Laufer et al. 2015). Given this scenario, we identified three main variables that are important for the evaluation of the population viability of anurans – the abundance of the species, the difficulty of capturing specimens and the quality of the environment inhabited by the population.

While we identified an additional 10 anuran species during the survey of the scientific collections available in the SpeciesLink database, we did not include these species in our inventory of the anurans of the state of Paraná. The record of *Allobates brunneus*, for example, is almost certainly erroneous, given that the genus *Allobates* is not found in Paraná (Verdade & Rodrigues 2007). While it may be possible that *Vitreorana eurygnatha* occurs in Paraná, we found only a single specimen in the Brazilian National Museum of Natural History (NMNH), so at this time, we prefer to exercise caution, and not confirm that the species occurs in Paraná. Similarly, we have classified *Aplastodiscus cochranae*, *A. leucopygius*, *Crossodactylus gaudichaudii*, *Leptodactylus chaquensis* and *Physalaemus biligonigerus* as tentative occurrences, pending the confirmation of the taxonomic identification of the specimens. In the specific case of *Boana polytaenia*, we consider the identification doubtful, given that Cruz & Caramaschi (1998) did not refer to the occurrence of the species in Paraná in their review [the only species of this complex known unequivocally to occur in the state of Paraná, at the present time, and that was included in our list, is *Boana jaguariavensis* (Caramaschi, Cruz & Segalla, 2010)]. It seems likely the record of *Ololygon brieni* does in fact refers to *O. littoralis* (Pombal & Gordo 1991). Finally, it does seem likely that *Pseudopaludicola falcipes* occurs in Paraná, given that this species is cited by Frost (2017).

The inventories of anurans in the state of Paraná have been concentrated primarily in the metropolitan region of the Curitiba city, in the eastern extreme, and the central-northern region of the state, while there are severalgeographic distribution gaps in most of the state. This concentration of research in the north and east of the state reflects the proximity of its principal universities, including the Federal University of Paraná, in the Curitiba city, and the State universities of Londrina and Maringá, both located in the central-northern region. The metropolitan region of Curitiba, which extends as far as the Atlantic coast, also encompasses several other institutions, including universities and museums, and a major portion of the largest continuous remnant of Atlantic Rainforest found in Brazil. This region also contains a number of protected areas, which may stimulate the interest of researchers seeking well-preserved environments and faunal communities. It is interesting to note that there has been no inventory of anurans in the Foz do Iguaçu micro-region, in western Paraná, which includes the Iguaçu National Park, one of Brazil's first national parks, created in 1939.

This study was the first attempt to compile a comprehensive inventory of the anuran fauna of the Brazilian state of Paraná. We consider our list of species to be a relatively reliable estimate of the anuran diversity of the state, although we do expect new species to be added to the list, in particular because many areas have yet to be sampled adequately. In addition to the need for more extensive surveys, especially in areas that have yet to be sampled adequately, a number of forms require taxonomic review. We also emphasize the need for the conservation of the state's remaining natural habitats, as well as further research on the invasion of the region by the exotic frog, *Lithobates catesbeianus,* especially in relation to the escape and dispersal of animals from local frog farms.

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Author Contributions

Manuela Santos-Pereira: Substantial contribution to the conception and design of the work; Contribution in the acquisition of the data; Contribution in the analysis and interpretation of the data; Contribution in the writing of the work; Contribution in the critical review appending intellectual content.

José P. Pombal Jr.: Substantial contribution in the acquisition of the data; contribution in the analysis and interpretation of the data; contribution in the writing of the work; contribution in the critical review appending intellectual content.

Carlos Frederico Duarte Rocha: Substantial contribution to the conception and design of the work; contribution in the acquisition of the data; contribution in the analysis and interpretation of the data; contribution in the writing of the work; contribution in the critical review appending 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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The importance of protected areas for conservation of bare-faced curassow (*Crax fasciolata* spix, 1825) (Galliformes: Cracidae) in the São Paulo State, Brazil

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Abstract: The Bare-faced Curassow *Crax fasciolata* (Cracidae:Cracinae) is a vulnerable species that was classified as critically endangered by the São Paulo State -Brazil, in 2008. Specialists recommended "searches for areas where there may be a few remaining populations and protection of the last remaining semideciduous forest in the State". Protected areas were investigated in the Cerrado and semideciduous forest in São Paulo State by camera trapping to evaluate the presence of the Bare-faced Curassow. Eight protected areas in Cerrado biome were investigated, but the Bare-faced Curassows was recorded only at Furnas do Bom Jesus State Park (2,069 ha), São Paulo State despite the large sampling effort in larger protected areas such as the Santa Bárbara Ecological Station (2,712 ha) and Jataí Ecological Station (9,074 ha); the largest protected area of Cerrado biome in São Paulo State. This record of *C. fasciolata* is important for the Conservation Action Plan for the Cracid family and shows the importance of monitoring biodiversity and conserving even relatively small remnants of native vegetation for the conservation of this species.
Keywords: *Cerrado, camera trap, conservation, endangered birds.*

A importância das áreas protegidas na conservação do mutum-de-penacho (*Crax fasciolata* spix, 1825) (Galliformes: Cracidae) no estado de São Paulo, Brasil

Resumo: O mutum-de-penacho *Crax fasciolata* (Cracidae:Cracinae) é uma espécie ameaçada de extinção e foi considerada criticamente em perigo em 2008 no estado de São Paulo. As recomendações dos especialistas para a conservação da espécie no estado foram "a pesquisa por áreas onde poderia existir populações remanescentes e a proteção de remanescentes de floresta semidecidual". Neste trabalho, oito Unidades de Conservação foram investigadas em áreas com vegetação de Cerrado e de floresta semidecidual por meio de armadilhas fotográficas digitais para verificar a presença do mutum-de-penacho (*Crax fasciolata*). Entretanto, o mutum-de-penacho foi registrado apenas no Parque Estadual Furnas do Bom Jesus, que possui 2.069 ha, apesar do maior esforço amostral em áreas maiores, como a Estação Ecológica de Santa Bárbara (2.712 ha) e Estação Ecológica de Jataí (9.074),- a maior Unidade de Conservação do bioma Cerrado no estado de São Paulo. O registro do mutum-de-penacho no Parque Estadual Furnas do Bom Jesus é muito importante para o Plano de Ação e Conservação da família Cracidae e mostra a importância do monitoramento da biodiversidade e a conservação de remanescentes de vegetação nativa, mesmo pequenos, para a conservação dessa espécie.
Palavras-chave: *armadilhas fotográficas, aves ameaçadas, Cerrado, conservação.*

Introduction

Among groups of birds quite affected by human activities is the Cracidae family, the most threatened among birds on a global level (Pinilla-Buitrago et al. 2014). This family is endemic to the Neotropics and consists of a group of large frugivorous birds and seed eating (Sick 1997, Brooks and Strahl 2000). Brazil has the second highest diversity of cracids in the world (22 species; Silveira et al. 2008) and the highest number of endemic species of any Neotropical country, with approximately half of the Guans and Curassows considered vulnerable or at risk (Brooks and Strahl 2000). Five of its six endemic species and two of its six endemic subspecies are on the priority list (Brooks & Strahl 2000). Of particular interest for conservationists are the eastern and central forests, where a number of species are now endangered or highly vulnerable (Brooks & Strahl 2000). The main threats to the species of cracids are poaching, habitat fragmentation, deforestation and predators such as domestic dogs *Canis familiaris* (Sick 1997, Brooks and Strahl 2000).

In the São Paulo State, there are five species of cracids (Silveira & Uezu 2011): *Ortalis guttata* (Spix1825), *Penelope superciliaris* Temminck, 1815 LC, *Penelope obscura* Temminck, 1815 LC, *Pipile jacutinga* (Spix 1825) and, *Crax fasciolata* Spix, 1825. The last two species are considered endangered and vulnerable, respectively (IUCN 2014). Absence of cracids in tropical forests can affect several ecological processes since they are important seed dispersers and are therefore very important component in the community to maintain forest ecosystem (Brooks & Fuller 2006, Galetti et al. 1997).

The bare-faced curassow *Crax fasciolata* (Cracidae:Cracinae) is a vulnerable species (IUCN 2014) that occurs in the eastern-central and southern Brazil, Paraguay, eastern Bolivia and Argentina (Delacour & Amadon 2004, Pereira & Brooks 2006). It is mostly terrestrial, occurring in semi-deciduous and gallery forests, but also on forest edges and forest clearings (Lowen et al. 1996, Wallace et al. 2001, White 2001, Pereira & Brooks 2006). The species is either solitary or live in pairs, although it has also been reported group of males (Pereira & Brooks 2006, Desbiez & Bernardo 2011). The bare-faced curassow feeds mostly on fruits, but also on seeds, flowers and invertebrates (Delacour & Amadon 2004, Muñoz & Kattan 2007), playing an important role as seed disperser and on forest regeneration, especially in forest clearings (Silva & Strahl 1991, Golçalves et al. 2010, Langanaro 2013). The main threats to *C. fasciolata* are habitat loss and poaching (Del Hoyo 1994, Caziani et al. 1997, Brooks 1999).

In Brazil, the bare-faced curassow has been reported from south of the Amazon River to the western State of São Paulo and Minas Gerais (Sick 1997). Despite having a wide distribution, the species is endangered in Minas Gerais State (Minas Gerais 2010) and critically endangered in Paraná State (Paraná 2004). In 1993, the species was considered disappearing species in São Paulo (Willis & Oniki 1993); later, other authors argued that the species was "practically extinct in the State" (Bressan et al. 2009, São Paulo 2010).

The IUCN Cracid Action Plan created in 2000 by the Cracid Specialist Group (CSG) highlights the importance of conserving the Cracid family and lists the *C. fasciolata* as of high conservation priority (BirdLife International 2009, Langanaro 2013), indicating the need of research analyzing the protected areas of Brazil, the role of cracids as environmental indicator species, and the conservation of priority species. In 2014, it was recommended guidelines for *C. fasciolata* conservation management in the São Paulo State (São Paulo, 2014). Despite such recommendations, knowledge of the species occurrences is still lacking in many regions, even in protected areas. In São Paulo State, Brazil, for instance, there are gaps of bird inventory in the north, west and southern regions (Silveira & Uezu 2011). In this study, we performed an inventory in eigth protected areas in the north of São Paulo State using camera-traps. We reported *C. fasciolata* for the first time in the Furnas do Bom Jesus State Park, showing the importance of this protected area for conservation of this species in the São Paulo State.

Material and Methods

The study was conducted in eigth areas of Cerrado vegetation included in the original distribution of *C. fasciolata*:

1) Furnas de Bom Jesus State Park (Furnas) (20° 11' S/47° 22' W) located in the northern São Paulo State, Brazil (Figure 1). The park (2,069 ha) is a Cerrado fragment surrounded by an altered matrix composed of pastures, coffee plantations, roads, and human settlements. The park does not have any recent avian inventory published;

2) Jataí Ecological Station (Jataí) located in the northeastern of São Paulo State (21° 30' S/47° 40' W) is one of the largest remnants of the Cerrado biome in the state (9,074 ha; Figure 1). The surroundings of the Jataí consists mainly of sugar cane plantations, forest plantation (*Eucalyptus* sp. and *Pinnus* sp.), citrus plantations, pastures and roads such as the SP-253 highway. There are 211 bird species recorded for the area (Almeida 2002);

3) "Augusto Ruschi" Biological Reserve (Augusto Ruschi) is located in the municipality of Sertãozinho, in northeastern São Paulo State (21° 10' S/48° 05' W; Figure 1). It has a total area of 757 ha divided into five forest fragments ranging from 55.07 ha to 189.21 ha that are 300-1000 m apart from each other. The Augusto Ruschi consists of pasture and forest fragments of seasonal semi-deciduous forest. In addition to pasture, the area is surrounded by sugar cane plantations and is crossed by the SP-333 highway;

4) Bebedouro State Forest (Bebedouro) is located in the municipality of Bebedouro, northeastern São Paulo State (20° 57' S/48° 27' W) (Figure 1). It has an area of 99.41 ha semi-deciduous forest and forest plantation (*Eucalyptus* sp. and *Pinnus* sp.) surrounded by sugar cane plantations and pasture. There are 37 species of birds recorded in this area (Lopes et al. 2007);

5) Santa Bárbara Ecological Station (Santa Bárbara) is located in the municipality of Águas de Santa Barbara, central-southern São Paulo State (22° 46' S/49° 10' W), and has a total area of 2,712 ha (Figure 1). It consists largely of Cerrado, being composed predominantly by grassland and savanna-type formations surrounded by forest plantations (*Eucaliptus* sp. and *Pinus* sp.), pastures, and sugar cane plantations. There are 226 species of birds recorded in this area (Lucindo et al. 2015).

6) Porto Ferreira State Park (Porto Ferreira) is located in the municipality of Porto Ferreira (21° 49' S and 27 ° 25' W) and has a total area of 637 ha. It is composed of remnants of semideciduous and closed seasonal forest surrounded by sugarcane cultivation, forest plantations (*Eucalyptus* sp. and *Pinus* sp.), citrus and pasture (Mendes et al. 2009). There are 186 species of birds recorded in this area (São Paulo 2003).

7) Experimental Station Santa Rita do Passa Quatro (Santa Rita do Passa Quatro) is located in the municipality of Santa Rita do Passa Quatro, SP (21º 44' S and 47º 29' W), and consists of three fragments

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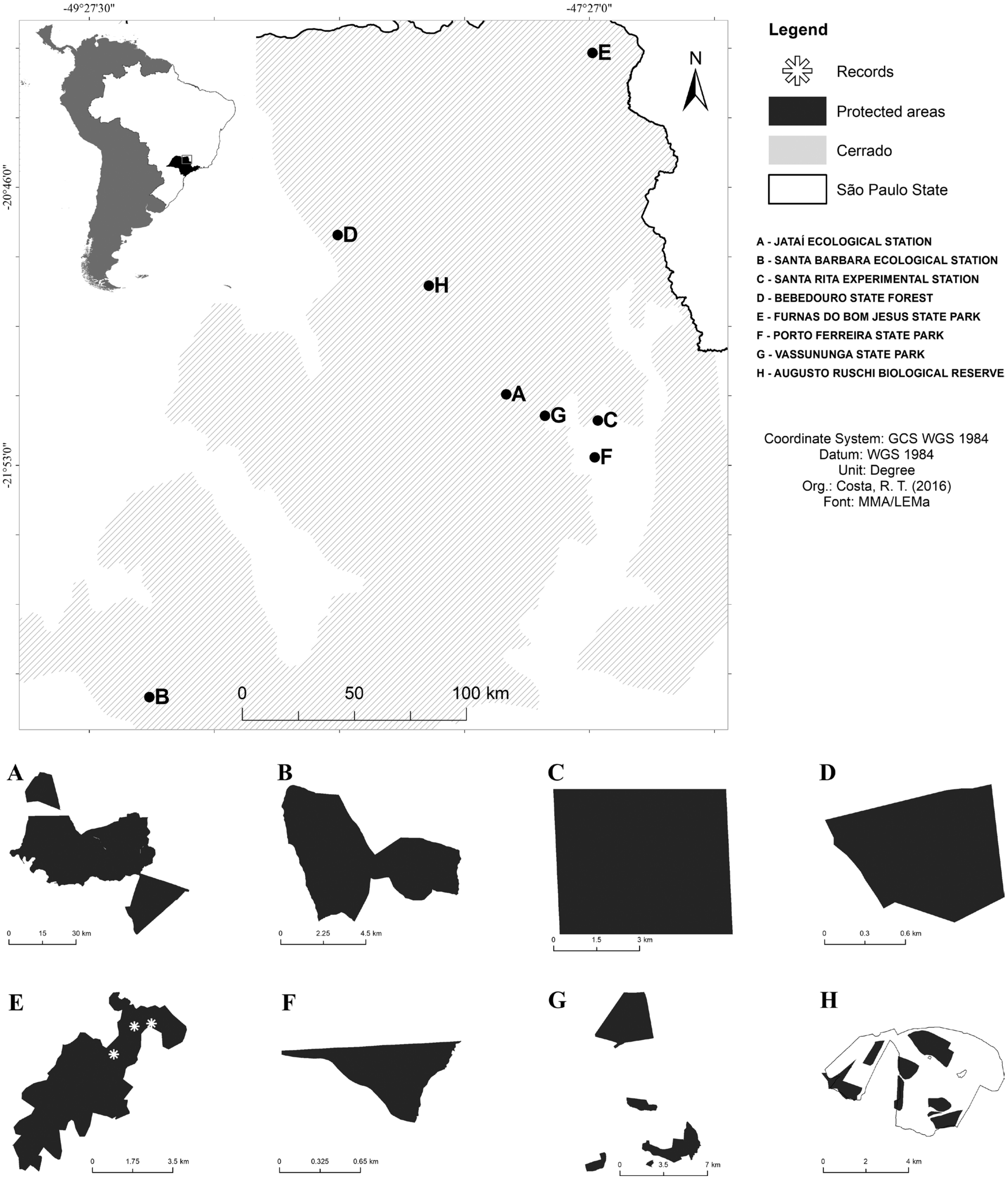

Figure 1. Maps of the areas sampled in São Paulo State, Brazil. (A) Jataí Ecological Station, Luiz Antônio, SP. (B) Santa Bárbara Ecological Station, Águas de Santa Bárbara, SP. (C) Santa Rita Experimental Station, Santa Rita do Passa Quatro, SP. (D) Bebedouro State Forest, Bebedouro, SP. (E) Furnas do Bom Jesus State Park, Pedregulho, SP. (F) Porto Ferreira State Park, Porto Ferreira, SP. (G) Vassununga State Park, Santa Rita do Passa Quatro, SP. (H) Augusto Ruschi Biological Reserve, Sertãozinho, SP.

with total areas of 9.1 ha, 19.1 ha and 51 ha. The vegetation of the smaller fragment is constituted by semideciduous seasonal forest and pinus plantations, the fragment with area of 19.1 ha consists of cerrado vegetation, semideciduous seasonal forest and pine plantations, and the largest fragment by plantations of different species of pine and eucalyptus with some sparse native trees. The Station is divided by two highways, Zequinha de Abreu (SP-241) and Luiz Pizetta (SP-328), and its surroundings are predominantly composed of sugar cane.

8) Vassununga State Park (Vassununga) is located in the municipality of Santa Rita do Passa Quatro, and consists of six fragments which together correspond to 2,071.42 ha. For this work, five fragments (130 ha, 169 ha, 231 ha, 329 ha, and 1,217.13 ha) were sampled, all of them consisting of semideciduous seasonal forest, with the largest fragment being partially composed of cerrado vegetation. The park is divided by the Anhanguera Highway and its surroundings consists predominantly of sugarcane and forest plantations (*Eucalyptus* sp. and *Pinus* sp.), citrus and pasture. There are 259 species of birds recorded in this area (São Paulo 2009).

All areas were sampled using camera traps (Scoutguard®-SG560C and Bushnell®-Trophy Cam) distributed 0.5-1km apart from each other. When triggered, cameras took pictures with 10 to 30 second intervals. The Furnas de Bom Jesus State Park was sampled twice: in 2011 it was sampled using 36 trapping stations baited with bobcat urine (*Lynx rufus* BobcatPee®) bacon, banana, pineapple and salt, adding up a total sampling effort of 1189 trap-days; in 2013, the park was sampled with 16 trapping stations without bait and a total sampling effort of 1385 trap-days (Table 1).

The Augusto Ruschi was sampled from October 2012 to May 2013 using a total of 20 trapping stations spaced 500 m apart from each other in the five fragments comprising the reserve. The fragments were sampled one at a time, and the number of trapping stations in each fragment varied from two to six. The cameras were baited with bobcat urine (*Lynx rufus*, BobcatPee®), banana and bacon. Total sampling effort was 562 trap-days.

In all other areas we did not use bait and trapping stations were located 1 km apart from each other. From October 2014 to December 2014, Bebedouro was sampled with four stations and a total sampling effort of 190 trap-days. From January 2014 to January 2015, we established a grid of 41 stations in Jataí with total sampling effort of 2,758 trap-days. In Santa Barbara, cameras were placed in 26 stations from December 2014 to March 2015 totaling 1891 trap days. Porto Ferreira was sampled from July to October 2015 with total sampling effort of 639 trap-days and nine stations. Vassununga was sampled from November 2015 to April 2016 with total sampling effort of 1585 trap-days and 25 trap stations. Santa Rita do Passa Quatro was sampled from March to June 2016 with total sampling effort of 395 trap-days and five trap stations (Table 1).

We also performed a literature search for studies of avian inventory in São Paulo State in Web of Science and Scielo using the keywords “birds of São Paulo State”. We also used records available in the book “Aves do Estado de São Paulo” where there are informations of bare-faced curassow in museum, field and literature. We also performed a search in Scielo and Google with the same keywords in Portuguese to look for additional papers, as well as thesis, dissertations or monographs. Finally, the management plans of protected areas of Cerrado bioma in São Paulo State were verified for their respective lists of avian species (Fundação Florestal e Instituto Florestal).

We did not use records from the Wikiaves Platform because the geographic coordinates of records were not available. Besides, Wikiaves shows records of *C. fasciolata* in cities such as Bebedouro, an area with few remaining native areas; in this case, we considered the presence of this species as a possible individual translocation from other areas because is the photographed individual had leg rings. Moreover, there were no official records of *C. fasciolata* in other areas that have been well inventoried, such as São Carlos e Itirapina, but Wikiaves shows records on them (Table 2). Considering such incongruences, we decided not to use information available on that platform; only records published on scientific journals, thesis, dissertation and management plans were considered.

Results

Our total sampling effort was 10,131 trap-days, which is equivalent to 243,144 hours, from 2011 to 2016 (Table 1). Despite the large sampling effort, we recorded bare-faced curassows only at Furnas, one of the areas with 2,069 ha and for which the sampling effort was 2,574 trap-nights. Two individuals – a male and female-were recorded during six days in August and September 2011 at two trapping stations; in

Table 1. Sampled areas, size (ha), time period sampled, sampling effort (number of camera-traps ×number of nights), number of trapping stations and areas where the bare-faced curassow (*Crax fasciolata*) was recorded.

Area	Size (ha)	Time period sampled	Sampling effort in trap-days	Sampling effort in hours	Number of trapping stations	Records of *C. fasciolata*
Furnas	2,069	Jul/11 to Oct/11	1,189	28,536	36	6
Furnas	2,069	Jul/13 to Nov/13	1,385	33,240	16	3
Augusto Ruschi	720	Oct/12 to May/13	562	13,488	20	No
Bebedouro	99.41	Oct/14 to Dec/14	112	2,688	04	No
Jataí	9,074	Jan/14 to Jan/15	2,768	66,432	41	No
Santa Bárbara	2,700	Dec/14 to Mar/15	1,891	45,384	26	No
Porto Ferreira	637	Jul/15 to Oct/15	639	15,336	09	No
Vassununga	2,071	Nov/15 to Apr/16	1,585	38,040	25	No
Santa Rita do Passa Quatro	79.2	Mar/16 to Jun/16	395	5,688	05	No

Table 2. Studies of avian inventory in State of São Paulo. City = indicate where the study was conducted; Record of Wikiaves = record of bare-faced curassow in Wikiaves Platform in the same city where avian inventory was conducted; Presence of Cracide = species of Cracidae in avian inventory; Sampling method = sampling method applied in each study; Sampling effort = sample effort applied in each study; Author = authors of each study.

City	Record of Wikiaves	Presence of Cracidae	Sampling method	Sampling effort	Author
Águas de Santa Bárbara	No	*Penelope superciliaris*	Transects, engravers and observations	5 years	Lucindo et al. (2015)
Águas de Santa Bárbara		*Penelope superciliaris*	Literature review	-	São Paulo (2011)
Anhembi	No	*Penelope superciliaris*	Transects, observations and point count	8 years	Antunes (2008)
Anhembi	No	-	Transects, observations and point count	960 hours	Antunes (2007)
Bauru	No	*Ortalis supercilliaris, Penelope supercilliaris, Aburria cumanensis*	Literature review	-	Cavarzere et al. (2011)
Bauru		-	Point count	15 days	Cavarzere & Moraes (2010)
Bauru	No	*Penelope superciliaris*	Transects	50 hours	São Paulo (2010)
Bebedouro		-	Observation points were randomly distributed between the quadrants	3 months	Lopes (2007)
Brotas	No	-	Qualitative sampling by the points method	11 months	Pozza & Pires (2003)
Cajuru	No	*Penelope superciliaris*	Rapid assesment (Mackinnon method)	33 hours	Antunes (2014)
Campinas	No	*Penelope superciliaris*	Observation and point count	23 months	Aleixo & Vielliard (1995)
Gália	No	*Penelope superciliaris*	Point count (with modifications)	110 hours	Cavarzere et al. (2009)
Gália	No	*Penelope superciliaris*	Transects for counting species and individuals and recordings	14 months	Cavarzere et al. (2012)
Gália	No	*Penelope superciliaris*	Point count	15 months	Cavarzere et al. (2009)
Gália	No	*Penelope superciliaris*	Literature review	-	São Paulo (2005)
Itapetininga	No	*Penelope superciliaris*	Point count	80 hours	Donatelli et al. (2007)
Itirapina	Yes	*Penelope superciliaris*	Observations not systematized, systematic fixed points	580 hours	Motta-Junior et al. (2008)
Itirapina	No	*Penelope superciliaris*	Fixed point and observation	12 months	Telles & Dias (2010)
Itirapina	Yes	-	Transects and points count	4 months	Kanegae (2011)
Itirapina	Yes	-	Systematized transects, observation and point count	12 months	Fieker et al. (2013)
Jardinópolis	No	*Penelope* sp.	Observation	27,8 hours	Chiarello (2000)
Junqueirópolis	Yes	*Crax fasciolata*	Non-linear transects, observations, audition and recording of vocalizations	-	São Paulo (2010)
Lençóis Paulista	No	*Penelope superciliaris*	Point count	11 months	Donatelli et al. (2004)
Luís Antônio		*Penelope superciliaris*	Transects, observations and point count	920 hours	Almeida (2002)
Luiz Antônio	No	*Penelope superciliaris*	Transects, observations and point count	920 hours	São Paulo (2013)
Paraibuna	No	-	Observation and fixed point	80 hours	Rossano & Almeida (2002)
Patrocínio Paulista	No	-	Qualitative sampling by the points method	11 months	Pozza & Pires (2003)
Porto Ferreira		*Penelope superciliaris*	Observations, audition and recording of vocalizations	35 hours	São Paulo (2003)
Rio Claro	No	*Penelope superciliaris*	Play-back	353 hours	Gussoni (2007)
Santa Rita do Passa Quatro		*Penelope superciliaris*	Observations and literature review	-	São Paulo (2009)
São Carlos	Yes	*Penelope superciliaris*	Observations through transects, point count, net captures and sporadic visits	10 years	Motta-Junior & Vasconcellos (1996)
São Carlos	Yes	-	Observation and photographic records	72 hours	Mercival & Galleti. (2001)
São João do Pau D'Alho		*Crax fasciolaata*	Non-linear transects	-	São Paulo (2010)
Teodoro Sampaio		*Penelope superciliaris*	Observations, transects and literature review	-	São Paulo (2006)

September and October 2013; a female and a male were also recorded together in three distinct days (Figure 2). The individuals were recorded early in the morning (from 06h27 to 07h35) and at sunset (17h26) in 2011, as well as in the afternoon (from 16h36 to 18h01) in 2013. All records were in the same trapping stations (20° 12' 10" W, 47° 25' 02" S and 20° 11' 58" W, 47° 24' 34" S). In addition, in the early morning of November 2013, a male Bare-faced Curassow was seen perching on a tree (approximately 20° 12' 49" E, 47° 25' 32" S). All records were obtained near a river in an area of closed canopy and understory, typical of Deciduous Alluvial Forest (Figure 3). This area is difficult to access, and are the most preserved area of the park.

Figure 2. Individuals of *Crax fasciolata* in the Furnas do Bom Jesus State Park. (a) A couple of *Crax fasciolata* recorded in 2011 and (b) a male recorded in 2011.

We found 23 studies about birds in Cerrado of state of São Paulo besides eight protected area manegment plans with birds species list (Table 2). We just record bare-faced curassow in two protected areas, at Aguapeí State Park and Rio do Peixe State Parque (Figure 4).

Discussion

We expected to find bare-faced curassow in larger protected areas. However, despite having used larger sampling effort in larger protected areas we did not recorded the species in Jataí, an area that, if considered together with the contigous Experimental Station of Luís Antônio, is almost 15.000ha. The bare-faced curassow was recorded only at the Furnas do Bom Jesus State Park, a relatively small park in the Cerrado biome (Rodrigues 2008).

It is therefore surprising to find this animal in such a small and relatively isolated area in the north of the State since this species seems to be positively influenced by forest size (Nunes 2015). The occurrence of bare-faced curassow in that park may be facilitated by the presence of several native vegetation remnant in the region which may allow individuals coming from other areas such as the nearby Serra da Canastra National Park to recolonize the area.

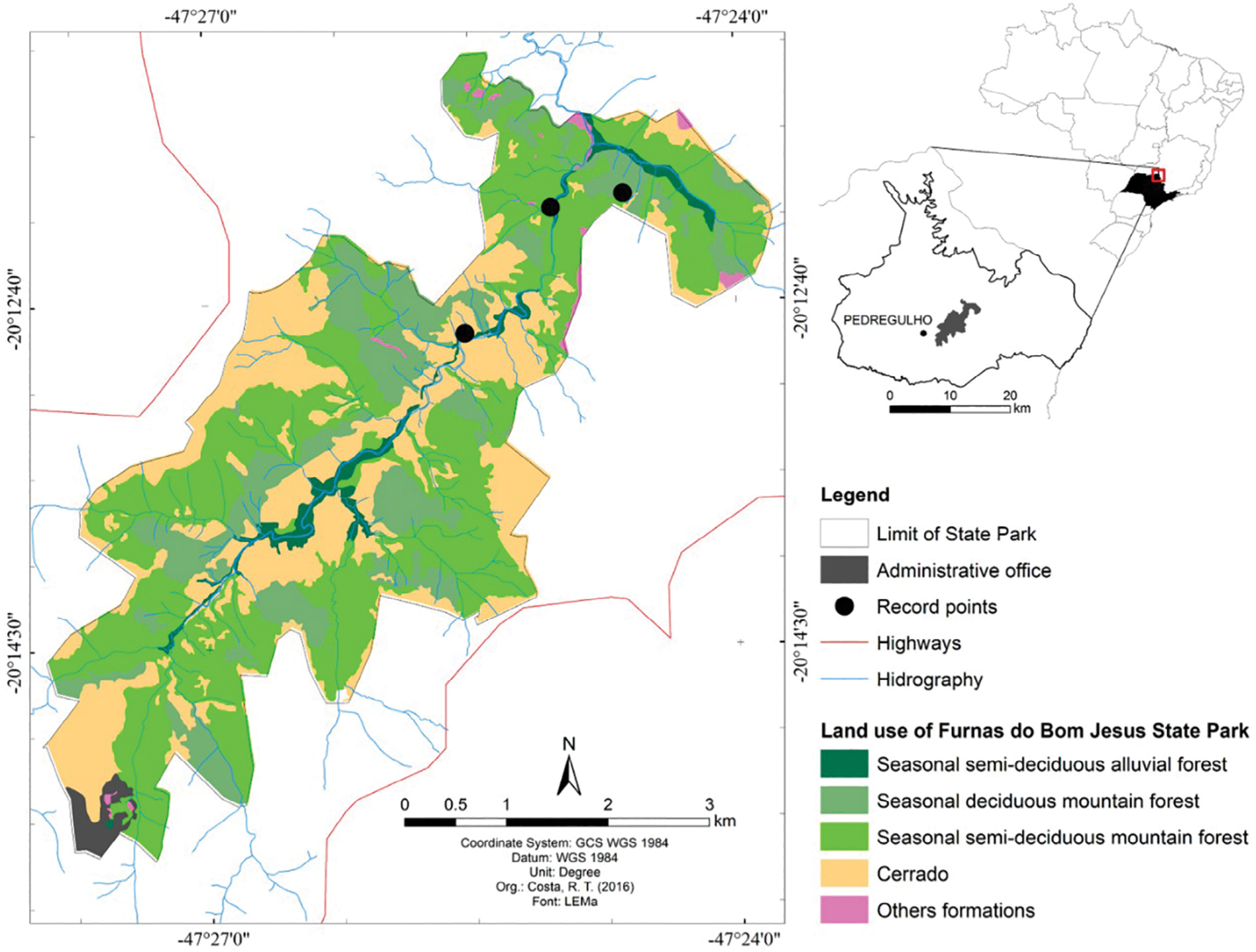

Figure 3. Record locations of *Crax fasciolata* in the Furnas do Bom Jesus State Park.

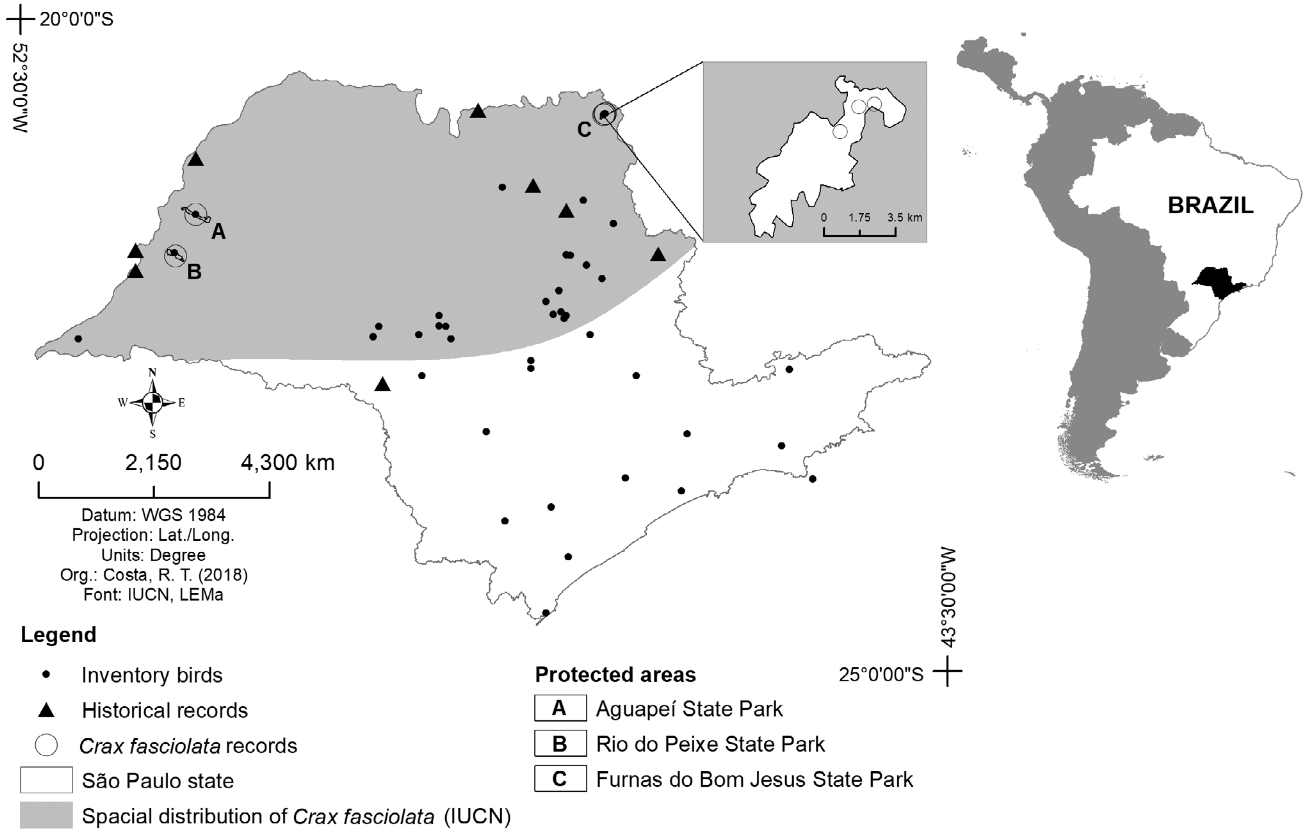

Figure 4. Confirmed records of bare-faced curassow in São Paulo State.

The assemblage of birds in the São Paulo State has been affected by forest fragmentation and poaching pressure. Poaching on cracids, for instance, has been considered a strong pressure to wildlife in the State of São Paulo (Uezu et al. 2005, Develey & Metzger 2006, Uezu 2006, Martensen et al. 2008). As a result of such anthropic pressures, São Paulo is the Brazilian state with the highest number of threatened and locally extinct bird species (Silveira & Uezu 2011).

Although we have opted not to use information of WikiAves (a Brazilian birds database), it is important to notice that there are 65 records of *C. fasciolata* in the state according to this database (http://www.wikiaves.com.br; accessed on 23 June 2016). Although these cannot be considered official records, they highlight the paucity of studies in the state. In addition, as the records are listed at the municipality level, in most cases it is not possible to identify whether they occurred in protected areas or not. Moreover, some of photographic records available on WikiAves show individuals in yards or having leg rings indicating that the animal may have been introduced to the area. This information is important, given that animals seen at unprotected areas are certainly under higher risk of dying due to poaching and roadkilling.

Furthermore, the fact that animals were recorded during two years (2011 and 2013) may indicate that a (small) population may be persisting locally at Furnas Park. It also raises the possibility of the species to occur in larger protected areas in the region, even though we have not detected it in the largest surveyed areas as Jataí and Santa Bárbara.

In 1993 the bare-faced curassow was considered a disappearing species in the São Paulo State (Willis & Yoshika Oniki1993); at that time, it was present in the Paulo de Faria Ecological Station, an area of 435.73 ha in the northwest area of the state, and nearby the Aguapeí State Park, a 9,043.97 ha reserve located at the northwest of São Paulo that encompasses part of a large floodplain and contains large portions of riparian forests. Despite being considered almost extinct in the state (Silveira et al. 2009), the occurrence of the bare-faced curassow was recently recorded in the Aguapeí State Park (Secretaria de Meio Ambiente de São Paulo 2010), which might possibly represent a remaining population.

The record of *C. fasciolata* in the Furnas do Bom Jesus State Park highlights the importance of conserving even relatively small remnants of native vegetation, especially considering that there is only 0.5% of Cerrado protected in the São Paulo State (Klink & Machado 2005). The Furnas do Bom Jesus State Park had 30% of its area destroyed by a fire in late 2011. Fires may be a threat to many cracids species (Brooks 2006), including locally rare ones such as the bare-faced curassow; this is especially true in small protected areas where curassow populations are probably small. Other threats in the area are feral pigs (*Sus scrofa*) and free-ranging dogs (*Canis familiaris*) that were also recently recorded in our surveys. These species can prey on juvenile and adult birds (Ritchie et al. 2014). In addition, the records were obtained in the most preserved area of the park, which might be important for the conservation plan and management of the park. Although surveys of avian fauna in this region are still relatively scarce (Cavalcanti & Joly 2002, Silva & Bates 2002, Develey et al. 2005, Piratelli & Blake 2006) additional studies focusing on mapping the present occurrence of the bare-faced curassow are urgent. Moreover, estimating numbers, connectivity, and factors impacting the populations are the basis for any conservation effort.

The new record of *C. fasciolata* highlights the usefulness of camera traps for bird surveys, since one can obtain information regarding the life history of curassow, as reported recently by Srbek-Araujo et al. (2012) and Fernandez-Duque et al. (2013). Camera traps are usually used for recording medium to large-sized terrestrial mammals and occasional records of other groups might be put aside by mammalogists. Despite this, camera trapping are also apropriate for large, ground-dwelling birds, such as cracids and phesants (O'Brien & Kinnaird 2008). We recommend close collaboration between mammalogists and other specialists (e.g. ornithologists and herpetologists) so that non-mammal species recorded by camera traps can be promptly identified and records made available to the scientific community.

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Author Contributions

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Larissa Fornitano: Contribution to data collection. Contribution to data analysis and interpretation. Contribution to manuscript preparation. Contribution to critical revision, adding intellectual content.

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Conflict of Interest

We declare that we have no conflict of interest related to the publication of this manuscript.

Ethics

Approval by an ethical committee was not required, since this study was conducted using camera traps only. No invasive methods were used.

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Article

Diagnosis, presence of endoparasites, and local knowledge on the infestation of the exotic giant African snail (gastropoda: pulmonata: achatinidae), in the urban zone of Rio Branco, Acre, Brazil

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LIMA, M.S., GUILHERME, E. **Diagnosis, presence of endoparasites, and local knowledge on the infestation of the exotic giant African snail (gastropoda: pulmonata: achatinidae), in the urban zone of Rio Branco, Acre, Brazil**. Biota Neotropica. 18(3): e20170503. http://dx.doi.org/10.1590/1676-0611-BN-2017-0503

Abstract: The giant African land snail, *Lissachatina fulica* is considered to be one of the world's worst invader species. This snail can provoke major economic and public health problems in urban areas, in particular as a potential vector of nematodes that infect both humans and animals. In this context, the present study investigated the extent of the infestation of this exotic snail in the urban neighborhoods of the city of Rio Branco, verified the presence of endoparasites in these snails, and evaluated the knowledge of local residents with regard to the presence of this invader species. For this, daytime surveys were conducted between August 2015 and June 2016. For the analysis of endoparasites, 44 live specimens were sent to the National Reference Laboratory for Schistosomiasis-Malacology (LRNEM) of the Oswaldo Cruz Institute (FIOCRUZ). The knowledge of local residents in the infested neighborhoods was investigated using questionnaires with direct, closed questions. The exotic invader species (*L. fulica*) was detected in 24 of the 36 neighborhoods visited. A total of 858 *L. fulica* specimens were collected, of which, 329 were alive and 527 were dead. The total length of the shell ranged from 0.7 to 14.2 cm, with a mean±standard deviation of 4.8±2.21 cm. The density of snails in the areas surveyed varied from 0.34 individuals/m^2 to 3.54 individuals/m^2, while the mean density within the whole study area was estimated to be 0.54 individuals/m^2. Mature eggs were found in only 9 (2.7%) of the 329 specimens dissected. The endoparasitological analysis revealed the presence of rhabditiform larvae in 84% of the specimens examined, as well as the nematodes *Aelurostrongylus abstrusus* (22.7% of the specimens) and *Strongyluris* sp. (2.2%). A total of 39 local residents were interviewed, and while all were conscious of the presence of the mollusk, none knew its origin or the most adequate way of dealing with it. The results of the study indicate an ample infestation of the city of Rio Branco by *L. fulica*, and confirmed a complete lack of intervention on the part of the local authorities for the implementation of measures foe the control and management of this pest within the urban zone of Rio Branco.

Keywords: *Aelurostrongylus abstrusus; Lissachatina fulica; Strongyluris* sp.*; Southwest Amazon.*

Diagnóstico, presença de endoparasitas e conhecimento local sobre a infestação do caracol gigante exótico africano (gastropoda: pulmonata: achatinidae), na zona urbana de Rio Branco, Acre, Brasil

Resumo: Conhecido como caracol gigante africano a espécie *Lissachatina fulica* é considerada uma das piores espécies invasoras do mundo. Em áreas urbanas pode causar grandes problemas econômicos e para a saúde pública por ser um potencial vetor de nematódeos de interesse médico e veterinário. Desta forma, o presente trabalho teve como objetivos averiguar a extensão da infestação do caracol exótico nos bairros da zona urbana da cidade de Rio Branco; verificar a presença de endoparasitos, bem como avaliar o conhecimento da população local em relação a presença desta espécie invasora. Para isso, foram realizadas coletas diurnas no período de agosto de 2015 a junho de 2016. Para verificar a presença de endoparasitos foram enviados 44 indivíduos vivos ao Laboratório de

Referência Nacional para Esquistossomose-Malacologia (LRNEM) da Fundação Oswaldo Cruz (FIOCRUZ). A avaliação do grau de conhecimento dos moradores dos bairros infestados deu-se pela utilização de questionários com perguntas fechadas e diretas. A espécie exótica e invasora (*L. fulica*) foi detectada em 24 dos 36 bairros visitados. Dos 858 indivíduos coletados, 329 estavam vivos e 527 mortos. O comprimento total das conchas variou de 0,7 a 14,2 cm com uma média±desvio padrão de 4,8±2,21 cm. A densidade de indivíduos nas áreas amostradas variou do mínimo de 0,34 ao máximo de 3,54 indivíduos/m^2 enquanto que a densidade média de espécimes tendo como base a área total amostrada foi de 0,54 indivíduos/m^2. Somente em 2,7% (9) dos 329 espécimes dissecados foram encontrados ovos maduros. A busca por endoparasitos revelou a presença larvas rabditiformes em 84% dos espécimes investigados além dos nematódeos *Aelurostrongylus abstrusus* (22,7%) e *Strongyluris* sp. (2,2%). Ao todo foram entrevistados 39 moradores, onde verificou-se que eles têm consciência da presença do molusco mas desconhecem sua origem e a forma de manejá-lo adequadamente. Nossos resultados apontam para uma ampla infestação da espécie *L. fulica* na cidade de Rio Branco e constata a total ausência do poder público no que diz respeito a implementação de medidas de controle e manejo desta praga nos limites da cidade.

Palavras-chave: *Aelurostrongylus abstrusus; Lissachatina fulica; Strongyluris* sp.*; Sudoeste da Amazônia.*

Introduction

The giant African land snail, *Lissachatina fulica* (Bowdich 1822), is considered to be one of the world's 100 most important invader species (Sridhar et al. 2014, Gisd 2018). Native to eastern Africa, this snail was introduced to Mauritius in the 1800s, from where it spread rapidly throughout the islands of the Indian and Pacific oceans (Sankaran 2004, Tatayah et al. 2007). The species is now found in many countries throughout the world, including India, Sri Lanka, Malaysia, Australia, Japan, the United States, and almost all of South America, including Brazil (Fontanilla et al. 2014, Sridhar et al. 2014). In Brazil, *L. fulica* was introduced into the southern state of Paraná in the mid-1980s (Thiengo et al. 2007), with the aim of producing a commercially viable alternative to the smaller, edible European garden snail (*Helix aspersa* Müller, 1774), known as the "escargot", which is reared on a large scale for human consumption in many countries (Murphy 2001). Producers initially aimed to export the snail to Europe and, eventually, supply the Brazilian market, but while this operation was successful for a while, exportation eventually became unprofitable, and the producers were unable to establish a viable domestic market, given the lack of a culinary tradition in Brazil (Colley & Fischer 2009). The producers then released the remaining snails into the wild.

The capacity of the giant african land snail to adapt to a diversity of habitats, the absence of natural predators, and its generalist habits and high reproductive rates, have all contributed to its dispersal throughout the warm and humid regions of the globe, including the whole of Brazil (Fischer & Colley 2004, Coelho 2005, Fischer & Colley 2005, Thiengo et al. 2007, Fontanilla et al. 2014, Sridhar et al. 2014). As an invader species, *L. fulica* is a potential competitor of native mollusk species (Sankaran 2004). The giant african land snail is basically herbivorous, with no particular preference for any given plant species, which makes it a potentially important agricultural pest (Sankaran 2004). The species is a vector of nematode parasites to other animals, and also transmits parasites that threaten human health, such as nematodes of the genus *Angiostrongylus* Kamensky 1905 (Jarvi et al. 2012, Caldeira et al. 2007, Gisd 2018).

In the Americas, the first recorded case of eosinophilic meningitis in humans was registered in Cuba, in 1981 (Aguiar et al. 1981) and the disease spread rapidly to neighboring countries, such as the Dominican Republic, Haiti, Puerto Rico, and the United States (Lai et al. 1982, Andersen et al. 1986, Raccurt et al. 2003, Hochberg et al. 2007). In Brazil, eosinophilic meningitis spread by the nematode *Angiostrongylus cantonensis* (Chen 1935) was first reported in the state of Espírito Santo, in 2007 (Caldeira et al. 2007), and more recently in Pernambuco (Lima et al. 2009) and São Paulo (Espírito-Santo et al. 2013). In all three cases, the presence of *L. fulica* was confirmed in the areas adjacent to the residences of the individuals diagnosed with the parasite. Given its ample spectrum of neurological symptoms, eosinophilic meningitis has been commonly referred to as neural Angiostrongyliasis (Hung & Chen 1988). The incidental presence of the nematode *Angiostrongylus costaricensis* Morera and Céspedes 1971 has also been confirmed in a child in Espirito Santo, Brazil (Pena et al. 1995). It is important to note that a number of rat species are the definitive vectors of the nematodes *A. costaricensis* and *A. costaricensis*, which cause eosinophilic meningitis and abdominal angiostrongyliasis, respectively, in humans (Morera & Céspedes 1971, Morassutti et al. 2014). However, these nematodes are much less specific in relation to their intermediate hosts, and may parasitize a diversity of mollusks, including both terrestrial and freshwater species (Wallace & Rosen 1969 (b), Mota & Lenzi 1995, Thiengo et al. 2013 (b)). In humans, infection may occur through the ingestion of fruit, leaves or other foods which have come into contact with the mucous of the intermediate host, typically a terrestrial gastropod, and have been washed inadequately (Moreira et al. 2013).

In many studies of the exotic giant African land snail in the Americas, the species has been classified as *Achatina* (*Lissachatina*) *fulica* (*e.g.*, Thiengo et al. 2007). However, in a molecular study, Fontanilla (2010) found significant differences between the populations of East Africa, assigned to the subgenus *Lissachatina*, and those from Central and West Africa, classified as *Achatina*. Based on these findings, we consider *Lissachatina* to be a full genus, a recommendation adopted in many recent studies (*e.g.*, Smith et al. 2013, Fontanilla et al. 2014, Iwanowicz 2015).

The giant African land snail is currently found in 25 of the 26 Brazilian states, as well as the Federal District (Thiengo & Fernandez 2013 (a)). Few data are available on the occurrence of this species in Brazilian Amazonia. Thiengo et al. (2007) recorded the occurrence of *L. fulica* in the northern Brazilian states of Amazonas, Rondônia, Pará, and Mato Grosso. Oliveira et al. (2013) confirmed the presence of this species in the state of Amapá while newspaper reports from 2014, available on the internet, refer to its presence in Boa Vista, capital of Roraima. This meant that Acre was the only state in which the presence of *L. fulica* had not been recorded, although the presence of *L. fulica* in Rio Branco had been confirmed through the collection of a shell by EG in the Zoobotanical Park of the Federal University of Acre (UFAC). This shell was sent to the Malacology collection of the Zoology Museum of São Paulo University (USP), where it was deposited under catalog number MZSP-98749. In addition to Rio Branco, *L. fulica* is known to occur in other towns in Acre, including Acrelândia, Xapuri and Brasiléia (E.G. pers. obs.). While this invader species appears to be relatively widespread in the state, there have been no previous studies in Acre.

Given this, the present study aimed to (a) verify the occurrence of *L. fulica* in the urban neighborhoods of the city of Rio Branco, capital of Acre, (b) determine whether this population includes individuals infected with the larvae of parasites of medical importance to humans or animals, and (c) verify whether the residents of the affected neighborhoods recognize *L. fulica* as an invader species that poses a public health risk, and the appropriate management measures.

Materials and Methods

1. *Sampling area*

The present study focused on the urban zone of the municipality of Rio Branco, in the Brazilian state of Acre (Figure 1), which borders Peru and Bolivia, and the Brazilian states of Amazonas and Rondônia. The city is located on the Acre River, and the municipality has a total area of 8.835,541 km^2.

We visited 36 neighborhoods within the urban zone of Rio Branco to confirm the presence/absence of *L. fulica*. We searched vacant lots, household gardens and backyards, and public parks (Figure 1). Data were collected over an 11-month period, from August 2015 to June 2016, between 14h:00 and 18h:00, by a single collector. Each neighborhood was surveyed visually for signs of the presence of the animal, such as shells, shell fragments, feces, eggs or live animals. Local residents were also consulted for information on the presence of the mollusk within the area of each neighborhood. During each visit, the area was surveyed for mollusks during three hours, and local residents were interviewed during one hour. Each site was visited only once during the study period.

2. *Data collection*

When the presence of *L. fulica* was detected, all the specimens were collected, irrespective of their size or condition (dead or alive), using disposable gloves. During the collection, the biological data (body size, and the number of dead and living individuals) were noted on a standard form, together with information on the site (area of the site, and whether it was a residential property or a vacant lot) (Table 1). In the laboratory, all the specimens were washed, measured with a calliper and then deposited in the UFAC malacology collection. Population density was calculated by dividing the area of each site (m²) by the number of specimens (dead and alive) encountered.

3. *Presence of mature eggs*

All the live specimens with a shell longer than 5.5 centimeters were housed in terrariums until dissection. The specimens were dissected to confirm the presence of mature (encased) eggs. Reproductive activity was confirmed when mature eggs were found in the reproductive organs of the specimens (Teixeira et al. 2008). All the eggs found in each specimen were counted and measured using a ruler.

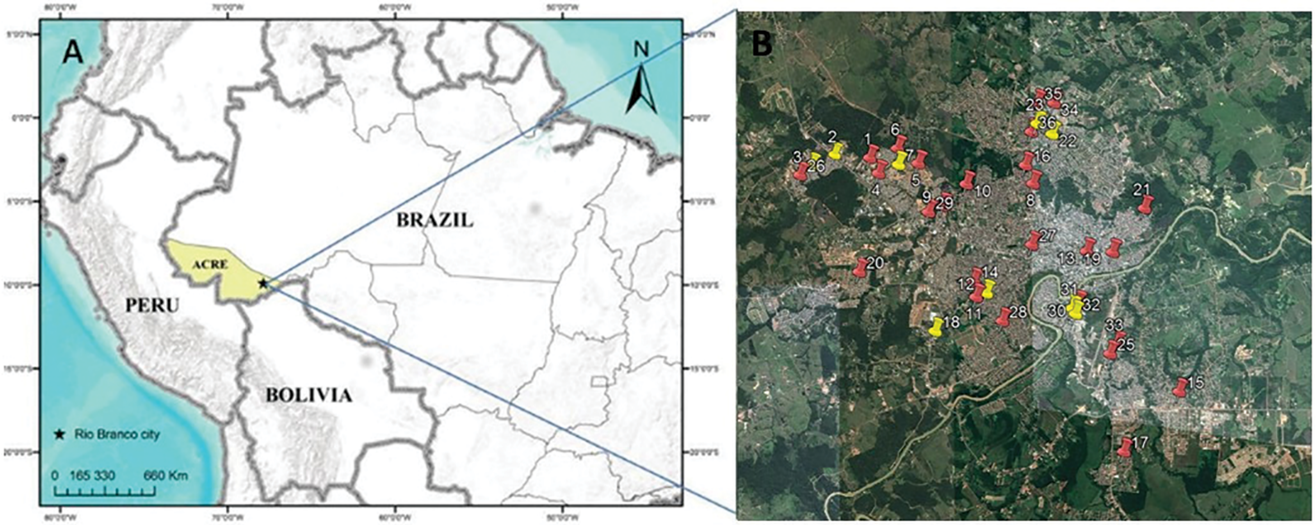

Figure 1. A – Location of Acre in Brazil. B – Satellite image of the city of Rio Branco. The numbered points indicate the neighborhoods visited during the present study, in chronological order. The red points indicate the neighborhoods infested with *L. fulica* and the yellow points, the neighborhoods in which the mollusk was not found during the survey: 1-Distrito Industrial; 2- Universitário II; 3- Universitário I; 4- Tucumã; 5-Bairro Rui Lino; 6- Mocinha Magalhães; 7-Conjunto Rui Lino; 8-Bairro da Paz; 9- Esperança; 10- Geraldo Fleminger; 11- João Eduardo II; 12- Betel I; 13- Capoeira; 14-Betel II; 15-Belo Jardim; 16- Vila Ivonete; 17- Santa Helena; 18- Floresta; 19- Habitasa; 20- Calafate; 21-Morada do Sol; 22-Vitória; 23-Chico Mendes; 24- Eldorado; 25-Recanto dos Buritis; 26-Universitário III; 27-Abrahão Alab; 28-Bahia Nova; 29- Conjunto Mariana; 30-Triângulo; 31-Triângulo Velho; 32- Triângulo Novo; 33-Jacarandá; 34-Parque dos Sábias; 35-Adalberto Sena; 36-Placas. Source: GoogleEarth®.

Table 1. Neighborhoods of the city of Rio Branco surveyed for the presence of the invader mollusk *L. fulica*.

Neighborhood	Street	Date	Number of specimens found*	Geographic coordinates	Area in which the specimens were collected (m^2)
Distrito Industrial	Rua das Acácias	08/20/2015	24	09° 56’ 41.0”S 067° 51’ 59.3”W	168,85
Universitário II	Not found	09/01/2015	0	09° 56’ 39.12”S 067° 52’ 34.2”W	0
Universitário I	Not found	09/02/2015	0	09° 56' 49.04"S 067° 52' 56.66"W	0
Distrito Industrial	Setor B	09/10/2015	20	09° 56 ' 37.5"S 067° 52' 15.70"W	50,1
Distrito Industrial	Setor C	09/23/2015	0	09° 56' 38.4"S 067° 53' 9.0"W	0
Tucumã	N-1	09/25/2015	6	09° 56' 58.18"S 067° 51' 50.1"W	14,64
Bairro Rui Lino	Not found	09/26/2015	0	09° 56' 49.13"S 067° 51' 10.15"W	0
Mocinha Magalhães	Not found	09/27/2015	0	09° 56' 33.83"S 067° 51' 30.65"W	0
Conjunto Rio Lino	Not found	09/28/2015	0	09° 56' 49.13"S 067° 51' 29.66"W	0
Tucumã	N-3	09/30/2015	13	09° 56' 54.44"S 067° 51' 45.8"W	68,0
Tucumã	RW-1	10/01/2015	8	09° 57' 12.4"S 067°51' 37.5"W	107,0
Tucumã	W-5	10/01/2015	8	09° 57' 6.4"S 067° 51' 37.2"W	20
Bairro da Paz	Travessa Natal	10/14/2015	31	09° 57' 09.7"S 067° 49' 14.7"W	74,21
Esperança	Siqueira Santos	10/14/2015	12	09° 57' 10.8"S 067° 50' 36.4"W	28,74
Geraldo Fleminger	Botafogo	10/26/2015	16	09° 57' 08.24"S 067° 50' 22.25"W	141,54
João Eduardo II	Beco sem nome	10/28/2015	51	09° 59' 0.91"S 067° 50' 13.9"W	34
Betel I	Not found	10/28/2015	0	09° 58' 56.23"S 067° 50' 07.19"W	0
Capoeira	Maria Amélia	11/05/2015	15	09° 58' 16.2"S 067° 48' 22.5"W	35,90
Betel II	25 de dezembro	11/10/2015	15	09° 58' 55.7"S 067° 50' 35.6"W	43,05
Belo Jardim	Das Flores	11/14/2015	11	10° 00' 33.98"S 067° 46' 51.30"W	32
Vila Ivonete	Serena nogueira	11/16/2015	33	09° 56’ 52.26” S 67° 49' 22.67"W	33
L. Santa Helena	Not found	11/18/2015	7	10° 1'35.52"S 67°47'46.42"O	85,59
Floresta	Not found	12/09/2015	0	10° 1' 30.29"S 067°47'41.81"W	0
Habitasa	Venezuela	12/29/2015	12	9°58'18.75"S 67°47'56.19"W	20

Continued Table 1.

Neighborhood	Street	Date	Number of specimens found*	Geographic coordinates	Area in which the specimens were collected (m^2)
Geraldo Fleminger	Padre Cícero	02/16/2016	15	09° 58' 15.0"S 067° 57' 51.8"W	100,03
João Eduardo II	Beco sem nome	02/17/2016	6	09° 57' 08.24"S 067° 50' 22.25"W	17,84
Habitasa	Peru	02/24/2016	14	09° 59' 02.5"S 067° 50' 14.2"W	44,81
Calafate	Envira	03/01/2016	26	09° 58' 35.06"S 067° 52' 09.63"W	69,70
Morada do Sol	Mercúrio	03/23/2016	32	09°57'35.63"S 067°47'23.67"W	15,8
Morada do Sol	Luna	03/23/2016	12	09° 58' 35.93"S 067° 52' 09.81"W	29,80
Bairro Vitória	Not found	03/23/2016	0	09°56'21.61"S 67°48'56.00"W	0
Chico Mendes	Not found	03/23/2016	0	09°56'10.38"S 67°49'11.13"W	0
Eldorado	Girassol	03/23/2016	11	09°56'14.64"S 67°48'31.79"W	17,9
Recanto Dos Buritis	Areal	04/04/2016	65	09°59'58.87"S 067°47'59.49"W	172,5
Universitário 3	Maranhão	04/05/2016	5	09°57'0.03"S 067°53'9.14"W	18,72
Universitário 3	Fran. L. Bezerra	04/05/2016	14	09° 59' 46.39"S 067° 47' 50.14"W	24,43
Abrahão Alab	Av. Ceara	04/18/2016	46	09° 59' 51.97"S 067° 47' 55.28"W	176,56
Bahia nova	São Pedro	04/18/2016	24	09° 57' 00.61"S 067° 53' 01.63"W	20,16
Conjunto Mariana	Vitória	04/18/2016	44	09° 57' 01.47"S 067° 53' 03.77"W	239,96
Conjunto Mariana	Liberdade	04/18/2016	20	09° 58' 09.8"S 067° 49' 15.5"W	152,01
Triângulo	Adolfo Barbosa	04/28/2016	158	09° 59' 35.26"S 067° 49' 50.64"W	44,87
Triângulo Velho	Not found	04/28/2016	0	09° 57' 36.6"S 067° 50' 47.7"W	0
Triângulo Novo	Not found	04/28/2016	0	09° 57' 36.0"S 067° 50' 49.9"W	0
Jacarandá	Osvaldo coelho	05/03/2016	58	09° 57' 37.0"S 067° 50' 49.4"W	35,96
Parque dos Sabias	Not found	06/08/2016	8	09° 59' 23.2"S 067 48' 34.7"W	55,69
Adalberto Sena	Mamoeiro	06/15/2016	15	09° 59' 15.99"S 067° 48' 40.76"W	16,46
Placas	Jarbas passarinho	06/15/2016	3	09° 59' 30.63"S 067° 48' 38.25"W	341,67

This column shows only the specimens collected for the morphometric measurements and dissection. The specimens obtained for parasitological analysis were collected specifically for this purpose on other days (Table 2).

4. *Presence of endoparasites*

To determine the possible infection of snails by nematodes of medical-veterinary relevance, 44 live specimens were collected in 11 different neighborhoods (four specimens per neighborhood) in the urban zone of Rio Branco. The neighborhoods were selected randomly by lottery for the survey. A subsample of 44 specimens was collected specifically for the analysis of the presence of endoparasites, and for this reason, they are not included in the total number of individuals measured and dissected (Table 1). The specimens collected were sent to the National Reference Laboratory for Schistosomiasis-Malacology (LRNEM) of the Oswaldo Cruz Institute (FIOCRUZ) in Rio de Janeiro, where they were digested artificially using the technique proposed by Wallace & Rosen (a) (1969).

5. *Interviews with residents*

During the collection of samples, residents of the neighborhoods in which the snail was found were interviewed using a questionnaire that investigated the knowledge of the residents on the giant African land snail, *L. fulica.* The name, age, and sex of each interviewee were recorded, as well as the length of time they had lived in the neighborhood. The questions referred to (i) the knowledge of the resident with regard to the animal, (ii) the name used to refer to the species, (iii) its origin, (iv) where and when the snail was observed most frequently, (v) what measures are taken, if any, when the animal is encountered in the proximity of the household, (vi) if the mollusk is known to transmit any diseases, (vii) whether the local public health authorities have alerted the resident to any risk associated with the species, (viii) if the interviewee knows how the species arrived in the state, and in particular, Rio Branco, and (ix) whether it is known to cause any problems for the environment or the local population. The knowledge of the infested neighborhoods was investigated using questionnaires with direct, closed questions. The responses to the questions were tabulated and organised in classes of answers. Following the interviews, the participants were alerted to the potential dangers associated with *L. fulica*, and the measures that can be taken to eliminate the species.

This study was conducted according to the current institutional norms governing animal research in Brazil. The project on which the study was based was approved by the UFAC ethics committee for the use of animals in research, under protocol number 59/2015. The collection of specimens of the exotic snail, *L. fulica,* is permitted by normative instruction 18/2005 of the Brazilian Federal Environment Institute (IBAMA), which authorizes the control and elimination of the species.

Results

The *L. fulica* was detected in 24 (66.6%) of the neighborhoods visited during the present study, with no individuals being detected in only twelve (33.4%) neighborhoods (Table 1). A total of 858 specimens (= 34.32±31.98 SD individuals per site; median = 26), were collected, including 329 live individuals (= 13.16±18.31 SD; median = 8), and 529 shells (= 21.08±20.07 SD; median = 12) per site. The largest number of individuals was recorded in the Triângulo neighborhood, where 158 specimens (90 alive), representing 18.41% of the total number of specimens, were collected, whereas only three dead shells, 0.34% of the sample, were collected in the Placas neighborhood (Table 1). No snails were detected during the survey in a number of neighborhoods, including Mocinha Magalhães, Conjunto Rui Lino, Floresta, Vitória, and Chico Mendes (Table 1). Density varied from a minimum of 0.34 to a maximum of 3.54 individuals/m^2, with a mean of 1.89±2.76 SD individuals/m^2. The mean density of snails over the whole study area was 0.54 individuals/m^2.

1. *Presence of mature eggs*

Only nine of the 329 live specimens presented mature eggs in the oviduct. A total of 526 eggs were found, which measured 4.3±0.57 mm, on average. The number of reproductive specimens represented only 2.7% of the live individuals encountered during the surveys.

2. *Morphometry (total length)*

In all, 760 of the 858 specimens collected had an intact (unbroken) shell, which was measured. Total length ranged from 0.7 to 14.2 cm, with a mean±standard deviation of 4.8±2.21 cm (Figure 2). Most (570 specimens; 75%) of the individuals measured between 2.1 to 7 cm in length (Figure 2). Smaller (shells smaller than 3 cm) individuals were found more frequently between August 2015 and March 2016 (Figure 2), and most of the individuals in all size classes were collected during this period.

3. *Presence of endoparasites*

None of the 43 *L. fulica* specimens analyzed (one specimen was dead on arrival at the LRNEM, which impeded analysis) from 11 different neighborhoods of Rio Branco were infected by larvae of the nematodes *A. cantonensis* or *A. costaricensis*. However, rhabditiform larvae were identified in almost all the specimens (n = 37 or 84%), in addition to two nematodes, *Aelurostrongylus abstrusus* (Railliet, 1898) (Nematoda:

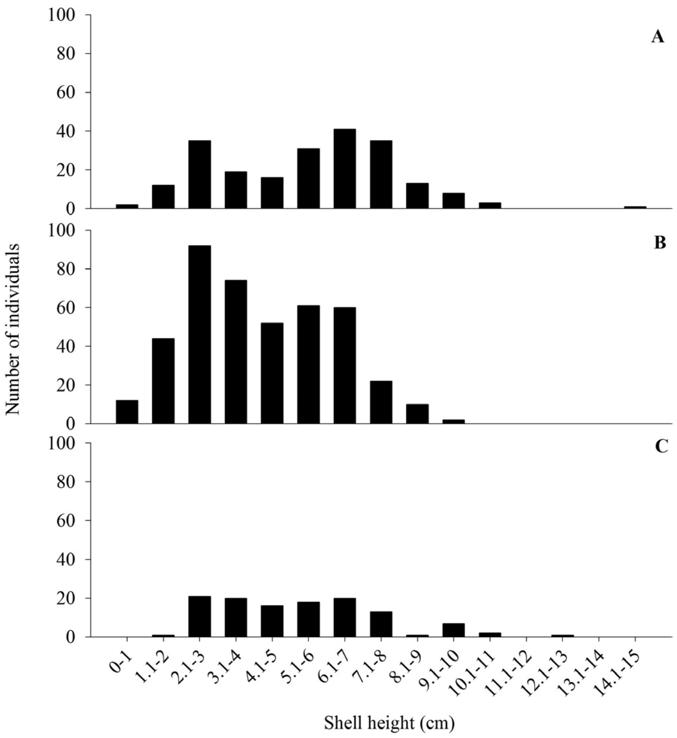

Figure 2. Size classes of total shell length (cm) of the *L. fulica* specimens collected in the city of Rio Branco between August 2015 and June 2016. A: August to November; B: December to March, and C: April to June.

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Table 2. Results of the analyses of the *L. fulica* specimens collected in the different neighborhoods of Rio Branco, Acre, Brazil.

Neighborhoods	Samples analyzed*	Endoparasites
Tucumã	4	Rhabditiform larvae (4)**
Belo Jardim	4	Rhabditiform larvae (3), *Aelustrongylus abstrusus* (2)
Betel I	4	Rhabditiform larvae (4), *Aelustrongylus abstrusus* (3)
João Eduardo II	4	Rhabditiform larvae (4), *Aelustrongylus abstrusus* (2)
Bairro da Paz	4	Rhabditiform larvae (2), *Aelustrongylus abstrusus* (1)
Geraldo Fleminger	4	Rhabditiform larvae (2), *Aelustrongylus abstrusus* (1)
Capoeira	4	Rhabditiform larvae (3)
Distrito industrial	4	Rhabditiform larvae (4)
Morada do Sol	4	Rhabditiform larvae (4), *Aelustrongylus abstrusus* (1)
Vila Ivonete	4	Rhabditiform larvae (4), *Strongyluris* sp. (1)
Habitasa	3	Rhabditiform larvae (3)

* Only 43 of the 44 specimens sent for analysis were alive when they arrived at the laboratory and could be analyzed. ** The values in parentheses are the number of the *L. fulica* specimens analyzed that were infected by the respective nematode species.

Metastrongylidae) (n = 10 or 22.7%) and *Strongyluris* sp. Mueller, 1894 (Nematoda: Heterakidae) (n = 1 or 2.2%) (Table 2).

4. *Knowledge of the local population on the exotic snail*

Thirty-nine residents were interviewed in the different neighborhoods of Rio Branco, of which, 29 (74.3%) were female. The mean age of the 39 interviewees was 47.4±16.0 years (range: 17–79 years old). Just under half (n = 18 or 46.1%) of the interviewees had lived in their current neighborhood for 11–20 years, nine (23.0%) for 1–9 years, five (12.8%) for 21–30 years, five (12.8%) for more than 30 years, and two (5.1%) for less than one years. The oldest memories of the presence of the African land snail in Rio Branco data back to 2005, that is, 11 years prior to the present study, while the largest proportion (30.7%) of the interviewees only perceived the presence of the species in the neighborhood from 2014 onward.

None of the interviewees were familiar with the vernacular name by which *L. fulica* is known in Brazil ("*caramujo gigante africano*" = giant African land snail) or its scientific name. Most (n = 26 or 66.6%) of the interviewees referred to the animal as a *caracol* (snail), seven (17.9%) named it "*buzu*", five (12.8%) as a *caramujo* (conch or water snail), and one (2.1%) as a *lesma* (slug).

The vast majority of the interviewees (n= 31 or 79%) were unaware of the origin of the species, while the other 21% (n =8) believed that it is native to the Amazon region. The vast majority (33 or 84.6%) of the interviewees confirmed that the species is observed more often during the rainy season months. Around a third (n=10 or 25.6%) of the interviewees confirmed that the animal was typically found after rainfall, 11 (28.2%) in the morning, 10 (25.6%) at night, and eight (20.5%) at any time of day.

Most interviewees (n = 14) referred to the walls of houses as the location where the greatest concentrations of *L. fulica* could be found. Others indicated the most humid areas of the garden (n = 6 interviewees), vegetable gardens (n = 5), on waste ground (n = 4), vacant lots (n = 3), near water tanks (n = 3), and in piles of rubble (n = 2) or streams (n = 2).

When asked where the animal seeks refuge from the sun and high temperatures, most of the interviewees (74%) confirmed that they were unfamiliar with the ecological strategies of the species.

More than half of the interviewees admitted not knowing how the animal arrived in the state or the city of Rio Branco. The others confirmed that the species came "crawling", by plane or truck, and so on.

When asked whether the animal was a vector of diseases, most (58.9%) of the interviewees believed that *L. fulica* does contribute to the transmission of diseases, but were unable to identify these diseases. A considerable percentage of the interviewees confirmed that the snail does not transmit disease. The residents who referred to specific diseases mentioned Ascites (n = 2 interviewees), schistosomiasis (n = 1), "worms" (n = 1), and skin infections (n = 1).

The interviewees were clearly inconvenienced by the presence of the mollusks in their residences and adjacent areas, and a majority (71%) dealt with these animals by killing them. Other individuals reported dealing with the mollusks in a number of other ways. All of the interviewees confirmed that they had never been visited by a public health agent or other government official with information on the snail or the risks of contact with this animal.

Discussion

The exotic giant African land snail, *L. fulica*, is found throughout the urban area of the Brazilian city of Rio Branco, capital of the Brazilian state of Acre. The presence of a large number of individuals of all sizes, including juveniles and (sexually active) adults in the neighborhoods visited indicates that the *L. fulica* populations are fully established in the metropolitan region of this city.

In the present study, *L. fulica* was found to be abundant in some areas of certain neighbourhoods, whereas in others, it was quite rare. A similar pattern of variation in the distribution of the mollusk was observed by Fischer & Colley (2004) in Guaraqueçaba, on the northern coast of the Brazilian state of Paraná. The mean density of *L. fulica* recorded in the areas surveyed in Rio Branco was lower than that registered in Puyo, Ecuador (3.7 ind/m^2; Gołdyn et al. 2016), but higher than that recorded in some Brazilian cities (*e.g.*, Miranda et al. 2004). Some authors (*e.g.*, Thiengo et al. 2007, Gołdyn et al. 2017) consider high densities of this species to be a characteristic of the initial stages of infestation. Only a small number of live individuals contained mature eggs. This finding was similar to that of Roda et al. (2016), who found eggs in only a small proportion (5%) of the *L. fulica* specimens they analyzed. Fisher & Colley (2005) and Roda et al. (2016) reported finding adults laying eggs throughout the year. In Rio Branco, however, specimens containing eggs were only collected between December and March, the rainiest part of the year in this region (Duarte 2006). This difference may reflect the influence of the climate of the Southwestern Amazon basin on the reproductive patterns of the species, although more detailed, long-term data will be required to confirm any such effect, and its determinants.

The morphometric parameters recorded for the *L. fulica* populations in Rio Branco were compatible with those registered in other studies (Fisher & Colley 2005, Albuquerque et al. 2009, Roda et al. 2016, Gołdyn et al. 2017). In Guaraqueçaba, in the Brazilian state of Paraná, Fischer & Colley (2005) recorded a mean *L. fulica* shell length of 5.2 cm. In Lauro de Freitas, in the state of Bahia, *L. fulica* specimens of up to 10.25 cm in length were collected by Albuquerque et al. (2009), with a mean of 4.2 cm, values very similar to those recorded in the present study, i.e., a mean of 4.82 cm and a maximum of 14.2 cm. Most of the specimens collected in Rio Branco were between 2.1 to 7.0 cm in length, indicating a relatively young population, with individuals being yet to reach the maximum size of the species. Older individuals (with shells of more than 10 cm in length) make up only 0.95% of the total sample. In a similar study in Puyo, Ecuador, Gołdyn et al. (2017) obtained similar results to those of the present study, i.e., a predominance of subadults, and concluded that the reproductive biology of *L. fulica* is of the r strategist type. While plausible, this hypothesis requires more conclusive data, given that Roda et al. (2016) recorded gestating specimens with shell lengths of between 4.8 to 12.8 cm, even though individuals of less than 6.5 cm contributed fewer eggs than those larger than 9.0 cm.

The movements and dispersal of *L. fulica* vary according to the stage of the life cycle and the season (Tomiyama & Nakane 1993, Raut & Barker 2002). Young animals are generally more capable of dispersing than adults, although the absolute dispersal capacity is still limited. Tomiyama & Nakane (1993) found that *L. fulica* can disperse as much as 500 m in six months. This indicates that juvenile individuals can reach and colonize adjacent neighborhoods, although the species did not reach Rio Branco through a natural process of dispersal.

It is unclear how the species arrived in Acre. As no evidence was found of a deliberate process of introduction, the most probable source of the infestation was the accidental transportation of the snail in cargos from other regions of Brazil or the neighboring countries of Peru and Bolivia, where *L. fulica* is also known to occur (Sridhar et al. 2014).

The establishment of the giant African land snail in Rio Branco is a cause for concern, given that this generalist species has an enormous potential for proliferation (Fischer & Colley 2005). There are few reports of predation by native Brazilian animals (Martins & Donatelli 2014), which implies that there is little potential for natural control mechanisms of the species populations. This was confirmed in the present study through the detection of the larvae of *A. abstrusus*, which causes pneumonia in cats (Silva et al. 2005) and *Strongyluris* sp., a parasite of wild lizards (Santos et al. 2013). The larvae of *A. abstrusus* have also been found infecting *L. fulica* in a number of Brazilian states (Thiengo et al. 2008, Ohlweiler et al. 2010), although there is no evidence of the transmission of these larvae to domestic cats. A number of the specimens analyzed presented co-infection between *A. abstrusus* and rhabditiform larvae of other helminths (possibly *Strongyluris* sp.). The co-infection of *L. fulica* by multiple helminth species was reported by Oliveira et al. (2015) in the region of São Gonçalo, in the state of Rio de Janeiro. The co-infection of mollusks by helminths and other parasites may indicate a severe and chronic level of local infestation (Bonfim et al. 2013), which cannot be ruled out in the case of the *L. fulica* population of Rio Branco. The specimens collected in the present study in Rio Branco did not contain the larvae of nematodes that cause diseases in humans. It is nevertheless possible that snails infected with the larvae of *A.cantonensis* may still be found in the *L. fulica* populations of Rio Branco, given that snails infected with the larvae of this nematode have been collected in other region of Brazil (Morassutti et al. 2014).

The ethno-ecological data (interviews) indicate that the local population is aware of the presence of this exotic mollusk in the city. However, few of the residents interviewed were aware of the origin of the animal or the risks it represents for the health of human populations and domestic animals or family agriculture (kitchen gardens). The relatively recent memories of the interviewees with regard to the presence of *L. fulica* in Rio Branco implies that the species arrived in the city very recently. However, *L. fulica* arrived in Acre much earlier, given that one of the authors (E.G. pers. obs.) obtained reports of its presence in the town of Acrelândia, in the eastern extreme of the state, in 2008.

A number of the interviewees referred to *L. fulica* as the "*buzu*", which is the vernacular name used by the region's rubber-tappers and other forest dwellers to refer to the mollusk *Megalobulimus* sp. Miller, 1878 (Megalobulimidae) which is native to Southwestern Amazonia, and is relatively common in the local forests. By confusing the invader mollusk with the native species, the local residents are overlooking the potential risks that the presence of this species represents for the local environment and the health of local human populations. In general, the population is aware of the presence of the animal when it appears in large numbers in houses and on waste ground, which typically occurs after torrential downpours or during major floods. This result is similar to the findings of Fischer & Colley (2005) on Ilha Rasa, an island in the municipality of Guaraqueçaba, on the northern coast of Paraná, where 83.3% of the interviewees associated the appearance of *L. fulica* with the heavy summer rains. Carvalho Junior & Nunes (2009) recorded a similar situation in Várzea Grande, in the Brazilian state of Mato Grosso, where 90.9% of the population associated the appearance of the mollusk with the rainy season.

None of the residents interviewed were aware of techniques for the eradication of the species. This indicates that the city's administration has not followed the recommendations of IBAMA normative instruction number 18/2005 (IBAMA 2005), which authorizes local authorities to employ measures of collection, control, and elimination of *L. fulica* as a means of impeded the expansion of its populations to local rural areas and other Brazilian regions. Colley (2010) concluded that the first stage in the successful management of *L. fulica* is the diagnosis of the occurrence and distribution of this invasive snail within the target area, prior to the selection of the most appropriate measure, which should then be adapted to the local context. The rapid advance of the species through the different neighborhoods of the city, and to other areas of the state, together with the lack of adequate knowledge on the management of this problem by local residents, is an alarm call for the public authorities of the state, which have, up to now, neglected the presence of the species in Acre. It will be necessary to implement urgent measures to control the invasive populations of *L. fulica* in order to avoid infestation in the near future.

Final considerations

Much of the city of Rio Branco is infested with the giant african land snail (*L. fulica*). However, while the population of Rio Branco is aware of the presence of *L. fulica* in its houses and neighborhoods, there is a general lack of knowledge on the adequate procedures for the management of its populations. Municipal and state authorities should thus adopt immediate preventive measures, such as informative leaflets and announcements in the local media, to increase the awareness of the local population and encourage the management and control of the *L. fulica* population.

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Author Contributions

Marcos Silva de Lima: Collected and analyzed the data, and drafted the first version of the manuscript.

Edson Guilherme: Formulated the original research project, contributed to the data analyses, and wrote the final version of the manuscript.

Conflicts of interest

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

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Article

Distribution and conservation of *Aniba* Aubl. (Lauraceae Jussieu) species in Brazil

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Abstract: This study conducts a biogeographical review of *Aniba* in Brazil, including its actual and potential distribution, and a review on the species conservation status. The potential distribution of each *Aniba* species was modeled using the Brazilian Plants and Fungi Biogeography system, which is interconnected with the SpeciesLink network. A consensus model based on up to five algorithms was used to model the potential distribution of each species. The endemism, conservation status and rarity of each species in Brazil were also analyzed. A total of 3059 records of 30 Brazilian *Aniba* species were found. In total, 16.1% of the records were excluded because of the lack of valid names, and 53.4% were excluded because of the lack of georeference and name of the person who identified the record. The consensus model showed good results for species with 20 or more pixels. The other species had many errors, and their models could only be used to determine new areas for collections. Nine species were endemic to Brazil, and only three species showed no indicator of rarity. The geographical distribution of Brazilian *Aniba* species shows a predominantly Amazonian pattern. Only four species are not actually found in the Amazon. Some Amazonian species may also be found in Atlantic Forest areas, and many other species have the potential to live there. Our results indicate that only three species have no conservation problems, whereas 14 species have conservation problems, and 13 other species require further population studies to be best evaluated.

Keywords: *Amazonian, Atlantic Forest, Endemism, Models, Rarity.*

Distribuição e conservação das espécies de *Aniba* Aubl. (Lauraceae Jussieu) no Brasil

Resumo: Este estudo objetiva apresentar uma revisão biogeográfica de *Aniba* no Brasil, incluindo a distribuição real e potencial, além de uma revisão sobre o estado de conservação das espécies. A distribuição potencial de cada espécie de *Aniba* foi modelada através do sistema Biogeografia da Flora e Fungos do Brasil (*Biogeo*), interligado à Rede *SpeciesLink* que integra em tempo real dados primários de coleções científicas com os quais foram estabelecidas a distribuição real das espécies. Um modelo de consenso, baseado em até cinco algoritmos, foi usado para modelar a distribuição potencial de cada espécie. Também foram analisadas a situação de endemismo no Brasil, o estado de conservação e raridade de cada espécie. Foram encontrados 3059 registros de 30 espécies de *Aniba* no Brasil. Em média houve uma perda 16,1% dos registros por falta de nomes válidos e 53,4% dos registros por falta de georreferenciamento e identificador. A avaliação do modelo consenso mostrou bons resultados para as espécies com 20 ou mais pixels, as demais espécies apresentaram muitos erros; sendo que seus modelos servem apenas para orientar novas áreas para coletas. Nove espécies são endêmicas do Brasil. Apenas três espécies não apresentaram nenhum indicador de raridade. A distribuição geográfica das espécies de *Aniba* no Brasil mostra um padrão predominante amazônico. Apenas quatro espécies não possuem ocorrência conhecida na Amazônia. Algumas espécies amazônicas também podem ser encontradas em áreas da Mata Atlântica e muitas outras apresentam potencial para ocorrer lá. Nossos resultados indicam que apenas três espécies não possuem nenhum problema referente à conservação, 14 espécies possuem problemas e 13 outras espécies necessitam de mais estudos populacionais para melhor serem avaliadas.

Palavras-chave: *Amazônia, Mata Atlântica, Endemismo, Modelos, Raridade.*

Introduction

Aniba is the largest Lauraceae genus. It encompasses 92 registered species, with *96, 125 and 99 valid names in the Index Kewensis, The Plant List and TROPICOS, respectively*. The *Aniba* species are concentrated in the South American region and are rarely found in Central America and the Antilles (Quinet 2005, Barbosa et al. 2012). Central Amazon and Guyana forests are considered dispersal centers of the genus (Kubitzki & Renner 1982).

In general, *Aniba* species are commonly known as laurel, with many variations, mainly according to their odors, colors, exudates and similarities with other species: pichurim bean, oleander, cayenne rosewood, bois de sassafras, moena and itauba. The Tupi Indians referred to laurel plants in general as "ajuva", "aijuba" and "aniuba" (Pio-Corrêa 1926-1978).

Several *Aniba* species are exploited for the production of timber and essential oils used in perfume industries and traditional medicine. The oils contain flavonoids, riparins, pyrones, linalool, allylbenzene and benzoate (Maia & Andrade 2009, Alcântara et al. 2010). Studies on the phytotherapeutic use of some species have proliferated in recent years, involving bioassays for some species, including *A. riparia* (Nees) Mez, *A. canelilla* (Kunth) Mez, *A. rosiodora* Ducke and *A. hostmanniana* (Nees) Mez (Simić et al. 2004, Lahlou et al. 2005, Nunes et al. 2014). However, the best-known species is *A. rosiodora*, commonly known as Brazilian rosewood; a linalool-rich essential oil is extracted from this species and has been used for decades as a fixative, including in the famous perfume Chanel No. 5 (Maia et al. 2007, Fonseca 2014).

Predatory exploitation and habitat destruction, particularly of species with restricted distributions, has prompted the inclusion of several species in the Red List of Threatened Species (IUCN, 2015) and/or in the Brazilian Flora Red List (BFRL 2015), including: *A. ferrea* Kubitzki, *A. intermedia* (Meisn.) Mez, *A. pedicellata* Kosterm*.*, *A. santalodora* Ducke and *A. rosiodora* Ducke.

The morphological and taxonomic complexity of Lauraceae, particularly *Aniba*, places them in a group that is difficult to identify (Kubitzki & Renner 1982, Quinet 2005, 2015). Consequently, identification errors in herbarium collections are very common. The use of common names of species, which do not always correspond to the scientific name, also hinders identification. Many forest, ethnobotanical, phytosociological or floristic inventories are key sources of information on species occurrence. However, fertile specimens are rarely collected, which would enable accurate identification, either for comparison in herbaria or by experts. Common names are usually given by a parataxonomist at the collection site, and these common names are used to search for the corresponding scientific name published in the literature, often referring to several species that are sometimes from very different taxonomic groups (Ferreira & Hopkins 2004, Baraloto et al. 2007, Procópio & Secco 2008). Species commonly known as laurel, with different variations (oleander, bois de sassafras, cayenne rosewood), may correspond to at least 50 species of the Lauraceae family and may belong to different genera, including *Aniba*, *Ocotea* and *Mezilaurus* (Quinet 2006, Ferraz et al. 2009). All of those problems lead to low numbers and a low quality of collection records in herbaria, which are considered the main problems for the analysis of spatial distribution of many plant species (Gaston & García-Viñas 2010, Hassemer et al. 2016).

The establishment of multi-national information exchange between collections and the development of data networks has improved the accuracy, standardization and updating of identifications of specimens registered in herbaria (Canhos et al. 2015). For example, the SpeciesLink network (http://splink.cria.org.br) database integrates primary data on Brazilian species registered in 179 national and international herbaria. However, those databases still require experts to characterize the taxa, check synonyms, update the nomenclature and certify (or not) the person who identified the species, generating more accurate data that may support studies on species diversity and geographical distribution (Siqueira & Durigan 2007).

Potential distribution models can be gerenated by correlating species occurrence points with environmental data (Soberón & Peterson 2005). Such models may benefit from more accurate data becoming available through species occurrence data networks, and they may help guiding new fieldwork to obtain more records of a particular species (Bourg et al. 2005, Guisan et al. 2006, Siqueira et al. 2009). These models may also guide conservation actions, including the establishment of new priority conservation areas (Marco & Siqueira 2009, Siqueira et al. 2009, Miller & Morgan 2011), planning and conservation actions (Giannini et al. 2012, Addison et al. 2013) among other.

Recently, the Reference Center for Environmental Information (CRIA) and the Virtual Herbarium of Plants and Fungi (HVFF) of the National Institute of Science and Technology (INCT) created the Brazilian Plants and Fungi Biogeography system (Biogeo 2015 - http://biogeo.inct.florabrasil.net/) for expanding knowledge on the biogeography of plant and fungal species in Brazil. Biogeo provides an open-access tool to model potential distributions using up to five different algorithms, in addition to a consensus model.

Using that tool, this study aimed to conduct a biogeographical review of *Aniba* in Brazil, including the actual and potential distribution, in addition to a review on the species conservation status.

Materials and Methods

1. *Survey of species and synonyms*

The names and synonyms of Brazilian *Aniba* taxa were compiled based on the latest taxonomic review of the genus (Kubitzki & Renner 1982), the List of Brazilian Plant Species (BFG, http://www.floradobrasil.jbrj.gov.br/) and the International Plant Names Index (http://www.ipni.org); the latter two are included in the main dictionaries and indexes of scientific names of angiosperms; both were consulted in December 2015.

2. *Species geographical distribution*

The actual distribution of each *Aniba* species was initially analyzed. For such purposes, all of the records of valid names existing in the SpeciesLink network database were considered. Those records were georeferenced either using the source collection or the dataCleaning (DC) app that was developed by CRIA (http://splink.cria.org.br/dc). That app identifies possible spelling mistakes in species names and facilitates data standardization; the app does not change the information, and no data are modified. The app only identifies suspicious records and recommends that the author choose between correcting or not correcting the information. Subsequently, the selected data were analyzed using another tool that was also developed by CRIA, called geoLoc (http://splink.cria.org.br/geoloc), that aims to help with the biological collection work through georeferencing the collections by assigning municipality coordinates to records that lack longitude and latitude data in voucher labels.

In addition to all of the records (Rt) available at SpeciesLink, we also examined all of the labels and scanned images of the voucher specimens from virtual herbaria that were included or not in the collection data integration networks and publications in the fields related to our study, including plant ecology, forest and floristic inventories and, in particular, papers reporting the list of species and flowers from different locations. However, that material was only analyzed when the voucher specimen record was cited in an herbarium registered in the Index Herbariorum (http://sweetgum.nybg.org/science/ih/).

The potential distribution of each *Aniba* species was modeled using the Biogeo system, which is directly interconnected to the SpeciesLink, from where species occurrence records are retrieved. Biogeo uses openModeller software to generate potential distribution models (http://openmodeller.cria.org.br/). Also developed by CRIA, openModeller openModeller is free, open-source, multi-algorithm software that supports multiple data formats; the software is specifically used to generate species potential distribution models based on environmental variables assigned to each training point or pixel (Muñoz et al. 2011).

Thus, the following three steps were required before generating species potential distribution models: (1) the application of filters to select the records to be included; (2) the search for environmental variables; (3) and the models productions. Lastly, the models were evaluated using a set of indicators.

3. *Record selection filters*

All of the data from the samples found, including valid synonyms, were individually revised based on all records (Rt) of each *Aniba* species found in SpeciesLink. Two records selection filters with the best data quality were applied as described in Giovanni et al. (2012). Only records with valid names (Rv) passed through the first filter (F1). Those records were then analyzed using Biogeo, wherein they passed through the second filter (F2), which only included records with different geographic coordinates and that had been identified by experts in the respective taxonomic group (Rgi). After the BioGeo system applied the filters all records were manually revised before being used to generate models.

Each record with a different coordinate was considered a training point inside a ~1 km^2 pixel. The records were aggregated into a single pixel to generate the models because many collections have the same coordinates or were performed very close to one another. Only the most recent records were considered in the case of duplicate records in the same collection or with conflicting identification between two or more collections. Samples lacking the name of the person who identified it or samples identified as derived from crops were not considered.

4. *Environmental variables*

Eight environmental variables were used, including one topographic variable (elevation) and seven bioclimatic variables from WorldClim (Hijmans et al. 2005) that have demonstrated no correlation in the Brazilian territory and that knowingly affect the distribution of great part of the vegetal species: (1) average daily temperature variation; (2) maximum temperature in the warmest month; (3) minimum temperature in the coldest month; (4) rainfall in the wettest quarter; (5) rainfall in the driest quarter; (6) rainfall in the warmest quarter; and (7) rainfall in the coldest quarter.

The elevation was directly obtained from the Global Climate Data WorldClim database (http://www.worldclim.org/current) to indirectly represent other factors, including incident solar radiation and atmospheric pressure, which usually strongly affect the distribution of plant species. The bioclimatic variables were also extracted from WorldClim (http://www.worldclim.org/bioclim) at an approximate spatial resolution of 1 km^2. WorldClim provides data on bioclimatic variables derived from the monthly rainfall and temperature values, which may be selected and applied to any region of the planet and are commonly used in species distribution modeling (Giannini et al. 2012, Peterson & Soberón 2012, Yanga et al. 2013).

5. *Potential distribution models production*

The potential distribution models produced are based on species presence data and on the environmental variables assigned to each pixel (Muñoz et al. 2011). In BioGeo, the number of pixels available determines which algorithm(s) may be processed and, depending on this number, up to five algorithms may be used, thereby generating up to five models, in addition to a consensus model (BioGeo 2015).

If there are less than five pixels, the environmental dissimilarity (ED) model was generated using the Euclidean distance in relation to the nearest point of occurrence, according to Carpenter et al. (1993). If there are five to nine pixels, maximum entropy (MaxEnt) was used, according to Phillips et al. (2006). The following three models were used if there are 10 to 19 pixels: 1) MaxEnt; 2) Genetic Algorithm for Ruleset Prediction – Best Subsets (GARP-BS), according to Anderson et al. (2003); and 3) a consensus model between the first two, as proposed by Biogeo. The following six models were used if there are 20 or more pixels: 1) MaxEnt; 2) GARP-BS; 3) Mahalanobis Distance (MD), according to Farber & Kadmon (2003); 4) Ecological-Niche Factor Analysis (ENFA), according to Hirzel et al. (2002); 5) Support Vector Machines (SVM), according to Schölkopf et al. (2001); and 6) a consensus model between the five models above.

All of the models other than the ED model were transformed into binary models using a cutoff point based on the minimum training presence, also known as the lowest presence threshold. That method identifies and selects the lowest value of environmental suitability assigned by the model to a presence point of the set of pixels; that is, pixel values higher than the cutoff point are considered possible areas of presence (Phillips et al. 2006, Pearson et al. 2007).

The consensus model in BioGeo shows in each pixel the number of models predicting environmental suitability for the species. In the consensus model, the different levels of agreement between algorithms are described using different colors: red (for five algorithms), orange (four), yellow (three) and blue (less than three). Consensus has been helpful to support decision making on the distribution of various species (Chen 2009, Hassemer et al. 2016).

6. *Model evaluation*

Model quality evaluation also depends on the number of pixels used from Biogeo. Models with less than five pixels of occurrence are not tested; they only serve to indicate relatively close and environmentally similar areas where new botanical collections should be performed.

Models with five to 19 pixels of occurrence were evaluated using the intrinsic omission rate and were tested using the leave-one-out procedure, subsequently calculating the probability associated with the number of successful predictions; that is, a new model is generated for each point using every other point and is subsequently tested using the point in question. After performing this process for all of the points, the probability of obtaining the number of successful predictions is calculated using a one-tailed Poisson-binomial test. In addition to the intrinsic and extrinsic omission rates, the area under the curve (AUC) was also analyzed in models with 20 or more pixels.

Omission is a type of error that occurs when the model fails to predict areas that are environmentally suitable for the species. The intrinsic omission rate was calculated using the same points used in model generation, whereas the extrinsic omission rate was calculated using points that were not used in model generation. The extrinsic omission rate was not calculated for the MaxEnt model. The intrinsic omission rate is usually suggested to not exceed 5%, whereas the extrinsic omission rate should not exceed 20% (Peterson et al. 2008). The AUC is the relationship between the area from the model and the study area for each curve threshold; the values were calculated using 5-fold cross-validation, which is recommended for species with 20 to 199 pixels (Phillips et al. 2006). The AUC values were considered very good (AUC > 0.9), good (> 0.8) and reasonable (> 0.7), as advocated by Swets (1988).

7. *Endemism, conservation and rarity*

The species endemism in Brazil was analyzed by comparing the BFG (2015) classification with the national and international virtual herbarium data. The conservation status was evaluated based on the following three criteria: (1) presence on the IUCN Red List of Threatened Species (2015); (2) presence on the Brazilian Flora Red List (2015); and (3) presence in the classification of rarity by Rabinowitz et al. (1986).

The classification of rarity by Rabinowitz et al. (1986) suggests eight categories of rarity based on the simultaneous data of geographic range (wide or narrow), habitat specificity (broad or narrow) and local population size (abundant or sparse). The criteria by Serrato et al. (2004) were modified to categorize each *Aniba* species; the geographic range was defined by the number of pixels in which the species were found. Species with a narrow geographic range were found in less than 26 pixels, equivalent to a maximum of 25% of the number of pixels occupied by *A. firmula* (104 pixels, highest number of pixels among the *Aniba* species). The number of vegetation types in which the species were found was analyzed to determine the habitat specificity category; species with a narrow habitat only occurred in one type of vegetation. The data on habitats were gathered from Quinet et al. (2015) and herbarium voucher specimen labels. The local population size category was defined by the total number of records found in SpeciesLink (Tr); species with small populations had fewer than 172 records, which was equivalent to a maximum of 25% of the number of records of *A. firmula* (689 records, highest number of records among *Aniba* species).

The following are abbreviations of Brazilian states: Acre (AC), Alagoas (AL), Amapá (AP), Amazonas (AM), Bahia (BA), Ceará (CE), Distrito Federal (DF), Espirito Santo (ES), Goiás (GO), Maranhão (MA), Mato Grosso (MT), Mato Grosso do Sul (MS), Minas Gerais (MG), Pará (PA), Paraíba (PB), Paraná (PR), Pernambuco (PE), Piauí (PI), Rio de Janeiro (RJ), Rio Grande do Sul (RS), Rio Grande no Norte (RN), Rondônia (RO), Roraima (RR), Santa Catarina (SC), São Paulo (SP), Sergipe (SE) and Tocantins (TO).

Results

1. *Names, synonyms and records of Aniba species in Brazil*

A total of 30 *Aniba* species were identified in the Brazilian territory; 48 valid synonyms were found for 19 of those species, and no synonyms were found for 11 species (Table 1).

In total, 3,538 *Aniba* records were found in Brazil, 479 of which were pre-excluded because they were only identified to the genus level. Thus, a total of 3059 records were analyzed using SpeciesLink, 345 of which were excluded because they had no valid names, leaving 2,714 records. Of these, 1,449 records were excluded because they were not georeferenced, and they lacked the name of the person who identified it. Finally, 1,265 records were considered that were grouped in 551 pixels (Table 2).

Considering the 30 species, the rate of loss was very high (56.4%), with 11.3% lost in the first filter (valid names) and 53.4% lost in the second filter (georeferencing and name of the person who identified the record; Table 3). The mean loss of the 30 species was 16.1% in the first filter and 53.4% in the second filter, corresponding to a total loss of 64.1%.

Six species had too few records and failed to pass through the filters (*A. excelsa* Kosterm*, A. jenmanii* Mez, *A. kappleri* Mez, *A. muca* (Ruiz & Pav.) Mez, *A. pedicellata* and *A. permolliis* (Nees) Mez*). A. kappleri*, *A. pedicellata* and *A. permolliis* had too few records and were not georeferenced; *A. excelsa*, *A. muca* and *A. jenmanii* had very few records and were derived from the same samples. Some species (*A. rosiodora* and *A. lancifolia* Kubitzki & W.A. Rodrigues) had very high loss rates in the first filter. *A. rosiodora* was the only species without losses in the second filter (Table 2).

2. *Model evaluation*

The MaxEnt model, which was applied to species with five to nine pixels (*A. ferrea*, *A. lancifolia* and *A. santalodora)*, failed to show good results because the intrinsic omission rate values were well above 5% (Table 3), although the probability values were < 0.01 (Poisson-binomial test). The same result was observed in most of the 12 species with 10 to 19 pixels. Only the species *A. megaphylla* Mez, *A. riparia*, *A. terminalis* Ducke and *A. viridis* Mez had models with good quality, and no differences occurred between the algorithms used among that group of species, except GARP-BS, which was more stringent in *A. riparia.*

The intrinsic omission rate of the eight species with more than 20 pixels was lower than 5% and therefore was acceptable. Among the six models applied to that group of species, the SVM and Mahalanobis models had AUC values lower than 0.9 and average extrinsic omission rate values higher than 20% for most species. The consensus model adequately described the results from the ENFA, GARP-BS and MaxEnt models. Only the species *A. burchellii* Kosterm., *A. canelilla* and *A. williamsii* O.C. Schmidt had higher extrinsic omission rates and lower AUC values, which are nonetheless classified as good (Table 3).

Table 1. Names and synonyms of Brazilian Aniba species in SpeciesLink.

1. *Aniba affinis* (Meisn.) Mez
   = *Aydendron affine* Meisn.
2. *Aniba burchellii* Kosterm.
   No synonyms
3. *Aniba canelilla* (Kunth) Mez
   = *Aniba elliptica* A.C. Sm.
   = *Cryptocarya canelilla* Kunth
   = *Cryptocarya pretiosa* (Martiuse ex Ness)
   = *Laurus canelilla* Willdenow*
   = *Mespilodaphne pretiosa* (Martiuse ex Ness) Ness var. *angustifolia* Ness
4. *Aniba citrifolia* (Nees) Mez
   = *Aniba muelleriana* Mez
   = *Aniba trinitatis* Mez
   = *Aydendron citrifolium* Nees
   = *Cryptocarya pachycarpa* Gleason
5. *Aniba cylindriflora* Kosterm.
   No synonyms
6. *Aniba excelsa* Kosterm.
   No synonyms
7. Aniba desertorum (Nees) Mez
   No synonyms
8. *Aniba ferrea* Kubitzki
   No synonyms
9. *Aniba firmula* (Nees & Mart.) Mez
   = *Aniba laevigata* (Meisn.) Mez
   = *Aniba panurensis* (Meisn.) Mez
   = *Aydendron firmulum* Nees & Mart.
   = *Aydendron laevigatum* Meisn.
   = *Aydendron panurense* Meisn.
10. *Aniba guianensis* Aubl.
   = *Aniba salicifolia* (Nees) Mez
   = *Aniba tessmannii* O.C. Schmidt
   = *Aydendron salicifolium* (Sw.) Nees
   = *Cedrota longifolia* Willd.
11. *Aniba heringeri* Vattimo-Gil
   No synonyms
12. *Aniba hostmanniana* (Nees) Mez
   = *Aniba gigantifolia* O.C. Schmidt
   = *Aniba pittieri* O.C. Schmidt
   = *Aydendron hostmannianum* Nees
13. *Aniba intermedia* (Meisn.) Mez
   = *Aniba puchury-minor* var. *intermedia* (Meisn.) Kosterm.
   = *Aydendron intermedium* Meisn.
14. *Aniba jemannii* Mez
   = *Aniba castanea* C.K. Allen
15. *Aniba kappleri* Mez
   = *Aniba riparia* (Nees) Mez
16. *Aniba lancifolia* Kubitzki & W. A. Rodrigues
   No synonyms
17. *Aniba muca* (Ruiz & Pav.) Mez
   = *Aydendron muca* (Ruiz & Pav.) Nees
   = *Laurus muca* Ruiz & Pav.
   = *Ocotea oblanceolata* Rusby
18. *Aniba megaphylla* Mez
   = *Aniba anisosepala* Sandwith
   = *Aniba koumaroucapa* Kosterm.
19. *Aniba parviflora* (Meisn.) Mez
   = *Aniba fragrans* Ducke
   = *Aydendron parviflorum* Meisn.
20. *Aniba permollis* (Nees) Mez
   No synonyms
21. *Aniba panurensis* (Meisn.) Mez
   = *Aniba gonggrijpii* Kosterm.
   = *Aniba mas* Kosterm.
   = *Aydendron panurense* Meisn.
22. *Aniba pedicellata* Kosterm.
   No synonyms
23. *Aniba puchury-minor* (Mart.) Mez
   = *Aniba amazonica* (Meisn.) Mez
   = *Aniba tessmannii* O.C. Schmidt
   = *Aydendron amazonicum* Meisn.
   = *Nectandra puchury-minor* (Mart.) Nees
   = *Ocotea puchury-minor* Mart.
24. *Aniba riparia* (Nees) Mez
   = *Aydendron riparium* Nees
25. *Aniba rosiodora* Ducke* In:
   = *Aniba duckei* Kosterm.
   = *Aniba rosiodora* var. *amazonica* Ducke
26. *Aniba santalodora* Ducke
   No synonyms
27. Aniba taubertiana Mez
   = *Aniba simulans* C.K. Allen
28. *Aniba terminalis* Ducke
   No synonyms
29. *Aniba viridis* Mez
   No synonyms
30. *Aniba williamsii* O. C. Schmidt
   = *Aniba gigantifolia* O.C. Schmidt
   = *Aniba murcana* C.K. Allen
   = *Aniba williamsii* R.L. Brooks

(*) The specific epithet *rosaeodora* was corrected to *rosiodora* (ICN Art. 60.8 e Rec. 60G1 (Melbourne Code, 2011).

3. *Actual and potential distribution of Aniba species in Brazil*

Below, the actual occurrence of *Aniba* species is initially described based on Quinet et al. (2015) and Tropicos (http://www.tropicos.org/); thus, the results from the potential distribution found after applying the consensus model are shown. Data regarding habitats were gathered from Quinet et al. (2015) and from the herbarium voucher specimen labels. Environmental variables resulting from each species are shown in Appendix 1.

A. affinis (Meisn.) Mez.

Distribution: Model available at http://biogeo.inct.florabrasil.net/proc/17806 (miniature map in Figure 1a). Amazon: AC, AM, RO, RR and MA. MG, wherein we found one record in the Rio Doce State Reserve (Reserva Estadual do Rio Doce), should also be included (E.P. Heringer 14000, NY). The consensus model indicates a higher probability for AM, AC, PA, AP, RR, MA, MT and TO; a lower probability for the Atlantic Forest from RJ to RN (area currently very destroyed and fragmented); and a significant - albeit unlikely - area in RS (Pampas) on the border with Uruguay.

Table 2. Total number of records (Rt), records with valid names (Rv), georeferenced species records and the name of the person who identified it (Rgi), number of pixels (Px), percentage of loss in filter one (F1), percentage of loss in filter two (F2) and percentage of total loss found in databases used to build the models of Brazilian *Aniba* species distribution.

Species	Rt	Rv	Rgi	Px	Loss (F1) (%)	Loss (F2) (%)	Total Loss (%)
A. burchellii	125	109	44	28	12.8	59.6	64.8
A. canelilla	229	206	69	29	10.0	66.5	69.9
A. desertorum	112	107	61	30	4.5	43.0	45.5
A. firmula	689	609	220	104	11.6	63.9	68.1
A. heringeri	167	153	80	46	8.4	47.7	52.1
A. panurensis	335	309	196	52	7.8	36.6	41.5
A. rosiodora	166	92	92	22	44.6	0.0	44.6
A. williamsii	116	106	70	36	8.6	34.0	39.7
A. affinis	93	83	35	19	10.8	57.8	62.4
A. citrifolia	92	84	48	19	8.7	42.9	47.8
A. cylindriflora	55	52	18	13	5.5	65.4	67.3
A. guianensis	76	71	35	14	6.6	50.7	53.9
A. hostmanniana	83	79	37	18	4.8	53.2	55.4
A. intermedia	106	93	55	19	12.3	40.9	48.1
A. megaphylla	45	45	29	11	0.0	35.6	35.6
A. parviflora	111	109	34	19	1.8	68.8	69.4
A. riparia	71	61	31	14	14.1	49.2	56.3
A. taubertiana	49	46	25	14	6.1	45.7	49.0
A. terminalis	50	46	25	11	8.0	45.7	50.0
A. viridis	108	99	18	13	8.3	81.8	83.3
A. ferrea	58	54	21	5	6.9	61.1	63.8
A. lancifolia	42	27	7	5	35.7	74.1	83.3
A. santalodora	13	13	5	5	0.0	61.5	61.5
A. puchury-minor	33	33	8	4	0.0	75.8	75.8
A. excelsa	3	3	2	1	0.0	33,3	33,3
A. jenmanii Mez.	2	2	0	0	0	100	100
A. kappleri Mez.	5	3	0	0	40	100	100
A. muca	6	6	0	0	0	100	100
A. pedicellata	2	0	0	0	100	-	100
A. permolliis	21	20	0	0	4,8	100	100
TOTAL	3059	2714	1265	551	11,3	53,4	56,4

Habitat: Igapó forest (blackwater-flooded Amazonian forests), terra firme forest (flood-free Amazonian forest) and várzea forest (seasonally whitewater-flooded Amazonian forest), near the transition to terra firme. Sandy, clayey and clayey-sandy soils.

A. burchellii Kosterm.

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17794 (miniature map in Figure 2a). Amazon: AC, AM and PA. In the model, areas with high species occurrence probability are found in the Central Amazon, AC, AP, PA and RO. Low-probability and scattered areas are found in PE, AL, BA SP and PR and in the borders between GO, MS and MG.

Habitat: Campinarana (dryland forest on white-sand soil), várzea forest, rain forest, secondary forest in advanced stage of succession. Sandy soils.

A. canelilla (Kunth) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/16941 (miniature map in Figure 2b). Amazon: MT, AC, AM, AP, RO and RR, the Atlantic Forest and the Cerrado (Brazilian savanna; MS, ES and MG). The record found in MG is a sterile sample that lacks the name of the person who identified it; therefore, MG should be excluded from the actual distribution. The model indicates a high probability for the Amazon (AC, AM, AP, PA, RO and RR) and the Atlantic Forest

Table 3. Quality indicators of the models applied to the Brazilian *Aniba* species: intrinsic omission rate (Oi), medium extrinsic omission rate (Om) and area under the curve (AUC). The probability value, derived from one-tailed Poisson-binomial test, is shown for species with less than 20 pixels.

Species	**Oi (%)**	**Model/Algorithm**											
		Consensus		**SVM**		**ENFA**		**GARP BS**		**MaxEnt**		**Mahalanobis**	
		AUC	**Om**	**AUC**	**Om**	**AUC**	**Om**	**AUC**	**Om**	**AUC**	**Om**	**AUC**	**Om**
	Species with 20 or more pixels												
A. burchellii	0	0.89	20.7	0.84	31.3	0.93	4.0	0.91	20.7	0.91	14.7	0.79	30.7
A. canelilla	0	0.89	20.7	0.84	30.7	0.93	10.0	0.87	24	0.92	10.0	0.81	27.3
A. desertorum	3.3	0.93	10.0	0.8	36.7	0.86	6.7	0.9	6.7	0.71	6.7	0.83	23.3
A. firmula	1.9	0.96	4.8	0.78	20.2	0.9	1.9	0.88	5.8	0.92	6.7	0.9	2.9
A. heringeri	2.2	0.94	8.9	0.83	30.0	0.89	4.4	0.93	6.7	0.87	11.1	0.82	13.1
A. panurensis	0	0.93	11.6	0.83	31.3	0.94	0	0.92	9.5	0.93	9.8	0.83	11.6
A. rosiodora	4.5	0.93	13.0	0.83	33.0	0.97	4.0	0.97	9.0	0.9	8.0	0.77	37.0
A. williamsii	2.7	0.89	18.9	0.81	35.4	0.92	5.0	0.89	18.6	0.88	18.9	0.78	24.3
	Species with 10 to 19 pixels												
A. affinis	10.5	< 01		-	-	-	-	< 01		< 01		-	-
A. citrifolia	5.3	< 01		-	-	-	-	< 01		< 01		-	-
A. cylindriflora	15.4	< 01		-	-	-	-	= 01		< 01		-	-
A. guianensis	7.1	< 01		-	-	-	-	< 01		< 01		-	-
A. hostmanniana	5.6	< 01		-	-	-	-	< 01		< 01		-	-
A. intermedia	5.3	< 01		-	-	-	-	< 01		< 01		-	-
A. megaphylla	0	< 01		-	-	-	-	< 01		< 01		-	-
A. parviflora	5.3	< 01		-	-	-	-	< 01		< 01		-	-
A. riparia	0	= 0.01		-	-	-	-	= 0.06		< 01		-	-
A. taubertiana	7.1	< 01		-	-	-	-	< 01		< 01		-	-
A. terminalis	0	< 01		-	-	-	-	< 01		< 01		-	-
A. viridis	0	< 01		-	-	-	-	< 01		< 01		-	-
	Species with 5 to 9 pixels												
A. ferrea	20.0	-		-	-	-	-	-		< 01		-	-
A. lancifolia	20.0	-		-	-	-	-	-		< 01		-	-
A. santalodora	20.0	-		-	-	-	-	-		< 01		-	-
	Species with 1 to 4 pixels												
A. puchury-minor	-	Environmental Dissimilarity Model: Euclidean Distance, Maximum value = 0.1											
A. excelsa	-	Environmental Dissimilarity Model: Euclidean Distance, Maximum value = 0.1											
	Species with zero pixel												
A. jenmanii Mez.	-	No model											
A. kappleri Mez.	-	No model											
A. muca	-	No model											
A. pedicellata	-	No model											
A. permollis	-	No model											

(MG and ES); patches in MA, RJ and MT; and an extended distribution to extra-Amazonian environments in the Pantanal and Cerrado of MS, GO, MG and SP. Occurrence is even less likely in northern PR and northeastern patches (TO, SE, AL, PE, RN, CE and PI).

Habitat: Terra firme forest, seasonal semi-deciduous forest, várzea forest and vegetation on rocky outcrops. Poorly drained, clayey and sandy-rocky soils.

A. citrifolia (Nees) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17132 (miniature map in Figure 1b). Amazon: AM, AP, MA and PA. Our study detected actual occurrence in MT (R.L. Fróes, 1730, G). The consensus model extends the areas to MT, RR and TO (also in the Amazon) and CE and RN (in the Atlantic Forest).

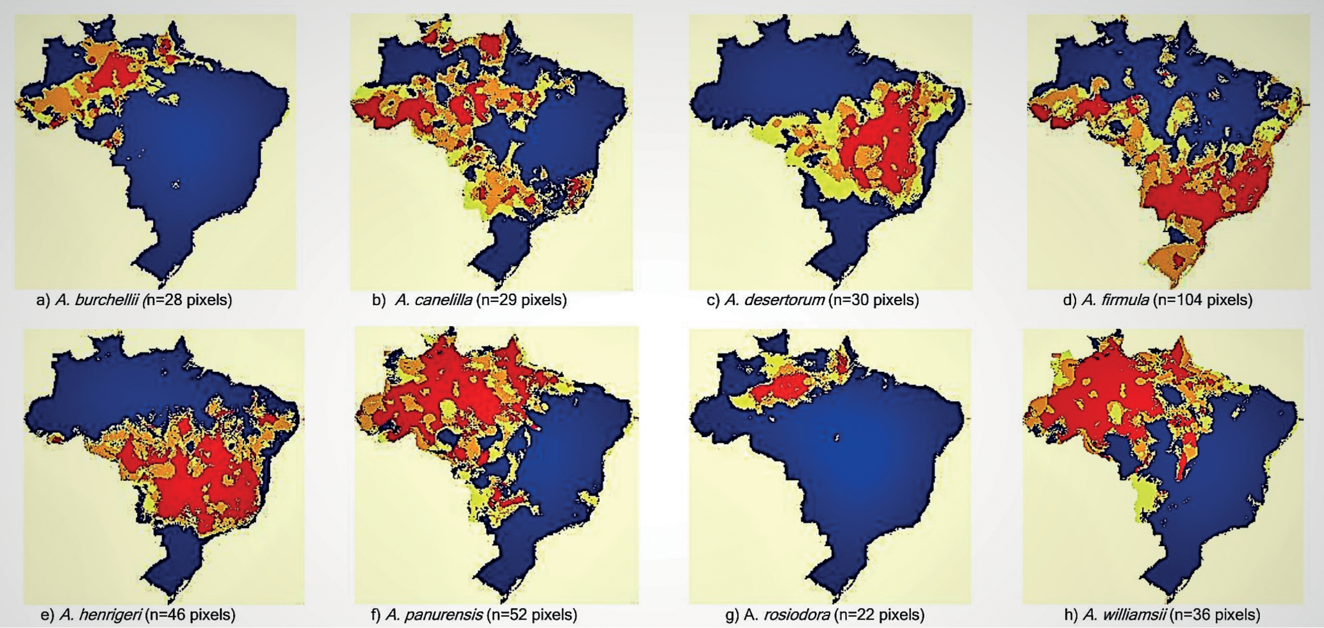


Figure 1. Potential distribution of *Aniba* species with 20 or more pixels. The colors represent areas of agreement between algorithms: red, 5 algorithms; orange, 4 algorithms; yellow, 3 algorithms, and blue, less than 3 algorithms.

Habitat: Terra firme forest, Várzea forest, transition vegetation between mangroves and sandy campina (shrubby vegetation with sparse trees), sandy savanna, secondary forest, Restinga (dunes) and mangroves. Sandy soils and terrain with granite rocks.

A. cylindriflora Kosterm.

Distribution: Model available at http://biogeo.inct.florabrasil.net/proc/17133 (miniature map in Figure 1c). Amazon: AM, AP, MT and RO. Our study detected actual occurrences in AC (L. Lima, 273, INPA) and RR (G.T. Prance, 4011, NY). The model extends the areas of potential species occurrence to the Amazon (AM, AP, MT and RO), Atlantic Forest (MA, RN, PB, PE, AL, SE and ES) and MS/TO (Bananal Island).

Habitat: Terra firme forest, Várzea forest, Igapó forest. Sandy and clayey-sandy soils.

A. desertorum (Nees) Mez

Distribution: Model available at http://biogeo.inct.florabrasil.net/proc/17134 (miniature map in Figure 2c). Caatinga (semi-arid vegetation) and Cerrado (savanna vegetation) including from grasslands to dense woodlands]): TO, BA, MA, PI, DF, GO and MG. The model expands the areas in the Cerrado and Caatinga, particularly in BA (which may be considered the distribution center of the species), DF and MT. CE, RN, PB and PE had low probability. There are no strong predictions for the Amazon, unlike the other *Aniba* species.

Habitat: Riparian forest, continental dunes, mesophytic forest. Sandy soils.

A. excelsa Kosterm.

Distribution: Model available at (http://biogeo.inct.florabrasil.net/proc/17859), (miniature map in Figure 3a). Amazon: AM. The species has only three records (duplicates) from a collection in the Amazon, near São Paulo de Olivença, on the border of Colombia (D.C. Daly, 4410, INPA, NY). The model generated using exclusively ED indicates the surroundings of the collection area as the most appropriate area.

Habitat: Rain forest, undulating terrain, sandy soil.

A. ferrea Kubitzki

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17135 (miniature map in Figure 1d). Amazon: distribution restricted to AM and AP, as indicated by the MaxEnt model. With lower probabilities, new collections may be planned for the Solimões riverbed, between Manaus (AM) and Macapá (AP), and other more random sites may be examined in the coastal strip of the Atlantic Forest.

Habitat: Terra firme forest. Clayey, clayey-sandy and yellow latosol soil.

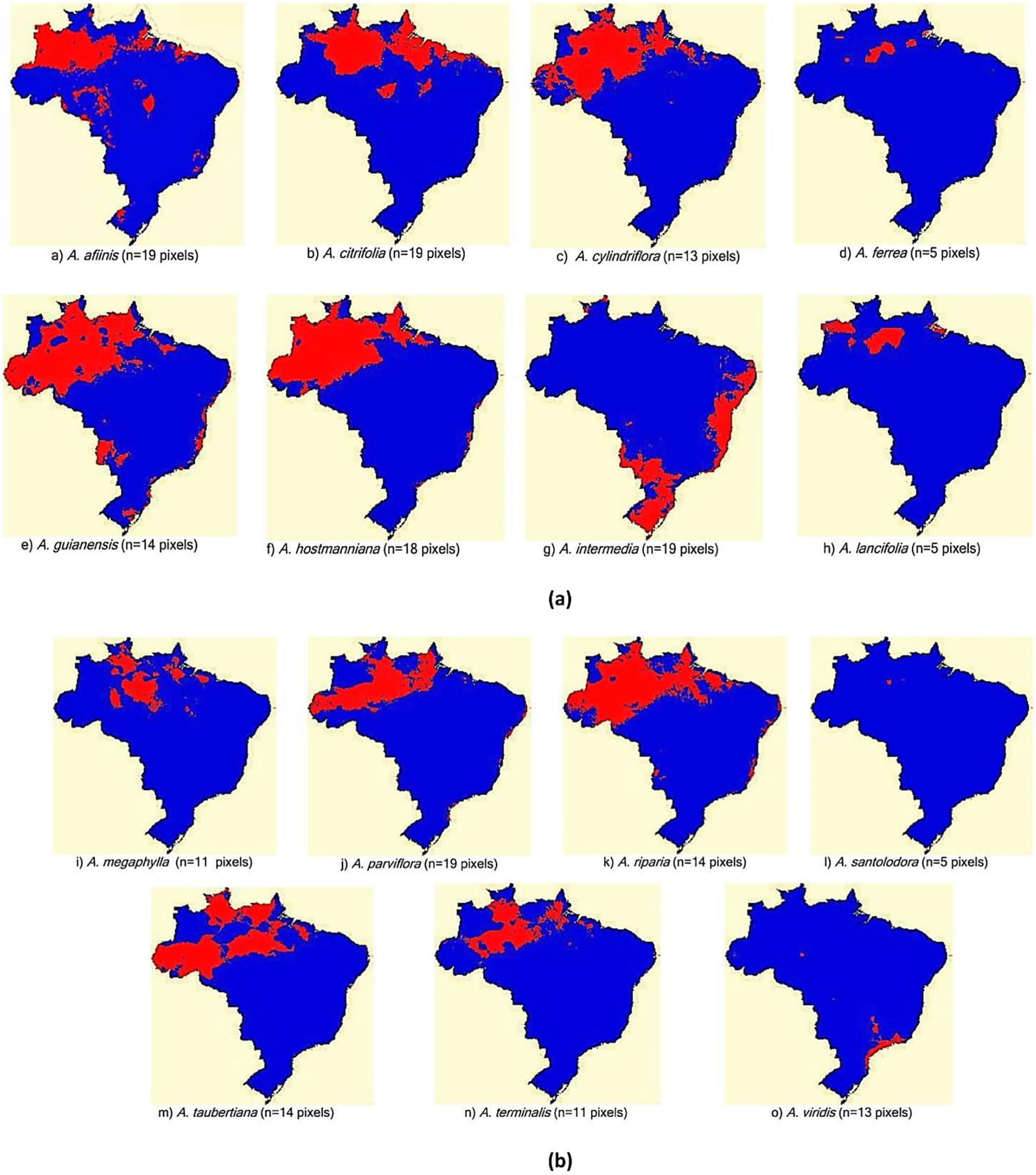
A. firmula (Nees & Mart.) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17136 (miniature map in Figure 2d). Amazon, Cerrado and Atlantic Forest (BA, MT, ES, MG, RJ, SP; PR and SC). We found species occurrence records in AM (A.R. Oliveira, n.n. [without number], INPA), RO (N.M. Ivanauskas et al. 2182, ESA), PA (A. Ducke, n.n., INPA) and PE (D.S. Correia, 79; E.M.N. Ferraz, 853, PEUFR). The model indicates species occurrence almost throughout the AC in a long strip of Atlantic Forest (from RS to CE). Patches are present in SP, MG, ES, RJ, MT, GO, BA and MA and in a large area in the Pampas in the mountainous region of Santa Cruz do Sul (RS). A lower probability of species occurrence is indicated for TO, MS, RO, RR and MA.

Habitat: Seasonal semi-deciduous forest, rain forest, low secondary forest, hygrophilous forest, upper restinga (tropical and subtropical moist broadleaf) forest. Clayey soil.

A. guianensis Aubl.

Distribution: Model available at http://biogeo.inct.florabrasil.net/proc/17137 (miniature map in Figure 1e). Amazon: AC, AM and AP.



a) *A. afiinis* (n=19 pixels)

b) *A. citrifolia* (n=19 pixels)

c) *A. cylindriflora* (n=13 pixels)

d) *A. ferrea* (n=5 pixels)

e) *A. guianensis* (n=14 pixels)

f) *A. hostmanniana* (n=18 pixels)

g) *A. intermedia* (n=19 pixels)

h) *A. lancifolia* (n=5 pixels)

(a)

i) *A. megaphylla* (n=11 pixels)

j) *A. parviflora* (n=19 pixels)

k) *A. riparia* (n=14 pixels)

l) *A. santolodora* (n=5 pixels)

m) *A. taubertiana* (n=14 pixels)

n) *A. terminalis* (n=11 pixels)

o) *A. viridis* (n=13 pixels)

(b)

Figure 2. Potential distribution of Aniba species with 5 to 19 pixels. The colors represent areas of agreement between algorithms: red, 2 algorithms, and blue less than 2 algorithms.

Our study extends the actual distribution to high Tapajós-PA (W.R. Anderson, 10800, MO), but no voucher specimen was found for Amapá (AP). However, the model indicates a large potential area in AP and almost all of AC; most of AM, RO, MS and ES; patches in RS, MA and PA; and small areas in the Atlantic Forest.

Habitat: Igapó forest, várzea forest, rain forest, secondary forest in a várzea area. Clayey, sandy, clayey-sandy and gleysol soil.

A. heringeri Vattimo-Gil

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17138 (miniature map in Figure 2e). Caatinga and Cerrado

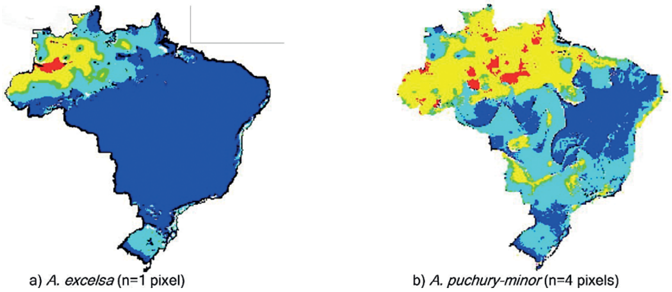


Figure 3. Potential distribution of Aniba species with 1 to 4 pixels.

(BA, TO, DF, GO, MS, MT, MG and SP). We detected actual species occurrence in MA (E.L. Taylor, 1271, HUEFS). The model indicates a high potential of species occurrence for much of MG, RJ, ES, PE, PB, CE, PI, AC, PA and RO. We may also find a lower probability of species occurrence in AM and AP.

Habitat: Riparian forest, seasonal semi-deciduous forest, Campos Rupestres (Montane savanna), várzea forest. Sandy-clayey soil with gravel.

A. hostmanniana (Nees) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17139 (miniature map in Figure 1f). Amazon: AC, AM, PA and RR. We found voucher specimens for AP (H.S. Irwin, 47982, NY; W. Egler, 4673, F). The model indicates a high potential of species occurrence in almost all of AM and much of RR and AP; it also expands into areas in PA and patches in the Atlantic Forest (PR, ES, BA and PE).

Habitat: Terra firme forest, várzea forest, secondary forest, forested campinarana, impacted Cerrado. Medium-texture, sandy, clayey and yellow latosol soil.

A. intermedia (Meisn.) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17140 (miniature map in Figure 1g). Amazon (PA) and Atlantic Forest (AL, BA, CE, PB and MG). We only confirmed actual species occurrence in PA, BA and MG. No records were found in the other states. The consensus model indicates appropriate conditions in large areas of RS, SC, PR, MS, SP and southern BA and a strip in the coastal region, starting from RJ, covering much of ES, PB, SE and AL, almost all of PE, patches in MT, AL, PB and RN and part of MG and ES.

Habitat: Seasonal semi-deciduous forest, rain forest, restinga, hygrophilous forest, riparian forest, vereda (palm swampy vegetation). Sandy-clayey and latosol soil.

A. jenmanii Mez

Distribution: No model is available. We found two georeferenced records and the name of the person who identified this species, referring to duplicates from a collection in the Ducke Forest Reserve (Reserva Florestal Ducke), in Manaus-AM (J.Pruski, 3242, INPA and MO). These records were included in SpeciesLink early in 2016 and, have not yet been recognized by Biogeo. This precluded generating the model of this species.

Habitat: Rain forest.

A. kappleri Mez

Distribution: No model is available. The species is only mentioned for PA; however, three well-identified records were found, indicating species occurrence in PA near Marabá (R.P. Solomões, 318, CVRD), in AM near Manaus, in the Ducke Forest Reserve (M.S. Costa et al., 319, MBM) and in AP (H.S. Irwin, 48104, SP). Thus, its actual occurrence should be corrected to PA, AM and AP. Despite these three quite distant records, they lack coordinates for pixel generation.

Habitat: Rain forest.

A. lancifolia Kubitzki & W.A. Rodrigues

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/13648 (miniature map in Figure 1h). Amazon: AM. Indeed, the species is underrepresented in Brazil, and the occurrence records are found in the campinas and campinaranas of the region near Manaus (AM). The MaxEnt algorithm extended the potential area of species occurrence with patches in the Upper Rio Negro in AM, in a narrow strip in southern AP and on Marajó island (PA). However, we believe that the Rio Negro basin is the most suitable location to collect the species because the species was only found in campinarana and campinas.

Habitat: Campinarana and campina.

A. megaphylla Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/13649 (miniature map in Figure 1i). Amazon: AM, AP, PA and RR. We found one record in MT (C. Soares et al., 2015, HERBAM). The model indicates the potential for species occurrence in a wide strip between Itaituba (PA) and the Viruá National Park (Parque Nacional do Viruá, RR), involving areas in the Amazon (AM).

Habitat: Terra firme forest, secondary forest, forested campinarana. Sandy, clayey and sandy-rocky soil.

A. muca (Ruiz & Pav.) Mez

Distribution: No model is available. Amazon: AM and AP. Only two very old records of the 125 that are available in Tropicos (MOBOT) refer to Brazil; one refers to AM (B.A. Krukoff, 4724, MO, 1933) and the other refers to AP (Rosa, 1825, MO, 1977). However, those samples lacked the name of the person who identified them. We suggest a review of the Brazilian records to re-evaluate the occurrence of *A. muca* in Brazil because all other species records refer to Andean (Bolivian, Colombian, Ecuadorian, Peruvian and Venezuelan) forests.

Habitat: Terra firme forest, várzea forests.

A. panurensis (Meisn.) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17141 (miniature map in Figure 2f). Amazon: AC, AM, AP, PA, MA and MT. We found no voucher specimens for MA. Our study extends the occurrence to RO (G. Vieira et al., 305, MO; C.A. Cid, 7377, NY) and RR (G.T. Prance, 4011 NY). The model indicates a high potential of occurrence for the entire Amazon, including the Amazon areas of MA. A lower probability of species occurrence is indicated for MS, RJ and MG in the Triângulo Mineiro and in the border with ES; a patch is also indicated in southeastern SP and in a strip of Atlantic Forest from BA to CE.

Habitat: Terra firme forest, secondary terra firme forest. Very clayey and yellow, sandy, clayey, clayey-sandy, and latosol soils.

A. parviflora (Meisn.) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17142 (miniature map in Figure 1j). Amazon: AC, AM, AP and PA. Our study indicated the occurrence of the species in MT at the Cristalino State Park (Parque Estadual do Cristalino; G.A. Gallo, 90, NY). The consensus model extended the areas of potential species occurrence towards the Upper Rio Negro, RR and AP and small strips of the Atlantic Forest from ES to PB. The consensus model indicated no area of potential species occurrence in MT, despite the record of actual species occurrence in this state. When the models were analyzed separately, only the MaxEnt model showed potential species occurrence in the region of MT. Therefore, in this case, we considered the MaxEnt model more appropriate than the consensus model.

Habitat: Terra firme forest, sandbank forest. Sandy, heavy-texture clayey, clayey-sandy, sandy-clayey, and clayey-silty soil.

A. pedicellata Kosterm.

Distribution: No model is available. Only two records were found, which were duplicates of a single individual collected in Teresópolis-RJ in 1933 (Kulman s/n, RB), which was typified as a holotype (RB 19.958). Although it was considered valid, the records were recently removed from the BFG, SpeciesLink and Tropicos (MOBOT). The lack of any other record for over 80 years suggests that the species is extremely rare or, even worse, is extinct.

Habitat: Igapó forest, as mentioned in the original label.

A. permollis (Nees) Mez

Distribution: No model is available. Amazon: AM. Records qualified by name of the person who identified them indicate that the occurrence is restricted to AM in the region of Tefé and Manaus (Ducke Forest Reserve). However, those records failed to pass through the filters because they lacked georeferencing to generate pixels.

Habitat: Rain forest.

A. puchury-minor (Mart.) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17097 (miniature map in Figure 3b). Amazon: AC and AM. The model generated using only ED indicates that the areas of AC, AM, RR, PA, AP and MA have high probabilities of finding populations. The Atlantic Forest and mesophilic forests of the interior of SP and MG represent less likely areas of potential occurrence.

Habitat: Terra firme forest, várzea forest.

A. riparia (Nees) Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17098 (miniature map in Figure 1k). Amazon: AM, RO and RR. Our study extends the actual species occurrence to PA (D.C. Daly, 1194, NY; N.T. Silva, 45, MO). The consensus model extends the areas of potential occurrence to AC, AM, AP, MA, PA, RO and RR and small areas in MS and in the Atlantic Forest, with a lower probability of finding the species in ES, SE, AL, PB and RN.

Habitat: Terra firme forest, terra firme secondary forest.

A. rosiodora Ducke

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/14748 (miniature map in Figure 2g). Amazon: occurrence restricted to AM, AP and PA. The consensus model extends the areas in these states and indicates the probability of occurrence in southern RR. This species has plantations in the Ducke Forest Reserve (AM) and in Curuá-Una (PA).

Habitat: Terra firme forest, in medium-texture sandy, heavy-texture clayey, sandy-clayey and yellow latosol soil.

A. santalodora Ducke

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17143 (miniature map in Figure 1l). Amazon: AM. Indeed, the species is restricted to a small area in the region of Manaus (AM). The MaxEnt model also merely suggests the area surrounding the Ducke Forest Reserve (Manaus – AM) for new botanical collections.

Habitat: Forested campinarana, terra firme forest, secondary forest at early stages of succession and the sandbank forests; sandy soils.

A. taubertiana Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17144 (miniature map in Figure 1m). Amazon: AM, PA, RO, RR and MT. However, we found no voucher specimen for MT in our study. The model extends the occurrence of the species to almost all parts of RR and AC, a large area in western AM that borders with AC and RO, the northern part of AP and the border with PA, a patch in MA and small strips in the Atlantic Forest.

Habitat: Terra firme forest, igapó forest, várzea forest, transition from field to forest. Clayey and rocky lateritic soil.

A. terminalis Ducke

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/13660 (miniature map in Figure 1n). Amazon: AM and PA. In this study, we extended the actual occurrence to AP (S.A. Mori, 16042, NY) and RO (G. Pereira-Silva, 16524, CEN). The consensus model expanded the probability of occurrence to the vicinity of Manaus, southern Roraima, part of AP, patches in the interior of PA and in the border with MA, in addition to strips in the Atlantic Forest.

Habitat: Terra firme forest, open rain forest. Clayey and yellow latosol soil.

A. viridis Mez

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17145 (miniature map in Figure 1o). Atlantic Forest: RJ and SP. The consensus model expands the areas of these states and also indicates patches in AM (Juruena National Park (Parque Nacional do Juruena - PARNA de Juruena)), a strip from the interior of SP to MG (Triângulo Mineiro), a patch in MT and a coastal strip in SC and PR.

Habitat: Restinga, dense rain forest, secondary forest, restinga forest, seasonal semi-deciduous forest and mixed rain forest. Peaty soil.

A. williamsii O.C. Schmidt

Distribution: Model available at: http://biogeo.inct.florabrasil.net/proc/17809 (miniature map in Figure 2h). Amazon: AC, AM, AP and PA. In this study, we detected the actual occurrence of the species in RO (G.

Pereira-Silva, 16160, CEN). The consensus model indicates large areas of potential species occurrence in regions of AM, AP and RO and parts of AC, PA and RR, with a lower probability in the borders between MT and TO (Bananal Island), TO and MA, and MT and GO. Areas of MS and the coastal strip of the Atlantic Forest had rather low probabilities.

Habitat: Terra firme forest, sandbank forest, forested campinarana, occasionally in campina, upper caatinga, impacted Cerrado, Amazon savanna. White-sandy, sandy-clayey, sandy-stony, yellow latosol soil.

4. *Endemism, conservation and rarity*

Most studied species (21) are not endemic to Brazil (Table 4). Four species (*A. canellila*, *A. desertorum*, *A. ferrea* and *A. lancifolia*) are mentioned as non-endemic to Brazil in the BFG (2015); however, no record of these species collected outside of Brazil was found. Eight other species (*A. burchellii*, *A. excelsa*, *A. henrigeri*, *A. intermedia*, *A. parviflora*, *A. pedicellata*, *A. rosiodora* and *A. santalodora*) are mentioned as endemic to Brazil in the BFG (2015); however, *A. burchellii*, *A. parviflora* and *A. rosiodora* records were found in other countries.

A. burchellii was found in Columbia (MO, S. Defler, 182, 183, 184); Peru (C.A. Grandez, 16178, MO) and Venezuela (A.H. Gentry & B.A. Stein, 46809, 47086, MO). *A. parviflora* was found in Guyana (H.D. Clarke et al., 7533, 7722, MO), French Guyana (J.J. Grenville, et al., 14302, CAY, K, MO, NY, P), Peru (R. Vásques et al., 16828, 16833, MO) and Suriname (J. Donselaar, 1484, MO). *A. rosiodora* was found in French Guyana (M.F. Prévost & P. Grenand, 4317, MO), Peru (A.H. Gentry et al., 26151; T.B. Croat, 18198, 18245,20749, MO) and Venezuela (G. Davidse & J.S. Miller, 27187, MO).

Five species were found in the IUCN Red List of Threatened Species and in the BFRL (*A. ferrea*, *A. intermedia*, *A. pedicellata*, *A. rosiodora*

Table 4. Endemism, conservation status and categories of rarity based on classification of Rabinowitz et al. (1986) of Brazilian *Aniba* species. (NE) not evaluated; (DD) data deficient; (VU) vulnerable; (NT) near endangered; (EN) endangered; (CR) critically endangered.

Species	**Endemism**		**Conservation Status**		**Categories of Rarity**		
	BFG 2015)	**This research**	**IUCN (2015)**	**BFRL (2015)**	**Geographical Distribution**	**Habitat Specificity**	**Local Populations Size**
A. affinis	No	No	NE	NE	Narrow	Broad	Scarce
A. burchellii	**Yes**	**No**	NE	NE	Wide	Broad	Scarce
A. canellila	**No**	**Yes**	NE	NE	Wide	Broad	Abundant
A. citrifolia	No	No	NE	NE	Narrow	Broad	Scarce
A. cylindriflora	No	No	NE	NE	Narrow	Broad	Scarce
A. desertorum	**No**	**Yes**	NE	NE	Wide	Broad	Scarce
A. excelsa	Yes	Yes	NE	NE	Narrow	Restricted	Scarce
A. ferrea	**No**	**Yes**	VU	VU	Narrow	Restricted	Scarce
A. firmula	No	No	NE	NE	Wide	Broad	Abundant
A. guianensis	No	No	NE	NE	Narrow	Broad	Scarce
A. henrigeri	Yes	Yes	NE	NE	Wide	Broad	Scarce
A. hostmanniana	No	No	NE	NE	Narrow	Broad	Scarce
A. intermedia	Yes	Yes	VU	NT	Narrow	Broad	Scarce
A. jenmani	-	No	NE	NE	Narrow	Restricted	Scarce
A. kappleri	No	No	NE	NE	Narrow	Restricted	Scarce
A. lancifolia	**No**	**Yes**	NE	NE	Narrow	Broad	Scarce
A. megaphylla	No	No	NE	NE	Narrow	Broad	Scarce
A. muca	No	No	NE	NE	Narrow	Broad	Scarce
A. parnurensis	No	No	NE	NE	Wide	Broad	Abundant
A. parviflora	**Yes**	**No**	NE	NE	Narrow	Broad	Scarce
A. pedicellata	Yes	Yes	CR	DD	Narrow	Restricted	Scarce
A. permollis	No	No	NE	NE	Narrow	Restricted	Scarce
A. puchury-minor	No	No	NE	NE	Narrow	Broad	Scarce
A. riparia	No	No	NE	NE	Narrow	Broad	Scarce
A. rosiodora	**Yes**	**No**	EN	EN	Narrow	Broad	Scarce
A. santalodora	Yes	Yes	VU	VU	Narrow	Broad	Scarce
A. taubertiana	No	No	NE	NE	Narrow	Broad	Scarce
A. terminalis	No	No	NE	NE	Narrow	Broad	Scarce
A. viridis	No	No	NE	NE	Narrow	Broad	Scarce

and *A. santalodora)*, and one species *(A. henrigeri*) is not present on the IUCN Red List but can be found in the BFRL and São Paulo State Red List (Table 4).

The 30 *Aniba* species were classified into four categories of rarity according to Rabinowitz et al., (1986; Table 4): 1) common, with wide geographic distribution, broad habitat and abundant populations (*A. canellila*, *A. firmula* and *A. panurensis*); 2) intermediate rarity, with wide geographic distribution and broad habitat, albeit with sparse populations (*A. burchellii*, *A. desertorum*, *A. henrigeri* and *A. williamsii);* 3) alarming intermediate rarity, with narrow geographic distribution, broad habitat and sparse populations (group wherein most species are concentrated: *A. affinis*, *A. citrifolia*, *A. cylindriflora*, *A. guianensis*, *A. hostmanniana*, *A. intermedia*, *A. lancifolia*, *A. megaphylla*, *A. muca*, *A. parviflora*, *A. puchury-minor*, *A. riparia*, *A. rosiodora*, *A. santalodora*, *A. taubertiana*, *A. terminalis* and *A. viridis*); and 4) highest-level rarity, with narrow geographic distribution, narrow habitat and sparse populations (*A. excelsa, A. ferrea*, *A. jenmanii*, *A. kappleri*, *A. pedicellata* and *A. permollis*).

Discussion

1. Database and filters

The BFG (2015) comprises 30 species of the genus. The record selection phase showed four different problems: 1) lack of synonyms in species databases; 2) invalid names in specimen labels, because the loss of data (11.3%) may be considered high; 3) the lack of geographic coordinates in the voucher specimen records; and 4) the lack of the name of the person who identified the specimen. The first three problems are linked to the database and information recorded by herbaria, which must take action to correct those problems and perform a taxonomic review and update to better define the *Aniba* species. Notably, the BFG (2015) has only one synonym (*Aydendron panurense* for *Aniba panurensis*) and therefore was not the best source to select species and synonyms. The third problem is related to the collectors who must record the geographic coordinates, even if only approximate coordinates are available, namely those of the municipality where the species was collected. The fourth problem, which is much greater, is related to the lack of expert taxonomists for a reliable identification of samples. The lack of taxonomists in Brazil has already been highlighted by other authors (Ferreira & Hopkins 2004, Hopkins 2007).

Six species (*A. excelsa*, *A. jenmanii*, A. *kappleri*, *A. muca*, *A. pedicellata* and *A. permolliis*) have few records, and none of those records passed through the filters applied. Plants may have few records in the collections for three reasons: 1) they have a narrow geographic distribution; 2) they have very sparse populations; and 3) they are seldom collected, which may lead to a false sense of species rarity, consequently resulting in erroneous assessments of the species conservation status (Siqueira et al. 2009) and limiting the identification of endemic areas because those areas may be confused with high-intensity collection areas (Murray-Smith et al. 2009).

In the Amazon, in particular, botanical collections are restricted to a few areas (Hopkins 2007, Schulman et al. 2007) and are sometimes confused with highly endemic areas (Nelson et al. 1990). However, even in areas with many botanical collections, including the region of Manaus-AM, only one individual each of *A. jenmanii* and *A. kapleri* were recorded, which suggests they are indeed rare; similarly, *A. pedicellata* only has one record from the municipality of Teresópolis (RJ), which is also a well-explored region. A different situation was observed for *A. excelsa* and *A. muca*, which have few records in poorly collected areas. This suggests the need for a greater collection effort. In turn, *A. permolliis* has 20 valid records, albeit none those were georeferenced or identified by an expert; that species could be prioritized by botanists to assess the identification and georeferencing.

2. Models

The number of records is directly related to the number of pixels used in *Aniba* models; the increase in the number of pixels or test points usually increases the accuracy (Stockwell & Peterson 2002) and the number of successful predictions (Siqueira et al. 2009) of the models. Our results also showed that species with the lowest number of pixels had the highest values of intrinsic omission (5 pixels – 20% intrinsic omission) and the species with the highest numbers of pixels had the lowest values of intrinsic omission (more than 20 pixels); however, the intrinsic omission of species with six to 19 pixels varied widely (from zero to 20%). In general, intrinsic omission seemed a good indicator to evaluate model performance across our experiments.

The GARP BS, MaxEnt and consensus models showed good performance when applied to species with five to 19 pixels. The MaxEnt model was only better than the consensus model for *A. parviflora* because the consensus model failed to identify the MT region as an area of potential occurrence of the species, although *A. parviflora* actually occurs in MT. Unlike the recommendation of Biogeo, which recommends the use of the above-mentioned models to predict areas of potential occurrence when < 20 pixels are available, we agree with Pearson et al. (2007) who recommended those models as tools to identify regions with similar environmental conditions; the identification of those regions is important for conducting new field surveys, thereby accelerating the discovery of unknown populations. Notably, that recommendation is already accepted for species with less than five pixels.

For species with 20 or more pixels, the results of intrinsic omission, average extrinsic omission and AUC were in agreement and indicate good models. Intrinsic omission < 5% and an AUC close to 1.0 were emphasized as good indicators by Anderson et al. (2003) and Peterson et al. (2008). Despite the known limitations to test potential distribution models that are generated with presence-only data, since there are no absence data or actual potential distribution to be used as a reference (see Pearson 2007 and Peterson et al. 2008 for more detailed discussions), predicting species potential distribution remains an important task, even when model accuracy cannot be perfectly measured (Lobo 2008, Lobo et al. 2008, Rupprecht et al. 2011)."

3. Aniba distribution in Brazil

The geographic distribution of *Aniba* species in Brazil shows a predominance in the Amazon. Only four species (*A. desertorum*, *A. henrigeri*, *A. pedicellata* and *A. viridis*) do not occur in the Amazon; however, *A. henrigeri* has great potential of occurrence in this biome. Some Amazonian species may also be found in Atlantic Forest areas (*A. canellila*, *A. firmula* and *A. intermedia*), and many others only have the potential to occur in the Atlantic Forest (*A. affinis*, *A. burchellii*, *A. citrifolia*, *A. cylindriflora*, *A. ferrea*, *A. guianensis, A. hostmanniana*, *A.*

panurensis, A. parviflora, *A. puchury-minor*, *A. riparia*, *A. taubertiana*, *A. terminalis* and *A. williamsii).* That potential of occurrence should be examined, particularly in some conservation units. Herein, we highlight the Rio Doce State Park, which has approximately 35,970 hectares of Atlantic Forest and crosses the municipalities of Marliéria, Dionísio and Timóteo in southwestern Minas Gerais. The natural vegetation extends beyond the boundaries of Minas Gerais, crossing part of the Linhares municipality in ES.

Some species of this study have been recorded in the Rio Doce State Park, and the model indicated suitable conditions for crop growth and conservation for other species. Furthermore, the park offers access to a research center to assist researchers, a nursery and an environmental police station, rendering it a key unit to implement and develop population conservation and recovery projects, particularly populations of threatened species or species regarded as rare.

However, the potential distribution in the Atlantic Forest is hampered by the reduction and fragmentation of that biome and associated ecosystems (Restinga salt marshes, mangroves and highlands), which originally encompassed approximately 1,300,00 km^2 in 17 Brazilian states. The native vegetation is currently reduced to approximately 22% of its original plant cover and is at different stages of regeneration. Only approximately 7% of the vegetation is well conserved in patches over 100 hectares in size (Ministério do Meio Ambiente do Brasil, http://www.mma.gov.br/biomas/mata-atlantica). Legal instruments determining strategies for the conservation and sustainable use of biodiversity in this biome, including promoting the recovery of degraded areas and the sustainable use of native vegetation (Law 11.428, of 2006 and Decree 6.660 of 2008), were only recently enacted. Similar to the Amazon, the Atlantic Forest has various endemic and threatened species and, even if reduced and fragmented, has environmental conditions suitable to house other threatened species, including some mentioned in this study.

The species *A. canellila*, *A. desertorum*, *A. firmula* and *A. henrigeri* occur in the Cerrado region of central Brazil, and *A. burchellii* and *A. panurensis* can potentially be found there. The species *A. desertorum* and *A. henrigeri* occur in the Caatinga region, and there is no high probability of occurrence of other species. Two Brazilian biomes have no records of *Aniba*, although a small potential of occurrence may be considered for Pantanal (*A. canellila*, *A. cylindriflora* and *A. williamsii*) and Pampas (*A. affinis* and *A. firmula*), mainly because of the wide ecological amplitude of those species; their occurrence in Pantanal and Pampas would be unprecedented because there are no records of *Aniba* in these biomes thus far.

4. Endemism, conservation and rarity

Nine *Aniba* species are endemic to Brazil (*A. canellila, A. desertorum, A. excelsa, A. ferrea, A. henrigeri, A. intermedia, A. lancifolia*, *A. pedicellata* and *A. santalodora*), and our result indicates the need to review the BFG (2015). Those species have characteristics that raise some degree of concern, except for *A. canellila*, which is present in several Brazilian biomes. The species *A. excelsa, A. Lancifolia* and *A. pedicellata* are endemic to Brazil and are found in well-located areas. *A. excelsa* is endemic to the Rio Negro basin, *A. lancifolia* is endemic to *campinarana* and *campina* areas near Manaus, and *A. pedicellata* occurs exclusively in Rio de Janeiro (RJ). The species *A. ferrea*, *A. intermedia*, *A. pedicellata* and *A. santalodora* are already included in the IUCN Red List of Threatened Species. However, *A. excelsa* and *A. lancifolia* are not yet included, and our results indicate the need to include those species in the Red Lists.

The only species with many records and no degree of rarity were *A. canellila*, *A. firmula* and *A. parnurensis*; the other species displayed some degree of concern regarding their conservation status. In addition to the species cited above, we consider the situations of *A. jenmanii, A. kappleri* and *A. permollis*, which have few occurrences in Brazil and more specifically in the Amazon areas, very alarming. Therefore, those species must also be included in the Red Lists.

The following 14 species have a lower degree of rarity, albeit no less alarming: *A. affinis, A. citrifolia, A. cylindriflora, A. guianensis, A. hostmanniana, A. megaphylla, A. muca, A. parviflora, A. puchury-minor, A. riparia, A. rosiodora, A. taubertiana, A. terminalis* and *A. viridis*, and only one is classified as endangered by the IUCN (*A. rosiodora)*. The others, which have not yet been evaluated by the IUCN, require further population studies to be better classified.

Supplementary material

The following online material is available for this article:

Appendix 1 - Environmental variables WorldClim recorded for the set of points of occurrence to 26 species of *Aniba*: (Pu+) rainfall in the wettest quarter; (Ps+) rainfall in the driest quarter; (Pq+) rainfall in the warmest quarter; (Pf+) rainfall in the coldest quarter; (Vt/d) average daily temperature variation; (Tq+) maximum temperature in the warmest month; (Tf -) minimum temperature in the coldest month; Elevation (topographic indirect variable).

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Author Contributions

Carlos Henrique Franciscon: substantial contribution in the concept and design of the study; contribution to data collection and interpretation; and, contribution to manuscript preparation.

Izildinha S. Miranda: contribution to data analysis and interpretation; 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

Reptile diversity of Sinos River Basin

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Abstract: This work aimed to catalog the species of reptiles of the Sinos River Basin based on records from scientific collections and data collected in the field. We recorded 65 species, including 46 snakes, nine lizards, five turtles, four amphisbaenians and one caiman. Snakes composed most of the recorded specimens (91.3%), and the three most representative are venomous and of medical importance. The most urban region of the basin (Lowland) has the highest number of records. This fact may be a reflection of the high human population density in this region, which would have favored the encounter of specimens and their sending to scientific collections and research centers. It is worth highlighting that most species with few specimens in the collections are also rarely observed in the wild, such as *Clelia hussani* and *Urostrophus vautieri*. This observation makes it feasible that these populations are small or that they are declining.

Keywords: *Chelonia, Crocodilia, Squamata, Vale dos Sinos, Atlantic Forest, Pampa.*

Diversidade de répteis da Bacia Hidrográfica do Rio dos Sinos

Resumo: Este trabalho teve como objetivo catalogar as espécies de répteis da Bacia Hidrográfica do Rio Sinos com base em registros de coleções científicas e dados coletados em campo. Registramos 65 espécies, incluindo 46 serpentes, nove lagartos, cinco tartarugas, quatro anfisbenas e um jacaré. As serpentes compuseram a maioria dos espécimes registrados (91,3%), e as três espécies mais representativas são peçonhentas e de importância médica. A região mais urbana da bacia (planície) possui o maior número de registros. Este fato pode ser um reflexo da alta densidade populacional humana nesta região, o que teria favorecido o encontro de espécimes e seu envio para coleções científicas e centros de pesquisa. Vale ressaltar que a maioria das espécies com poucos exemplares nas coleções também são raramente observadas na natureza, como *Clelia hussani* e *Urostrophus vautieri*. Esta observação torna viável que essas populações sejam pequenas ou que estejam em declínio.

Palavras-chave: *Chelonia, Crocodilia, Squamata, Vale dos Sinos, Mata Atlântica, Pampa.*

Introduction

The loss of natural habitats by human action is one of the main causes of the reduction of biological diversity (Sala et al. 2000). This effect extends to several taxonomic groups, including reptiles (Gibbon et al. 2000). In Brazil, one can notice that the knowledge regarding the impacts of habitat loss on the reptile fauna varies between different biomes and different localities. Regarding the Atlantic Forest, for example, the knowledge status for the Southeast region (e.g. São Paulo; Rio de Janeiro) is significantly higher than for its South portion (e.g. Rio Grande do Sul). This is an unsettling fact considering that in the last decades there was a loss of 20.7% of the natural vegetation cover at the southern limit of the Atlantic Forest and the Brazilian Pampas that was caused by human actions, which represents a mean loss of 845.04 km² per year (Cordeiro & Hasenack 2009). In Rio Grande do Sul, for example, only about 31.4% of the areas classified as natural or semi-natural regarding their original vegetation cover are remaining (Cordeiro & Hasenack 2009).

The Sinos River Basin (SRB), located in the northeast of the state of Rio Grande do Sul (RS), is composed of several ecosystems (forests, grasslands and wetlands) associated to the Pampa and Atlantic Forest biomes (Mauhs 2013). However, the natural ecosystems of this basin are drastically reduced and fragmented due to rural and urban occupation (Oliveira et al. 2013). This process has been intensified since the mid 19th century (Cordeiro & Hasenack 2009, Oliveira et al. 2013, Plano Sinos 2014) and today the region concentrates the largest cities of the state (IBGE 2010, Plano Sinos 2014). Considering the current context of the SRB, the acquisition of basic information on its biodiversity is a requirement for a good environmental management in its territory (Plano Sinos 2014). Although the reptile fauna of RS is well documented

(Lema et al. 1983, Lema 1994, Lema 2002), the knowledge of the species distribution in final scales is still superficial. On this regard, this work aimed to catalog the reptile species of the SRB, generating an unprecedented list of species of Squamata, Chelonia and Crocodilia of this region.

Materials and Methods

1. Study site

The SRB is located in the northeast of Rio Grande do Sul (29° 20' S a 30° 10' S e 50° 15' W a51° 20' O), covering an area of about 4000 km² (Plano Sinos 2014) (Figure 1). This basin includes 32 municipalities distributed among the Upland, Midland and Lowland portions. The Upland (with altitudes of more than 1000 m above the sea level) covers 48% of the basin, including six municipalities: Caraá, Osório, Riozinho, Rolante, Santo Antônio da Patrulha and São Francisco de Paula. The Midland (altitudes up to 900 m) covers 26% of the basin and includes 10 municipalities: Araricá, Canela, Glorinha, Gramado, Igrejinha, Nova Hartz, Parobé, Santa Maria do Herval, Taquara and Três Coroas. The Lowland (altitudes up to 200 m), also with 26% of the area, includes 16 municipalities**:** Cachoeirinha, Campo Bom, Canoas, Capela de Santana, Dois Irmãos, Estância Velha, Esteio, Gravataí, Ivoti, Nova Santa Rita, Novo Hamburgo, Portão, São Leopoldo, São Sebastião do Caí, Sapiranga and Sapucaia do Sul.

The SRB is under the influence of a temperate climate, with abundant rainfall throughout the year, hot (Cfa) or warm (Cfb) summer, the latter in the higher areas (Peel et al. 2007) and inserted mainly in the Atlantic Forest biome (ca. 80%) and the Pampa biome in its lower stretch. Its vegetation cover has varied phytophysiognomies distributed according to the topography. The Upland is characterized by mixed forests of conifers and angiosperms (Mixed Ombrophilous Forest) and grasslands (Steppes), the Midland by caducifolious forests (Semidecidual Seasonal Forest) and the lowland by caducifolious forests, grasslands (savanna) and pioneer formations in wide flood areas in the region of the river mouth (Maus 2013). Due to the intense human occupation, the natural ecosystems of the SRB were drastically converted to agricultural and urban ecosystems. Regarding the total area of the SRB in 2010, 49% was covered by grasslands (most of them derived from deforestation), 28% by native forests, 9% by agricultural land, 8% by urban centers, 5% by forest plantations and 1% by wetlands (Oliveira et al. 2013).

2. Data survey in scientific collections

We consulted four scientific collections: Reptile Collection of the Museu de Ciência e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCT-PUCRS), Reptile Collection of the Museu de Ciências Naturais da Fundação Zoobotânica (MCN-FZB), Herpetological Collection of the Universidade Federal do Rio Grande do Sul (UFRGS) and Herpetological Collection of the Universidade do

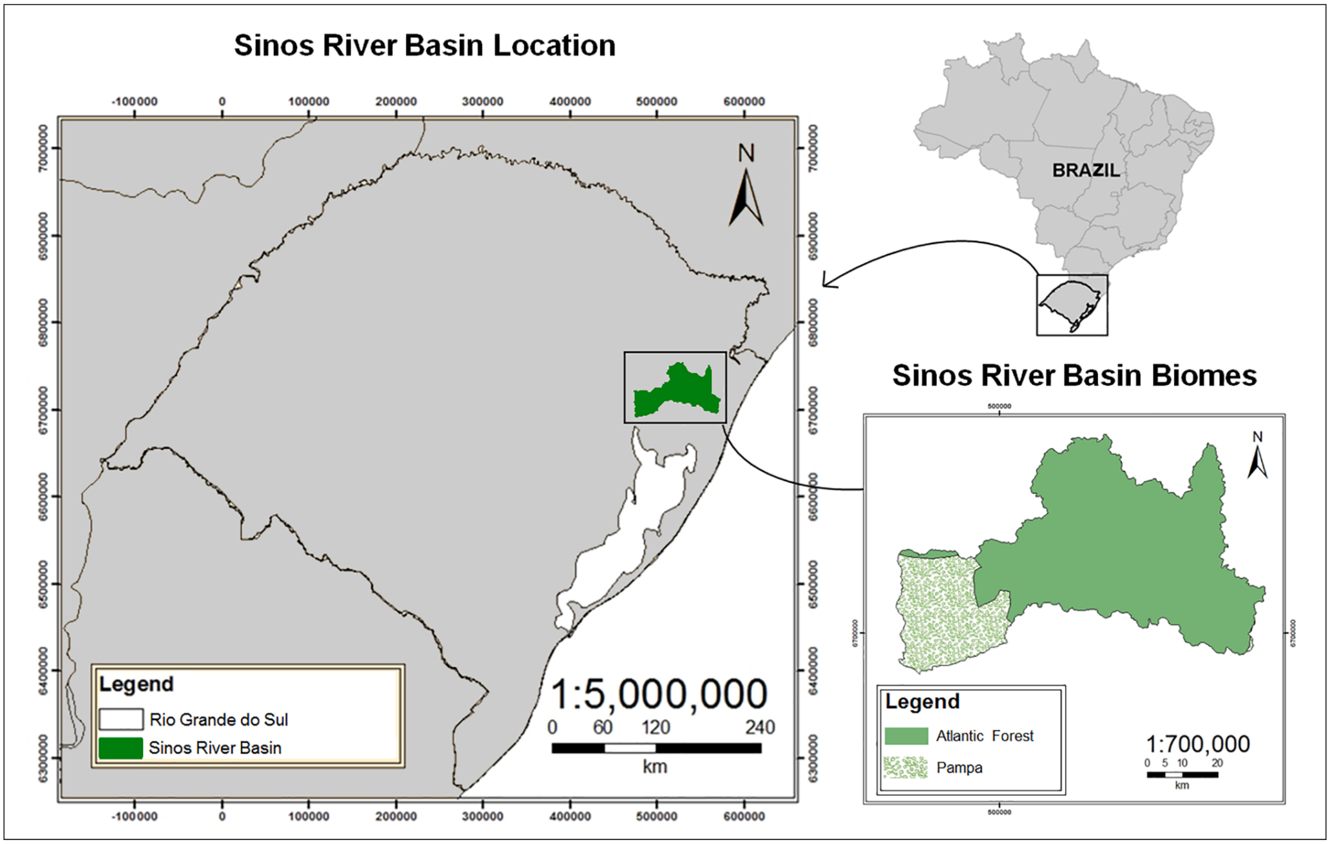

Figure 1. Map of the State of Rio Grande do Sul showing the location of the Sinos River Basin and the distribution of the Pampa and Atlantic Forest biomes within the limits of the basin.

Vale do Rio dos Sinos (UNISINOS). Species with a single specimen collected or with dubious identification were carefully examined for a double check of original identification.

3. *Occasional findings*

Reptiles found during other field activities within the limits of the basin were also considered, but only as a record of the species for the corresponding region of the basin, not being counted for abundance.

Results

The evaluated scientific collections presented 3154 reptile specimens (Table 1) distributed in 64 species (Figure 2). Of this total, 2876 (91.1%; N = 46) were snakes, 126 (4%; N = 9) were lizards, 122 (4%; N = 4) were amphisbenians and 30 (0.9%; N = 5) were turtles (Table 1). One species of caiman, *Caiman latirostris*, was recorded only by occasional findings.

Snakes are the species with the largest number of specimens represented in the collections, the three most abundant species belonging to the family Viperidae (*Bothrops alternatus*, with 613 specimens; *Bothrops jararaca*, with 339 specimens) and Elapidae (*Micrurus altirostris*, with 262 specimens).

Of the 65 recorded species, 27 were recorded in the three portions of the basin, while 8 species were exclusive of the Upland (6 snakes and 2 lizards), 8 of the Lowland (3 snakes, 1 lizard, 3 turtles and 1 caiman) and 1 of the Midland (1 snake) (Figure 3).

The portion of the basin with the largest record of specimens was the Lowland (1907), followed by Upland (682) and Midland (565). Regarding species richness, Lowland had a richness of 54, Upland of 52 species, while the Miland had 35 species.

Discussion

The Sinos River Basin (SRB) hosts more than half of the species that occur in the Brazilian Pampas and the Atlantic Forest of Brazil's extreme South. Of these species, only the turtle *Phrynops williamsi* was categorized in the regional list of threatened species as near threatened (NT; Fundação Zoobotânica 2014, Decreto Estadual Nº 51.797/2014). Other two, a snake (*Clelia hussami*) (Figure 2R) and a lizard (*Urostrophus vautieri*) deserve attention because they are rarely recorded and were classified as data deficient (DD) in the regional list of threatened species. The relatively high species diversity of the SRB may be related to the presence of two different biomes in the study area, the Pampa and the Atlantic Forest, which provide a combination of unique ecosystems (Bérnils et al. 2007, Verrastro & Borges-Martins 2015). There are some species that, although not recorded for the SRB, deserve to be mentioned as potential occurrences, since they were recorded in nearby areas in vegetal formations present in the basin. Some snakes, such as *Dipsas alternans*, *Tropidodryas striaticeps* and *Uromacerina ricardinii*, for example, have records in areas of Dense Ombrophilous Forest (Lema 1973, 1994, Di-Bernardo et al. 2003) relatively close to the Sinos River's headwaters. We cannot rule out the possibility that these species occur in the Upland portion, and a greater sample effort directed toward them is necessary since they have low encounter rates even where their occurrence is known (Lema 1994, Di-Bernardo et al. 2003). Likewise, the snake *Crotalus durissus* has a large number of records for grassland areas in the locality known as Cazuza Ferreira (28°55'53.09"S and 50°39'34.13"O), in the municipality of São Francisco de Paula, very close to our sample area. On the other hand, there are species previously recorded in the Basin but that were not included in the list. One of the excluded species is *Chironius laevicollis*, which was recorded by Lema et al. (1983) based on a specimen from the didactic collection of a school in São Leopoldo and later listed in the MCN collection as coming from this municipality. The fact that this species is frequent on the coast of the state of Santa Catarina, which receives a large number of visitors from Rio Grande do Sul, together with the absence of other records of this species in the state, led Di-Bernardo et al. (2003) to suspect that the specimen from São Leopoldo was actually collected in Santa Catarina and deposited in the school's didactic collection without a reference to its origin. We agree with the authors regarding the uncertainty of this record, this being the reason why it was not considered in our list. Lema (1994) recorded another species of this genus, *C. brazili* (referred as *C. flavolineatus*) from two embryos at the final stage of development removed from eggs found in the locality of Poço do Carvão, municipality of São Leopoldo, but the absence of the material as evidence led Di-Bernardo et al. (2004) to propose the exclusion of this species from the list of reptiles of RS. More recently, the occurrence of the species in RS was finally confirmed through two specimens collected in Santa Cruz do Sul and São Francisco de Assis, which were deposited in the MCP collection (Hamdan & Fernandes 2015). Abbeg et al. (2016) made a review of the species' distribution in RS, presenting new records from the western region, and considered the validity of the record by Lema (1994) to be highly likely. Although we do not rule out the possibility of the species to occur in this area, we did not considered the record of Lema (1994) mainly due to the lack of the material to serve as evidence, as proposed by Di-Bernardo et al. (2004) and also because the habitat of this record does not match the habitats in which the species has been recorded (Abbeg et al. 2016). Additionally, contrary to the areas in which the species has been currently found, the region of the SRB is better represented in scientific collections so that the absence of records is possibly a result of the species not occurring in this region since a number of recent records indicate that the species is not rare.

It is worth highlighting that the number of specimens of some species does not safely reflect their abundance in local populations. Regarding snakes, for example, the species with the largest number of records (*Bothrops alternatus*, *B. jararaca* and *Micrurus altirostris*) (Figure 2AX, 2AZ and 2AW) are venomous and of medical importance and are usually killed by local inhabitants and sent to universities (Barbo et al. 2008). The small number of specimens of lizards, turtles, amphisbaenians and caimans in the collections is likely the result of the population's lack of interest in these animals, as well as the scarcity of research groups dedicated to these organisms. Furthermore, storing large animals, such as crocodilians, turtles and some lizards is often impracticable due to the lack of available room in scientific collections. Despite these limitations, the collected data allow some considerations. The Midland portion, for example, had a considerably smaller amount of specimens and species than both the Upland and the Lowland (17.8% of the specimens and 35 species). This result most likely does not represent the real diversity of this portion, since the Midland includes a region with

Table 1. List of species recorded in the Sinos River Basin with the number of specimens in the examined collections, location in the basin and record method. Legend: Record in Scientific Collection (C) and Occasional Finding (OF); Portions of the basin: U (Upland), M (Midland) and L (Lowland); N (Number of specimens); Distribution in the basin: portions in which the species was recorded. Data regarding the number of specimens in the areas where each species was recorded were based exclusively on data from scientific collections.

Taxon	Record Method	Number of specimens in the collections				Distribution in the basin
		U	M	L	N	
CHELONIA						
CHELIDAE						
Acanthochelys spixii (Spix, 1824)	C	2	0	1	3	UL
Hydromedusa tectifera Cope, 1869	C	0	0	7	7	L
Phrynops williamsi Rhodin & Mittermaier, 1983	OF, C	0	0	1	1	L
Phrynops hilarii (Duméril & Bibron, 1835)	OF, C	1	0	3	4	UL
EMYDIDAE						
Trachemys dorbigni (Duméril & Bibron, 1835)	OF, C	0	0	15	15	L
LACERTILIA						
ANGUIDAE						
Ophiodes fragilis (Raddi,1826)	OF, C	1	7	22	30	UML
Ophiodes aff. *striatus* (Spix, 1824)	C	2	0	11	13	UL
GEKKONIDAE						
Hemidactylus mabouia (Moreau de Jonnès, 1818)	OF, C	0	0	3	3	L
LEIOSAURIDAE						
Anisolepis grilli (Boulenger, 1891)	C	8	0	1	9	UL
Enyalius iheringii Boulenger, 1885	OF, C	1	0	0	1	U
Urostrophus vautieri Duméril & Bribon, 1837	C	2	0	0	2	U
GYMNOPHTALMIDAE						
Cercosaura schreibersii Wiegmann, 1834	C	20	2	14	36	UML
TEIIDAE						
Salvator merianae (Duméril & Bibron, 1839)	OF, C	6	6	9	21	UML
Teius oculatus (D'Orbigny & Bibron, 1837)	C	1	0	10	11	UL
AMPHISBAENIA						
AMPHISBAENIDAE						
Amphisbaena darwini (Duméril & Bibron, 1839)	C	2	0	2	4	UL
Amphisbaena kingii (Bell, 1833)	C	0	1	46	47	ML
Amphisbaena prunicolor (Cope, 1885)	C	1	0	9	10	UL
Amphisbaena trachura Cope, 1885	C	27	1	33	61	UML
SERPENTES						
COLUBRIDAE						
Chironius bicarinatus (Wied-Neuwied, 1820)	C	19	7	26	52	UML
Chironius exoletus (Linnaeus, 1758)	C	3	0	0	3	U
Mastigodryas bifossatus (Raddi, 1820)	C	13	27	85	125	UML
Spilotes pullatus (Linnaeus, 1758)	C	1	4	6	11	UML
Tantilla melanocephala (Linnaeus, 1758)	C	5	0	1	6	UL
DIPSADIDAE						
Atractus reticulatus (Boulernger, 1885)	C	0	1	16	17	ML
Boiruna maculata Boulenger, 1896	C	3	0	12	15	UL
Clelia hussami Morato, Franco & Sanches, 2003	C	2	0	0	2	U
Echinanthera cyanopleura (Cope, 1885)	C	4	7	6	17	UML
Erythrolamprus almadensis (Wagler, 1824)	C	2	1	12	15	UML
Erythrolamprus jaegeri (Günther, 1858)	C	32	2	24	58	UML

Contined Table 1

Taxon	Record Method	Number of specimens in the collections				Distribution in the basin
		U	M	L	N	
Erythrolamprus miliaris (Linnaeus, 1758)	OF, C	21	21	2	44	UML
Erythrolamprus poecilogyrus (Wied – Neuwied, 1825)	OF, C	16	22	144	182	UML
Erythrolamprus semiaureus (Cope, 1862)	C	4	0	19	23	UL
Gomesophis brasiliensis (Gomes, 1918)	C	0	1	0	1	M
Helicops infrataeniatus (Jan, 1865)	OF, C	5	19	65	89	UML
Lygophis anomalus (Günther, 1858)	C	0	0	4	4	L
Lygophis flavifrenatus (Cope, 1862)	C	6	0	6	12	UL
Oxyrhopus rhombifer Duméril, Bibron & Duméril, 1854	OF, C	15	21	71	107	UML
Oxyrhopus clathratus Duméril, Bibron & Duméril, 1854	C	1	2	1	4	UML
Paraphimophis rustica (Cope, 1878)	C	1	0	5	6	UL
Phalotris lemniscatus (Duméril, Bibron & Duméril, 1854)	C	8	5	23	36	UML
Philodryas aestiva (Duméril, Bibron & Duméril, 1854)	C	17	6	5	28	UML
Philodryas arnaldoi (Amaral, 1933)	C	1	0	0	1	U
Philodryas olfersii (Liechtenstein, 1823)	C	4	13	33	50	UML
Philodryas patagoniensis (Girard, 1858)	OF, C	71	13	78	162	UML
Pseudoboa haasi (Boettger, 1905)	C	2	0	0	2	U
Psomophis obtusus (Cope, 1864)	C	0	0	1	1	L
Sibynomorphus neuwiedi (Ihering, 1911)	OF, C	5	16	18	39	UML
Sibynomorphus ventrimaculatus (Boulenger, 1885)	OF, C	1	3	41	45	UML
Taeniophallus affinis (Günther, 1858)	C	5	2	0	7	UM
Taeniophallus bilineatus (Fischer, 1885)	C	5	5	0	10	UM
Taeniophallus occipitalis (Jan, 1863)	C	1	0	0	1	U
Taeniophallus poecilopogon (Cope, 1863)	C	11	1	0	12	UM
Thamnodynastes hypoconia (Cope 1860)	C	4	0	8	12	UL
Thamnodynastes strigatus (Günther, 1858)	OF, C	8	7	15	30	UML
Tomodon dorsatus Duméril, Bibron & Duméril, 1854	C	9	20	33	62	UML
Xenodon dorbignyi (Duméril, Bibron & Duméril, 1854)	C	12	0	7	19	UL
Xenodon merremii (Wagler, 1824)	OF, C	34	81	117	232	UML
Xenodon neuwiedii Günther, 1863	OF, C	39	30	3	72	UML
DIPSADIDAE Incertae sedis						
ELAPIDAE						
Micrurus altirostris (Cope, 1860)	C	26	38	198	262	UML
Micrurus decoratus (Jan, 1858)	C	0	0	1	1	L
VIPERIDAE						
Bothrops alternatus Duméril, Bibron & Duméril, 1854	OF, C	82	19	512	613	UML
Bothrops cotiara (Gomes, 1913)	C	3	0	0	3	U
Bothrops jararaca (Wied, 1824)	OF, C	110	153	76	339	UML
Bothrops pubescens (Cope, 1870)	C	3	2	39	44	UML
CROCODYLIA						
ALLIGATORIDAE						
Caiman latirostris (Daudin, 1802)	OF	0	0	0	0	L
TOTAL		682	565	1907	3154	
Richness		54	35	52	65	
Number of exclusive species		8	1	8		

Figure 2. Reptile of Sinos River Basin: **A.** *Acanthochelys spixii*, **B.** *Hydromedusa tectifera*, **C.** *Phrynops hilarii*, **D.** *Trachemys dorbigni*, **E.** *Anisolepis grilli*, **F.** *Enyalius iheringii*, **G.** *Cercosaura schreibersii*, **H.** *Salvator merianae*, **I.** *Amphisbaena kingii*, **J.** *Amphisbaena prunicolor*, **K.** *Amphisbaena trachura*, **L.** *Chironius bicarinatus*, **M.** *Chironius exoletus*, **N.** *Mastigodryas bifossatus*, **O.** *Tantilla melanocephala*, **P.** *Atractus reticulatus*, **Q.** *Boiruna maculata*, **R.** *Clelia hussami*, **S.** *Echinanthera cyanopleura*, **T.** *Erythrolamprus almadensis*, **U.** *Erythrolamprus jaegeri*, **V.** *Erythrolamprus miliaris*, **W.** *Erythrolamprus poecilogyrus*, **X.** *Erythrolamprus semiaureus*, **Y.** *Gomesophis brasiliensis*, **Z.** *Helicops infrataeniatus*, **AA.** *Lygophis anomalus*, **AB.** *Lygophis flavifrenatus*, **AC.** *Oxyrhopus rhombifer*, **AD.** *Oxyrhopus clathratus*, **AE.** *Paraphimophis rustica*, **AF.** *Phalotris lemniscatus*, **AG.** *Philodryas aestiva*, **AH.** *Philodryas arnaldoi*, **AI.** *Philodryas olfersii*, **AJ.** *Philodryas patagoniensis*, **AK.** *Pseudoboa haasi*, **AL.** *Psomophis obtusus*, **AM.** *Sibynomorphus neuwiedi*, **AN.** *Taeniophallus affinis*, **AO.** *Taeniophallus bilineatus*, **AP.** *Taeniophallus poecilopogon*, **AQ.** *Thamnodynastes hypoconia*, **AR.** *Thamnodynastes strigatus*, **AS.** *Tomodon dorsatus*, **AT.** *Xenodon dorbignyi*, **AU.** *Xenodon merremii*, **AV.** *Xenodon neuwiedii*, **AW.** *Micrurus altirostris*, **AX.** *Bothrops alternatus*, **AY.** *Bothrops cotiara*, **AZ.** *Bothrops jararaca*, **BA.** *Bothrops pubescens*, **BB.** *Caiman latirostris*.

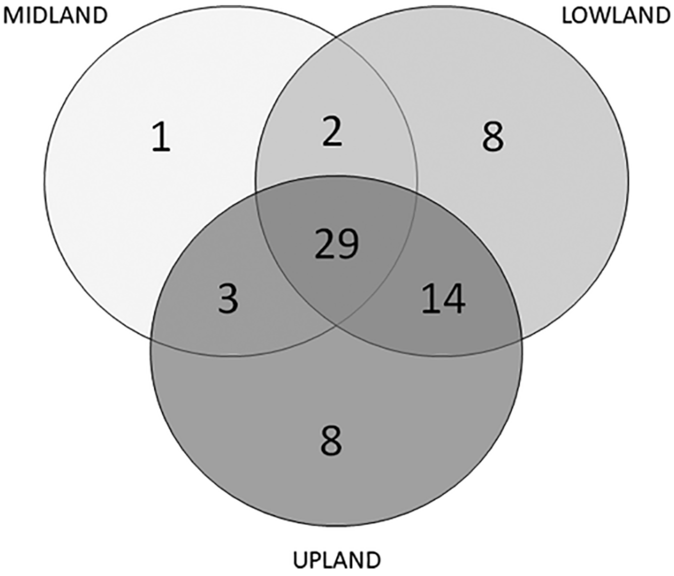

Figure 3. Diagram representing the number of species in the three portions of the Sinos River Basin. The intersections indicate the species shared by different portions. This diagram considered all record methods.

a relatively low population density and some of the largest remains of natural habitats, generating a high potential to harbor several species. A possible explanation for this result is the low human population density, which limits the encounters with the animals. The portion with the largest number of records (58.3% of the specimens) was the Lowland, a highly urban region, with a higher potential of impacts on the reptile fauna. Possibly this result is due to the geographic proximity to research centers and universities, facilitating logistic aspects of collections, and the proximity with the population, facilitating the receipt of specimens. Something similar may have occurred regarding the Upland that, despite being geographically distant from research centers, is a region with a high tourist appeal and has an important area of scientific interest (São Francisco de Paula National Forest) in which many studies were developed in the last two decades.

The species that were exclusive to Lowland (8) are all generalist species regarding habitat, have wide geographic distribution (Lema 1994, 2002, Borges-Martins et al. 2013) and it is likely that they were not collected in other localities due to the sample gaps, with the exception of *Micrurus decoratus*. This species has only one record in the state (Lema & Azevedo 1969) in the municipality of São Leopoldo, based on a specimen sent to the Instituto Pinheiros/SP and later donated to the MCN. In a review on the geographic distribution of *M. decoratus*, Gonzalez et al. (2014) did not consider this record due to the little information about it and its exclusivity for the state. However, we do not have justifications to disregard it, as the material with detailed information on the collection exists (see Lema & Azevedo 1969), even agreeing with the possibility of occurrence of an error when the specimen was placed in the Instituto Pinheiros/SP. We also include in our list the caiman species *Caiman latirostris* (the only crocodilian species of Rio Grande do Sul - Figure 2BB). This species was recorded occasionally (visual record by one of the authors) in the Lowland, in a marsh of the municipality of São Leopoldo. The difficulty in collecting specimens of this species, together with a possible low population density in the basin, are factors that explain its absence in scientific collections.

Only one snake was exclusive to the Midland, *Gomesophis brasiliensis* (Figure 2Y). This species inhabits areas associated with aquatic and muddy environments (Lema 2002) and may also occur in other localities of the basin. However, there is little available information on this snake. Seven species, *Bothrops cotiara*, *Chironius exoletus*, *Clelia hussami*, *Enyalius iheringii, Philodryas arnaldoi, Taeniophallus occipitalis* and *Urostrophus vautieri* (Figure 2AY, 2M, 2R, 2F and 2H) were exclusive to the Upland but *Taeniophallus occipitalis* and *Urostrophus vautieri* likely occur along the whole basin due to their generalist habits and wide geographic distribution (Lema 2002, Di-Bernardo et al. 2003, Quintela et al. 2011). *Chironius exoletus*, although more frequent in areas of Dense Ombrophilous Forest, has records in areas with other forest formations of the Atlantic Forest, both in the plateau and the coastal plain, thus it likely occurs in the three portions of the basin. *Bothrops cotiara* is a species whose distribution is limited to the Mixed Ombrophilous Forest and likely occurs in elevated areas of the Midland as well, where this forest formation is present. Lema (1980, et al. 1983) mentions the existence of two specimens of *Bothrops cotiara* collected in São Leopoldo and deposited in the collection of the Institute of Biogeography of the Saarland University in Saarbrücken, Germany, without citing the registration number. Checking this material would be necessary to confirm the presence of this species in the Lowland. Likewise, *Philodryas arnaldoi*, a rare species that is also associated with environments of dense forest in the plateau (Di-Bernardo et al. 2003), may occur in areas with these characteristics in the Midland. *Enyalius iheringii* has its distribution associated with the Dense Ombrophilous Forest (Verrastro & Borges-Martins 2015) and likely does not occur in other portions of the basin.

It is worth highlighting that the expansion of urban and agricultural areas, as well as the loss of natural landscapes in the SRB, is constant and intense, and some species listed here may be in an advanced process of population decline.

Supplementary material

The following online material is available for this article:

Appendix 1 - List of vouchers

Author Contributions

Camila Fernanda Moser: wrote the text, collected and revised the data.

Fernanda Rodrigues de Avila: contributed writing the text, collected and revised the data.

Roberto Baptista de Oliveira: contributed writing the text and revised the data.

Juliano Morales de Oliveira: contributed writing the text.

Márcio Borges-Martins: revised the text and provided the photos.

Alexandro Marques Tozetti: contributed writing 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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Short Communication

Nesting behavior of *Didelphis aurita*: twenty days of continuous recording of a female in a coati nest

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Abstract: Choosing the nest site to raise a litter has consequences on female fitness in mammalian species with no male participation in the parental care. We accidentally video recorded a coati's nest appropriation by a female opossum *Didelphis aurita*, at Parque Ecológico do Tietê, State of São Paulo, Brazil. For 29 days, from December 22, 2011, to January 19th, 2012, the activity of the female was video recorded 24h/day with a camera trap installed close to the nest. At her first appearance, she had infants in her pouch. After taking leaves to the nest twice on the first night, she kept a routine of going out after sunset and returning to the nest before dawn, carrying leaves on the tail on seven occasions. During the last days of recording, infants were seen attached to the female's body. Another episode of a female opossum with infants using a nest previously constructed by a coati was registered in 2013. To our knowledge, this is the first continuous description of the daily activity of opossums during the nesting phase.
Keywords: *Nasua nasua, marsupial, nest appropriation, nidification, common opossum, parental behavior.*

Comportamento de nidificação de *Didelphis aurita*: vinte dias de gravação contínua de uma fêmea em um ninho de quati

Resumo: A escolha de um ninho para criar uma ninhada tem consequências sobre o sucesso reprodutivo de fêmeas mamíferos que não compartilham com o macho o cuidado parental. Nós gravamos acidentalmente a apropriação de um ninho de quati por uma fêmea gambá *Didelphis aurita*, no Parque Ecológico do Tietê, São Paulo, Brasil. Durante 29 dias, de 22 de dezembro de 2011 a 19 de janeiro de 2012, a atividade da fêmea no ninho foi registrada 24h/dia. Em sua primeira aparição, ela tinha os filhotes ainda no marsúpio, e preparou o ninho, trazendo folhas para forração duas vezes na primeira noite. Depois, ela manteve uma rotina de sair após o pôr-do-sol e retornar ao ninho antes do amanhecer. Em sete dessas vezes ela trouxe folhas largas na cauda. Nos últimos dias, três filhotes foram vistos agarrados ao corpo da fêmea. Outra fêmea de gambá foi observada no PET usando um outro ninho de quati, em 2013. Esta é a primeira descrição contínua conhecida da rotina diária do comportamento de nidificação de gambás.
Palavras-chave: *Nasua nasua, marsupial, apropriação de ninho, nidificação, gambá, comportamento parental.*

Introduction

The Brazilian common opossum (*Didelphis aurita* Wied-Neuwied 1826) is a large marsupial species, smaller (body weight: 670-1800g; Cerqueira & Lemos 2000) and less versatile than the big opossum *D. albiventris*, and a forest-dweller species of the Atlantic forest (Emmons 1997; Paglia et al. 2012). It is an insectivorous-omnivorous animal that forages mostly during the early hours of the night, and whose diet is composed of insects (in 100% of collected feces, mainly Coleoptera, Diplopoda and Opiliones), solanaceous fruits (78%) and vertebrates (59%, primarily birds and mammals) (Cáceres & Monteiro-Filho 2001). Females almost exclusively maintain home ranges of 0.2 ha, a seemingly defensible territory (Cáceres 2003), selected according to resource availability (Loretto & Vieira 2005). The male home range is much more extensive (3.0 ha), and may vary in size during non-reproductive (larger) and reproductive seasons (smaller) (Cáceres & Monteiro-Filho 2001; Loretto & Vieira 2005).

The nesting behavior of didelphids is known for some species. For instance, the woolly mouse opossum (*Micoureus demerarae*) prefer to nest on a spiny palm tree of 4.66ft, approx. 1.42m, in height, during daylight (Moraes Junior & Chiarello 2005). *D. marsupialis* and *D. albiventris* both use shelters on the ground (during wet seasons), tree cavities or nests, also during the day (Sunquist et al. 1987; Vaughan & Hawkins 1999; Astúa et al. 2015). Finally, the Brazilian mouse opossum (*Monodelphis domestica*) adopts a wooden box that it fills with paper and uses during daylight, in captivity (Unger 1982; Harrison 1985; Faden et al. 1986).

All these species, and probably other didelphids, carry nest materials (dry leaves in nature or artificial material offered in captivity) to shelters that they opportunistically appropriate for themselves (i.e., dens of skunks and foxes, and nests of squirrels and birds: Vaughan & Hawkins 1999; Cáceres & Pichorim 2003; Loretto et al. 2005; Tortato & Campbell Thompson 2006; Turrin & Watts 2014). In some cases, nests are simultaneously used by didelphids and raccoons or skunks (Shirer & Fitch 1970; Beisiegel 2006). Dark anthropogenic spaces may also be used as nests (e.g., trash bins, cement boxes on the floor, Astúa et al. 2015). These shelters may serve to avoid predators (Moraes Junior & Chiarello 2005), to protect the young (Fadem et al. 1986), and to regulate body temperature (Unger 1982; Fadem et al. 1986). Nests or dens in anthropogenic environments would be preferentially chosen by didelphids as they are not usually visited by wild predators, and they provide food and water sources (Krause & Krause 2006).

Females with infants use the same nest for a longer period (Hossler et al. 1994; Allen et al. 1995) than males and non-reproductive females, and they fill it with more material (Fadem & Swartz, 1986; Moraes Junior & Chiarello 2005), evidently to hide infants from the time they exit the pouch until they are weaned (Fadem et al. 1986). In *D. aurita*, the fetus develops in the uterus for thirteen days, on average, after which they move to the pouch for the next 90 day developmental phase (Julien-Laferrière & Atramentowicz 1990). The infants are then ready to live outside the mothers body for a further 15 days, and are left in the nest when female goes out foraging overnight. Finally, at their 118th day of life (Julien-Laferrière & Atramentowicz 1990), the young may disperse from the natal nest and establish their home ranges, as suggested by their exploratory and moving behavior patterns (Julien-Laferrière 1995; Cáceres 2003).

Didelphis species, like other didelphids, were described as solitary (e.g., Cáceres & Monteiro-Filho 2001; Loretto & Vieira 2005), a hypothesis challenged by Astúa et al. (2015). Social behavior has only been studied in *D. virginiana*. In captivity and in the wild, they organize themselves in a hierarchical structure among males, positively correlated to body mass and testosterone concentration in plasma, and associated with scent-marking frequency and activity pattern, both of which are greater in higher status males (Holmes 1987; Ryser 1992). In nature, hours before the œstrus, females are followed by up to five males for several days (Ryser 1992). The same pattern was described in Philander opossum, the sister group of *Didelphis* (Guillemin et al. 2000).

Material and Methods

We describe here the nesting activity of a *D. aurita* adult female that we video recorded continuously for 20 days when monitoring coatis *Nasua nasua* nests at Parque Ecológico do Tietê (PET), State of São Paulo, Brazil. PET is a 1.400 ha area, composed of natural and exotic vegetation, artificial lakes, and a center for wildlife recovery and rehabilitation (Figure 1). There are dense populations of ring-tailed coatis and capuchin monkeys, and several bird species.

During the data collection campaigns that composed the Master Dissertation of AG, as we were interested in monitoring the nursing nests of coatis, we installed a camera trap (Bushnell trophy model 119436) in a *Senna multijuga* (Rich.) H.S. Irwin & Barneby (Fabaceae, Caesalpinioideae). On November 25th, 2011, a security guard saw two adult coatis inside a nest in that tree, in front of the administration building (Figure 1). On December 17th, the camera was installed and the recordings began at 15h07m. The appropriated nest was 7 meters above the ground in a 12 meter high tree of 1,570 m DAP (Figure 2 A-E), measured by a laser device (Rigid Micro LM-100) and an ordinary measuring tape. The camera trap was set to record for 30 seconds when triggered by movement. It did so until January 19th, 2012, when it was removed.

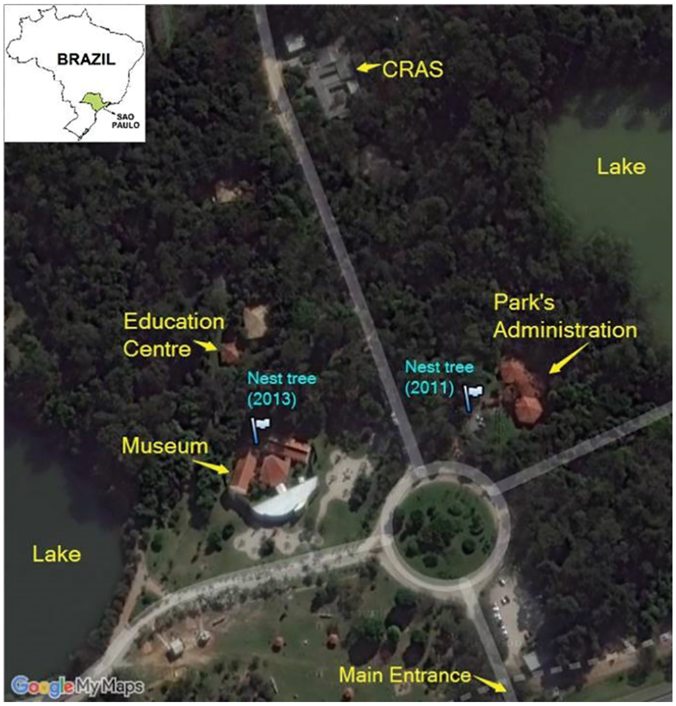

Figure 1. The position of the two trees, each containing a nest of ring-tailed coati appropriated by *Didelphis aurita* females in Parque Ecológico do Tietê, State of São Paulo, Brazil. The park was composed of artificial lakes, a center of wildlife reception and care (CRAS) and other facilities, such as the museum and the administration building. The distance between the two nest-trees was 214 m.

Results

The first observation of the opossum female occurred five days after camera installation, on December 22nd, 2011, at 23h58m (Figure 3). Table 1 shows the opossum activity during the following 29 days. On 7 out of 29 nights, she returned to the nest with dry leaves on tail, apparently for use as nesting material. The leaves were often broad. Two of these occasions occurred just after she appropriated the nest of the coatis (at 11h39m on December 22nd, and at 04h12m on December 23rd). During the subsequent days, the female's activity followed a circadian pattern. She left the nest around 7 p.m., in the light phase (it was summer, a hot and rainy season in São Paulo, Brazil), and returned before sunrise, between 3 and 4 a.m. She always returned to the nest in the dark phase, until January 13th, 2012, when she came back after the sunrise (Table 1). Judging from the volume of her pouch (Figure 3), it is possible the when she first visited the nest, her infants were already in her pouch. To the extent of our knowledge, there is no information in the literature about the stage of the reproductive cycle in which didelphid females select a place to serve as a nursing nest. January 11th, 2012, was when we first noticed the infants attached to the body of the mother (Figure 3), and they left the nest with her. So, that female and

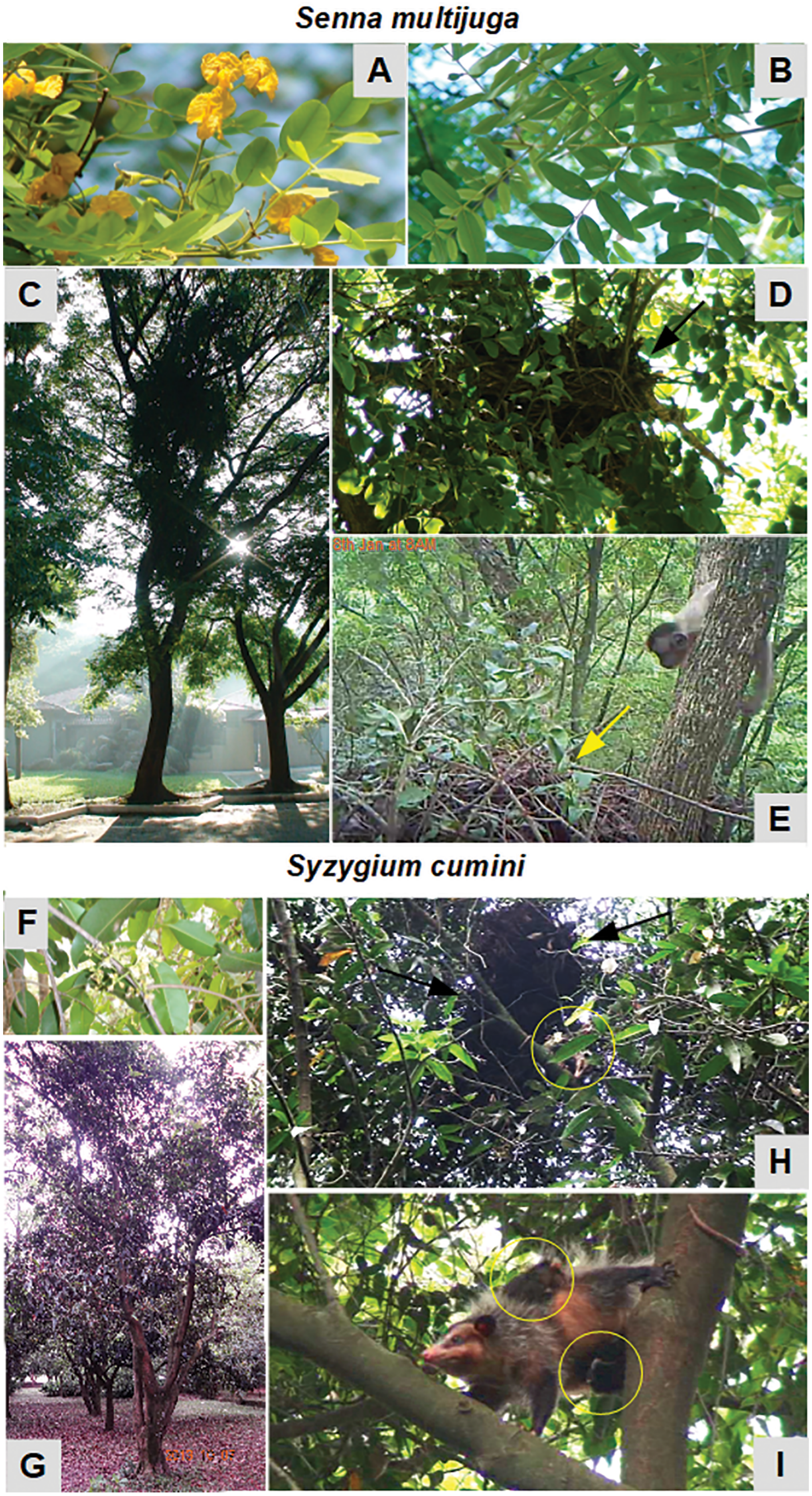

Figure 2. Details of two trees used by coatis to build nests, later appropriated by two females *D. aurita* in Parque Ecológico do Tietê: a *Senna multijuga* (A-E) and a *Syzygium cumini* (F-I), in 2011 and 2013, respectively. The first appropriated nest monitored (2011) is shown in D (from its bottom), and in E (overhead), when the camera registered the visit of a juvenile capuchin monkey. In 2013, a second female was seen coming out from a coati nest (H and I).

Figure 3. The nesting activity of the female opossum *Didelphis aurita* registered by a camera trap at Parque Ecológico do Tietê, in the city of São Paulo, Brazil. Her first appearance was on December 22nd close to midnight (top left). During that same night, she brought leaves on her tail (top middle). On December 26th, her pouch was voluminous. On the bottom, comparing the two consecutive photos taken on January 14th and 18th it is possible to see small tails moving against female's belly (red arrows) during grooming.

her offspring should not have reached that final stage of maternal care, in which nestlings are left in the nest during mothers' foraging activity, according to Julien-Laferrière & Atramentowicz (1990).

We spotted another female opossum, two years later, entering a coati nest in PET. It occurred on October 5th, in 2013, at 01:30 p.m., in a *Syzygium cumini* (L.) Skeels (1912) (Myrtaceae, Figure 2 F-I, and Suppl. Material). AG had a video camera (Sony DCR-DVD610 mini DVD Handy cam Camcorder) and followed the female from the time she climbed up the tree with infants attached to her body until she entered the nest. After 15 minutes, she went down the tree carrying the infants on her back. That second nest was about 127 meters away from the first one (Figure 1).

Discussion

Our report, although opportunistic, is a relevant contribution to the knowledge of the nesting behavior of *D. aurita*. Like other opossums, the observed Brazilian opossum female took cover in an abandoned nest built by a different species, the coati *Nasua nasua* (Caceres & Pichorim, 2003; Loretto et al. 2005; Tortato & Campbell Thompson 2006; Turrin & Watts 2014). The appropriation took place after the uterus developmental phase while infants were in the pouch.

In all the studied didelphids, nesting behavior was described as involving the transport of material on the tail. The opportunity of continuous recording provided the information about the frequency of nest improvement by the female. On the first night, when she first visited the nest (and presumably selected that place to be used as a nest), the female left and returned twice with leaves on her tail, and repeated this once more on the following day. Then the interval between nest material transport events increased (7 to a maximum of 11 days, Table 1). Unfortunately, the data does not indicate a precise association between the events of nest material transport and rainfall, lower temperatures or other environmental variables. For instance, the female returned to the nest with nest material on December 31st, 2011, a very wet day, but she did not do so on very windy days (January 2nd, 3rd, and 5th, 2012, when the camera trap fired several times during daylight).

To date, no one has ever seen opossums digging or folding branches to produce basket nests. Rather, all the literature offered describes of opportunistic appropriations of previously constructed nests, improved with collected material. If one considers improving appropriated nests with collected nest material as a nest-building behavior, it may be our contribution: to show that *D. aurita* females also construct nursing nests, as *D. virginiana* and *M. domestica* do, but not *D. albiventris* (Unger 1982; Fadem et al. 1986; Kimble 1997). Nevertheless, the behavior we describe here is very different from that of coatis (Gasco 2017). Coatis fold branches and straighten the leaves on the tree branches, stitching them all together and producing a basket (Gasco and Monticelli, in preparation). In addition, the juveniles carry materials to increase their nests.

Another contribution of our report relates to the nocturnal activity pattern of a female opossum during the pouch developmental phase. According to Caceres & Monteiro-Filho (2001), *D. aurita* forages mostly during the early hours of the night. The observed female spent about 14 uninterrupted hours out of the nest, returning only before sunrise. Would she stay out for so long during the next developmental stage, when the infants are left in the nest during the mother's foraging activity? We would expect not, because of the infants vulnerability to predators. In *D. virginiana*, infants with 70 days of life, younger than

Table 1. The activity of the female opossum in the video recordings taken from December 22nd, 2011 to January 19th, 2012. The last column shows when the female returns to the nest carrying leaves on her tail.

Date	Time (hh:mm)	Activity in the nest	Items on tail?
Dec 22nd and 23rd	22:58 to 01:16	First visit (Figura 3); smelling the surroundings; looking down to the nest, pulling a leaf with the mouth; leaving; arriving with nest material	yes
	04:12	Coming back and entering the nest with leaves	yes
	19:14	Leaving, going down the tree	
Dec 24th	03:14	Arriving, smelling nest before entering (infants at pouch opening)	
	22:01	Entering the nest with leaves	yes
Dec 25th	05:02	Entering the nest	
	18:59	Grooming in the nest	
Dec 26th	04:21	Entering the nest; voluminous pouch (Figure 3)	
	19:04	Grooming in the nest	
Dec 27th	04:53	Entering the nest	
	19:21	Leaving, going down the tree	
Dec 28th	03:40	Entering the nest and grooming	
	19:05	Smell nest, exit before going down the tree	
Dec 29th	03:58	Entering the nest; keeping tail up inside the nest	
	19:10	Leaving, going down the tree	
Dec 30th	04:33	Entering the nest	
	19:12	Sitting then grooming in the nest	
Dec 31st	04:26	Entering the nest	
	21:52*	Entering the nest with leaves	yes
Jan 1st	18:57	Leaving, going down the tree	
Jan 2nd***	04:28	Entering the nest	
	19:03	Smelling nest surroundings and going down the tree	
Jan 3rd	04:04	Entering the nest	
Jan 4th***	04:17	Entering the nest	
	19:15	Smelling nest surroundings and going down the tree	
Jan 5th***	18:00	Entering the nest? (just the final part of the tail is seen entering the nest)	
Jan 6th	18:45	Grooming	
Jan 7th***	23:18	Entering the nest with leaves	yes
	00:34	Leaving, going down the tree (just the tail is seen going down the tree)	
	04:22	Entering the nest	
	19:02	Grooming	
Jan 8th***	04:12	Entering the nest with leaves	yes
	19:14	Grooming	
Jan 9th	02:57	Entering the nest	
	19:17	Grooming	
Jan 10th	03:25 [08:09]	Arriving and smelling surroundings before entering [a monkey approaches and leaves]	
	19:20	Grooming and leaving; infant's tail is seen moving in its belly	
Jan 11th	00:48*	Entering the nest	
	19:24	Grooming; infant's tail is seen moving in its belly	
Jan 2nd***	04:10	Entering the nest	
	19:21	Grooming	
Jan 3rd***	05:15	Entering the nest	
	19:24	Leaving, going down the tree	
Jan 4th***	04:15	Entering the nest	
	19:21	Grooming	
Jan 5th***	05:21	Entering the nest	
	20:12*	Leaving, going down the tree	
Jan 16th	05:11	Entering the nest	
	19:19	Grooming	
Jan 17th	05:11**	Entering the nest	
	19:05**	Grooming and going down the tree	
Jan 18th	05:04	Entering the nest; infant's tail crossing her underbelly (Figure 3)	
	18:52 [21:49]	Grooming; 3 infants are seen [a smaller opossum appears]	
Jan 19th	05:06	Entering nest with wet leaves	yes

* Raining; ** Mistness; *** Wind.

in *D. aurita*, are left in the den during the mother's foraging activity. After weaning (93-103 days, in *D. virginiana*), the young start to forage outside the shelter and 60% are hunted by predators (Hossler et al. 1994). At this time, the quality of the habitat (e.g., food availability and protective vegetative cover) surrounding the weaning site was shown to affect juvenile survival rate (Hossler et al. 1994). Unfortunately, we have no information about predation on opossums or the habitat quality. The selected nest, a coati's basket built at 12 meters high *Senna multijuga* in front of the administration building, could offer protection against terrestrial predators. In fact, it had been used previously by more than one coati female and in subsequent years.

Supplementary material

The following online material is available for this article:

Video: Nesting behavior of common opossum *Didelphis aurita*.

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Author Contributions

Patrícia Ferreira Monticelli: installing the trap camera (DATA COLLECTION), analyzing videos (DATA ANALYSIS AND INTERPRETATION) and writing (MANUSCRIPT PREPARATION).

Aline Gasco: installing the trap camera (DATA COLLECTION), analyzing videos (DATA ANALYSIS AND INTERPRETATION) and writing (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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Isotopic niche of the catfishes *Bagre bagre* and *Genidens barbus* in a coastal area of south-eastern Brazil

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Abstract: The isotopic niche of *Bagre bagre* and *Genidens barbus* target of commercial fisheries in a marine coastal area from northern Rio de Janeiro State (~21ºS), south-eastern Brazil was compared to evaluate the feeding assimilation and the trophic relationship. The mean isotopic values of the catfishes and their food items in the $\delta^{13}C$-$\delta^{15}N$ space were coherent with their respective trophic positions. The relative contributions of the food items highlighted the shrimp *Xiphopenaeus kroyeri* as the most assimilated item in the diet of *B. bagre*, while for *G. barbus* the model highlighted the fish *Porichthys porosissimus*. The absence of niche overlap together with the trophic evenness point to a reduced feeding overlap between *B. bagre* and *G. barbus* in northern Rio de Janeiro State.

Keywords: *Ariidae, feeding assimilation, stable isotopes, trophic niche, tropical area.*

Nicho isotópico dos bagres Bagre bagre e Genidens barbus em área costeira do sudeste do Brasil

Resumo: O nicho isotópico de *Bagre bagre* e *Genidens barbus* alvos de pescarias comerciais em uma área costeira marinha do norte do estado do Rio de Janeiro (~21ºS), sudeste do Brasil, foi comparado para avaliar a assimilação alimentar e a relação trófica. Os valores isotópicos médios dos bagres e de seus itens alimentares em $\delta^{13}C$-$\delta^{15}N$ foram coerentes com suas respectivas posições tróficas. As contribuições relativas dos itens alimentares destacaram o camarão *Xiphopenaeus kroyeri* como o item mais assimilado na dieta de *B. bagre*, enquanto para *G. barbus* o modelo destacou o peixe *Porichthys porosissimus*. A ausência de sobreposição de nicho juntamente com a uniformidade trófica aponta para uma sobreposição alimentar reduzida entre *B. bagre* e *G. barbus* no norte do estado do Rio de Janeiro.

Palavras-chave: *área tropical, Ariidae, assimilação alimentar, isótopos estáveis, nicho trófico.*

Introduction

The catfishes are Siluriformes that show a wide and complex geographic distribution throughout tropical, subtropical and temperate waters, in lagoons, rivers, estuaries and marine environment (Diogo, 2004), being an important fish group in commercial fisheries worldwide (Marceniuk 2005, Froese & Pauly 2018). Among this primarily freshwater fish group, the family Ariidae is marine, generally more abundant in shallow coastal waters, in muddy or sandy bottoms (Marceniuk 2005, Silva et al. 2016). In general, the marine catfishes seek out river mouths and coastal lagoons during the spawning period, and they show reproductive adaptations as mouthbrooding (Chaves & Vendel 1996, Ferraris Jr. 2007).

The marine catfishes are generalistic benthophagous feeders, consuming fishes, crustaceans, molluscs and polychaetes (Mishima & Tanji 1982, Araújo 1984, Marceniuk et al. 2015). Tavares & Di Beneditto (2017) investigated the feeding habits of adult specimens of *Bagre bagre* (Linnaeus 1766) and *Genidens barbus* (Lacépède 1803) in northern Rio de Janeiro State, south-eastern Brazil. Both consumers are carnivorous with differences in feeding preference: the fish *Trichiurus lepturus* (Linnaeus 1758) and the shrimp *Xiphopenaeus kroyeri* (Heller 1862) were the most frequent prey species in the stomach contents of *B. bagre* and *G. barbus*, respectively. Both catfish species occur in Southwest Atlantic, being sympatric from northern to southern Brazil, where they support important resources to artisanal coastal fisheries (Froese & Pauly 2018). The conservation status of *G. barbus* deserves concern along Brazilian waters, since it is considered an "endangered" species of economic interest (Portaria MMA nº 445 de 17/12/2014, available at http://www.icmbio.gov.br/portal/images/stories/biodiversidade/fauna-brasileira/avaliacao-do-risco/PORTARIA_N%C2%BA_445_DE_17_DE_DEZEMBRO_DE_2014.pdf).

Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) provide data on marine vertebrates feeding ecology, understanding feeding preferences and food assimilation, and integrating diet over time (*e.g.* Cherel et al. 2005, Capelli et al. 2008, Di Beneditto et al. 2017, Navarro et al. 2017). The isotope values gradually increase with the trophic level, but the enrichment for $\delta^{15}N$ is more evident than for $\delta^{13}C$ (Hobson & Welch 1992, Fry 2008). The $\delta^{13}C$ values, in turn, are useful to indicate the carbon sources to the diet, as aquatic vs. terrestrial, pelagic vs. benthic and inshore vs. offshore (Peterson & Fry 1987). Isotopic models are particularly powerful when results of previous dietary studies are combined, reducing bias in data interpretation (Phillips et al. 2014). In tropical coastal areas where the availability of feeding resources is high, previous information on feeding preferences helps isotopic modelling (Di Beneditto et al. 2011).

The aim of this study is to compared the isotopic niche of adult specimens of *B. bagre* and *G. barbus* that are sympatric and target of commercial fisheries in a marine coastal area from northern Rio de Janeiro State, south-eastern Brazil, evaluating the feeding assimilation and the trophic relationship. We raised two assumptions: i) the main prey species recovered in the stomach contents are probably the most assimilated species by the consumers and ii) the isotopic niche of the consumers reflects their feeding preferences and assimilation.

Materials and Methods

1. *Sampling*

The sampling site is a marine coastal area from northern Rio de Janeiro State, southeastern Brazil (~21ºS; 41ºW) (Figure 1). The catfishes *B. bagre* and *G. barbus* are targets of commercial gillnet fisheries practised between 21º35'S and 22º00'S, from less than one to 10 nautical miles from shore, in depths varying from 10 to 30 m. The specimens captured in these fisheries are classified as adults according to its total length: the asymptotic or maximum length recorded for *B. bagre* is 55.0 cm (Marceniuk et al. 2015), while for *G. barbus* it is 120 cm, with the first maturity reached around 40.0 cm (Velasco et al. 2007, Froese & Pauly 2018).

In January 2016, 28 specimens of *B. bagre* (50.1 ± 4.2 cm of mean total length; 1,017.9 ± 248.0 g of mean total weight) and 16 of *G. barbus* (50.4 ± 4.4 cm of mean total length; 1,281.3 ± 263.9 g of mean total weight) were obtained from commercial fisheries. The back dorsolateral muscle samples (5 g) were collected for isotopic analysis.

Previous investigation about the local feeding habits of these catfishes done by Tavares & Di Beneditto (2017) guided the prey species selection for the present study. The prey species identified in the stomach contents of *B. bagre* were the fish *T. lepturus* (most frequent prey), *Gymnothorax ocellatus* (Agassiz 1831), *Paralonchurus brasiliensis* (Steindachner 1875) and the shrimp *X. kroyeri*. For *G. barbus*, the prey species recorded in the stomachs were the fish *Porichthys porosissimus* (Cuvier 1829) and *Conodon nobilis* (Linnaeus 1758) and the shrimps *X. kroyeri* (most frequent prey) and *Farfantepenaeus* sp. Besides the prey species identified in the stomach contents (excepting *G. ocellatus*, *C. nobilis* and *Farfantepenaeus* sp., whose sampling was not possible), bottom-associated fish species that are common along the sampling site (Di Beneditto et al. 2001, Gomes et al. 2003) were included in the isotopic analysis as potential prey to both catfishes: *Isopisthus parvipinnis* (Cuvier 1830), *Steliffer brasiliensis* (Schultz 1945) and *Symphurus plagusia* (Linnaeus 1766).

The prey species (or potential prey species) were collected by local fishermen through gillnet and bottom trawl net fisheries along the sampling site (Figure 1). A sample from the back dorsolateral muscle of fish (5 g) and abdomen muscle of shrimp (3 g) was removed from each prey (4-6 specimens) for isotopic analysis. All samples (consumers and prey species) were kept frozen (-20ºC) in acid washed vials, freeze-dried and grounded into a fine, homogeneous powder using mortar and pestle for isotope analyses.

2. *Isotopic analysis*

Stable isotope measurements were determined on fine powdered freeze-dried samples (1 mg) using a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific) interfaced with Conflo IV and linked to Elemental Analyzer Flash 2000 (Thermo Scientific) from the Laboratório de Ciências Ambientais - UENF. Stable isotope ratios were expressed in δ notation as parts per thousand (‰) deviations from the international calibration standards. Pee Dee belemnite carbonate and atmospheric nitrogen were standard values for carbon and nitrogen analyses, respectively.

Quality control for muscle tissue was performed through the reference standard Elemental Microanalysis Protein Standard OAS of certified isotopic composition $\delta^{13}C$= -26.98‰ and $\delta^{15}N$= 5.94‰. Reproducibility was tested with triplicate analyses at each five samples (± 0.3 ‰ for $\delta^{15}N$ and ± 0.2 ‰ for $\delta^{13}C$). Lipid content was not extracted from muscle samples prior to the analyses; however, the $\delta^{13}C$ interpretation was not compromised because C:N ratios were lower than 3.5 (low lipid levels) (Post et al. 2007).

A Bayesian approach with stable isotope mixing models in the SIAR (Stable Isotope Analysis in R) package (R Development Core Team 2016, Parnell & Jackson 2013) estimated the relative contribution of each food item to the diet of the catfishes. This package provides the probability density distributions, mean proportion and credibility intervals for each food item, incorporating uncertainty linked to elemental concentrations, isotopic signatures and discrimination factors (Parnell et al. 2010). Isotopic mixing models can have caveats when food items with similar isotopic values are included; however, Phillips et al. (2014) argued that when results of previous dietary studies are combined with isotopic approach the models are more powerful and the bias in data interpretation is reduced. Here, the previous description about the local feeding habits of *B. bagre* and *G. barbus* guided the isotope mixing models (Tavares & Di Beneditto 2017). Once the food items have been identified, stable isotope mixing models are consistent approaches to quantify the food assimilation (Phillips et al. 2014).

The Trophic Enrichment Factors (TEFs), or discrimination factors, are key parameters in isotopic mixing models, representing the isotopic differences between consumers' tissue and their food sources after they reached equilibrium (Parnell et al. 2010). In the absence of species-specific TEFs values from controlled diet experiments, these values can be obtained in meta-analyses for species phylogenetically related, considering the same tissue (Newsome et al. 2007). In this sense, we calculated $TEF^{15}N$ and $TEF^{13}C$ based on equations from a meta-analysis of isotopic studies that considered muscle of fish species

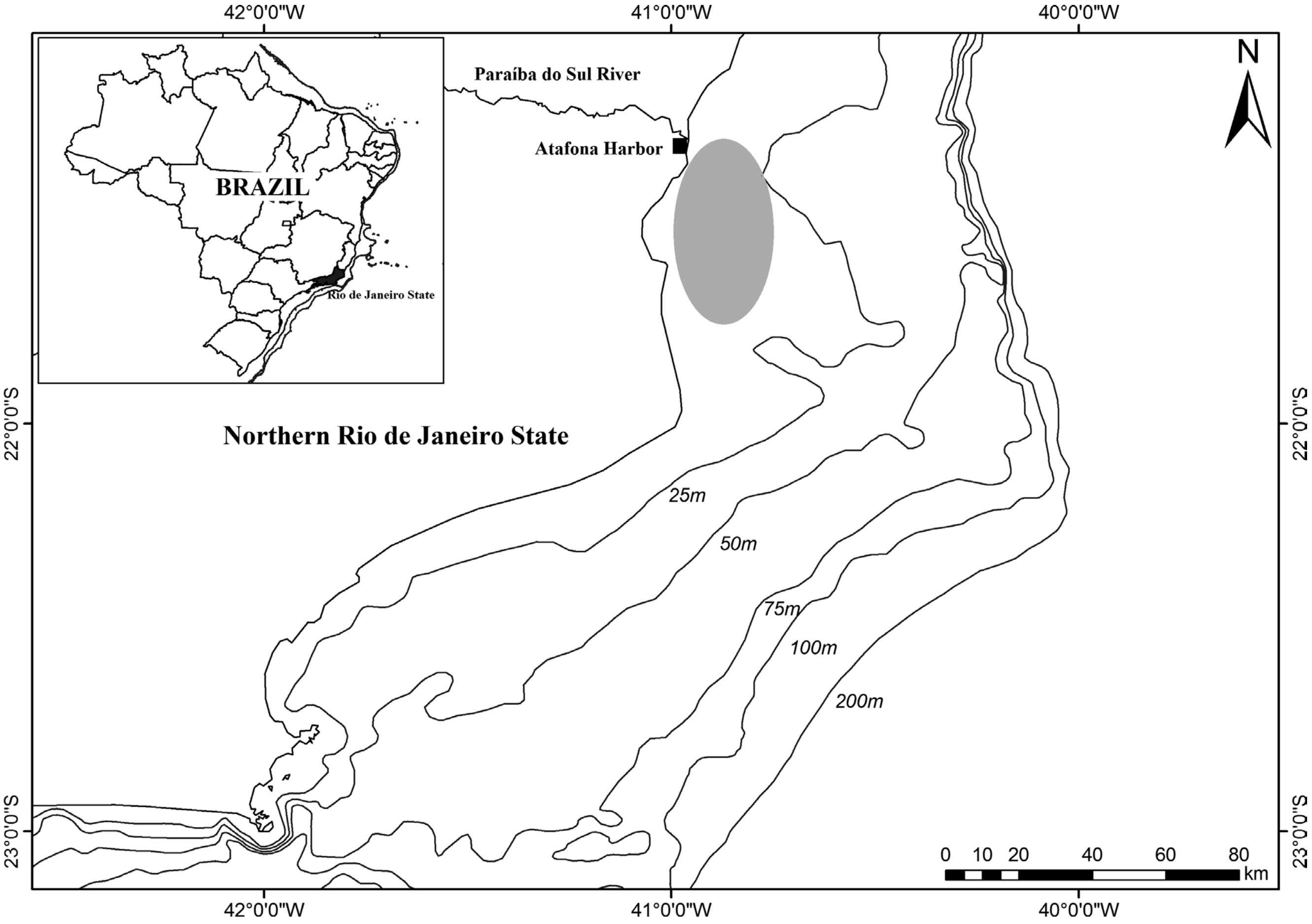

Figure 1. Map of Brazil, indicating the Rio de Janeiro State and its northern coast, where the sampling site of *Bagre bagre*, *Genidens barbus* and their prey species is located (grey ellipse).

(Caut et al. 2009) ($\Delta^{15}N = -0.281\delta^{15}N + 5.879$ and $\Delta^{13}C = -0.248\delta^{13}C - 3.4770$). The calculated values for our data were +2.1 ± 0.2‰ for TEF^{15}N and +0.7 ± 0.1‰ for TEF^{13}C.

3. *Niche width analysis*

The isotopic niche width of *B. bagre* and *G. barbus* was estimated by the quantitative metrics based on the position of individuals in the niche space ($\delta^{13}C$ x $\delta^{15}N$) (Layman et al. 2007, Jackson et al. 2011). These metrics were calculated by the functions for Stable Isotope Bayesian Ellipses in R (SIBER - Jackson et al., 2011). The Bayesian assessment for the comparison of isotopic niche metrics proposed by Jackson et al. (2011) is appropriate for small sample sizes (at least 10 samples), as in the present study (n= 28 for *B. bagre* and n= 16 for *G. barbus*).

Five quantitative metrics derived from stable isotope data compared the trophic structure of the catfishes populations in the study area: a) $\delta^{15}N$ range (NR) that is the distance between the two individuals with the highest and lowest $\delta^{15}N$ value within a population, providing an indication of the total nitrogen range exploited by a population (a large NR might suggest omnivory); b) $\delta^{13}C$ range (CR) that is the distance between the two individuals with the highest and lowest $\delta^{13}C$ value within a population, providing an indication of the total carbon range exploited by a population and representing the variability of food sources consumed (a large CR implies difference in basal resources within food webs); c) Standard ellipse area (SEA) or trophic niche width, where the standard ellipse is centred on the group centroid and scaled to encompass a 40% chance ($P = 0.40$) of including a subsequently sampled datum; d) Mean distance to centroid (CD) that is the average Euclidean distance of each individual of a population to the $\delta^{15}N$-$\delta^{13}C$ centroid for that population, used as a measure of population trophic diversity and e) Standard deviation of nearest neighbour distance (SDNND) that is the standard deviation of Euclidean distances of each individual to its nearest neighbor in the $\delta^{13}C$-$\delta^{15}N$ scatterplot space, used to infer population trophic evenness (a low SDNND indicates a more even distribution of individuals in the trophic niche space).

The differences between the catfishes regarding mean $\delta^{15}N$ and $\delta^{13}C$ values were assessed via t-tests. The SEAs of the catfishes were compared probabilistically with the posterior Bayesian distributions, calculating the proportion of ellipses for group 1 that was larger than ellipses for group 2 in the simulated draws (Jackson et al. 2011). The percent of overlapping SEA between the catfishes was the measure of isotopic niche overlap. Mean differences between species in CD were assessed by t-tests. The statistic SDNND, a standard deviation, was compared between groups by an F-ratio test. Here, the *P* values were interpreted as strengths of evidence toward null hypotheses, rather than on the dichotomic scale of significance testing (Hurlbert & Lombardi 2009).

Results

Considering *B. bagre* and *G. barbus*, the mean values for δ^{15}N and δ^{13}C were +13.6 ± 0.4‰ and +12.7 ± 0.4‰, and -16.8 ± 0.2‰ and -16.9 ± 0.2‰, respectively (Figure 2). The comparison between the mean values indicated that differences between δ^{15}N (P= 6.03^{e-09}) were more significant than δ^{13}C (P= 0.07). The mean values for δ^{15}N in the prey species identified from the stomach contents of *B. bagre* (*T. lepturus*, *P. brasiliensis*, *X. kroyeri*) were lower than those found in consumer, and the same pattern was noted for the prey species of *G. barbus* (*P. porosissimus*, *X. kroyeri*) (Figure 2).

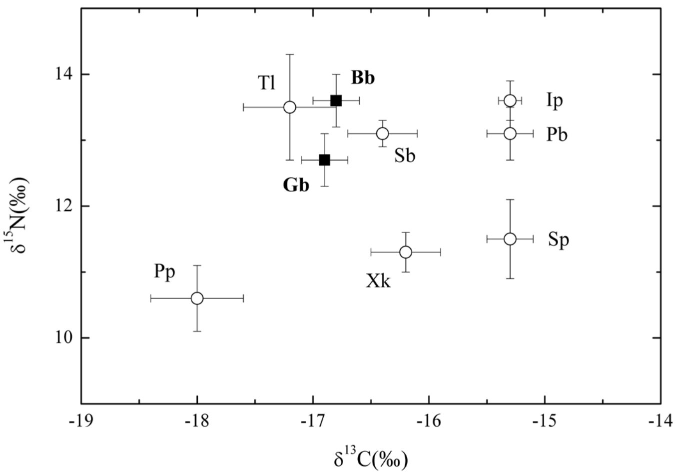

Figure 2. Relationship between δ^{13}C and δ^{15}N of the catfishes *Bagre bagre* and *Genidens barbus* and their prey species. Error bars are standard deviations. Bb: *Bagre bagre*, Gb: *Genidens barbus*, Tl: *Trichiurus lepturus*, Ip: *Isopisthus parvipinnis*, Pb: *Paralonchurus brasiliensis*, Sb: *Stellifer brasiliensis*, Sp: *Symphurus plagusia*, Pp: *Porichthys porosissimus*, Xk: *Xiphopenaeus kroyeri*.

The Bayesian mixing model incorporated isotopic signatures of consumers and prey species, and elemental concentrations and TEFs values of prey species in the feeding assimilation analysis. The relative contributions of the prey species highlighted *X. kroyeri* as the most assimilated item in the diet of *B. bagre*, while for *G. barbus* the model highlighted *P. porosissimus* (Figure 3).

The SEAs of the catfishes were rather similar in position in the δ^{13}C axis, but differences were noted in the δ^{15}N axis (Figure 4). A probabilistic comparison between the ellipse areas based on the posterior distribution of simulated ellipses indicated that 93% of the SEAs of *G. barbus* are larger than *B. bagre*. Moreover, no SEAs overlap was detected between the catfishes (Figure 4).

The quantitative metrics to estimate the isotopic niche width of the catfishes are presented in Table 1. Trophic preference measure (δ^{15}N range) indicated that *B. bagre* and *G. barbus* are comparable as consumers. The species are also comparable about variability of food sources (δ^{13}C range). The metric CD did not present a clear difference between the catfishes (CD t= 0.91, df= 33.06, P= 0.37), indicating similarities in the trophic diversity. Meanwhile, the SDNND value of *G. barbus* was 3.5 times lower than the estimate for *B. bagre* (F= 3.65, df1= 27, df2= 15, P= 0.01), showing a more even distribution of *G. barbus* in the trophic niche space.

Discussion

The most frequent prey species in the stomach contents of *B. bagre* and *G. barbus* were not the most assimilated species by the consumers, contradicting the first assumption of this study. Differences between ingestion and digestibility level (and assimilation) are not uncommon and can vary among different food items and consumers (Degani & Revach 1991, Fry 2008, Pereira et al. 2012), as within individuals of the same species (Di Beneditto et al. 2017). Considering fish species, for instance, the rainbow trout *Oncorhynchus mukiss* (Walbaum 1792) seems to digest better red algae, whereas the Nile tilapia *Oreochromis niloticus* (Linnaeus 1758) does better with green and brown algae (Pereira et al. 2012). Moreover, there are bias in traditional dietary studies performed only by stomach content analysis, such as sub- or superestimation of prey contribution to consumer's diet, due to differences in their digestion rates (Pierce & Boyle 1991). Regardless the relevance of these traditional studies that allow the identification of the taxonomic level of prey species (*e.g.*, Santos et al. 2002; Di Beneditto & Siciliano 2007; Krishnan et al. 2008), the evaluation of food items importance in the consumer's diet should take in account ingestion, digestion and assimilation rates.

The main assimilated prey by *B. bagre* (*X. kroyeri*) is the most capture species by local shrimp fisheries (Fernandes et al. 2011, 2014), while *P. porossissimus*, the main assimilated prey by *G. barbus*, is by-catch in these fisheries and it is also an important prey for a coastal dolphin that inhabits the study area (Di Beneditto & Lima 2003, Di Beneditto & Ramos 2004). All prey species recorded in the stomach contents of these catfishes are bottom-associated resources and common year-round along the coast of northern Rio de Janeiro State (Gomes et al. 2003, Fernandes et al. 2011, Tavares & Di Beneditto 2017). Thus, the local availability of these prey to the consumers is high.

The mean isotopic values of the catfishes and their prey species in the δ^{13}C-δ^{15}N space were coherent with their respective trophic positions; however, it might expect similar δ^{15}N values between the catfishes, instead of higher values for *B. bagre*. The δ^{15}N value has been widely considered a tool in establishing trophic levels and feeding interactions among species. Meanwhile, its efficiency as trophic marker is sensitive to consumers body size, nutritional status and excretion metabolism (Hobson et al. 1996, Jennings et al. 2002), as well as quantitative and qualitative differences in food ingestion and assimilation (Kurle & Worthy 2001, Das et al. 2003).

All catfish specimens analysed in the present study are comparable about size and nutritional status, measured as total body length and weight, respectively. The species *B. bagre* and *G. barbus* are probably similar regarding excretion metabolism because of physiological similarities since both belong to the Ariidae fish family. The biomass ingested of each prey species was not estimated to compare possible quantitative differences between the consumers (Tavares & Di Beneditto 2017), but differences in feeding assimilation were recorded in the present study (*X. kroyeri* was the most important to *B. bagre*, while *P. porossissimus* to *G. barbus*). The mean δ^{15}N value of *X. kroyeri* is higher than *P. porossissimus*, as showed in Figure 2, and it is reflecting in the nitrogen isotope value of the consumers. It is worth to say that the catfishes' δ^{15}N values did not represent differences in their trophic positions, but differences in their prey isotopic values. The interpretation

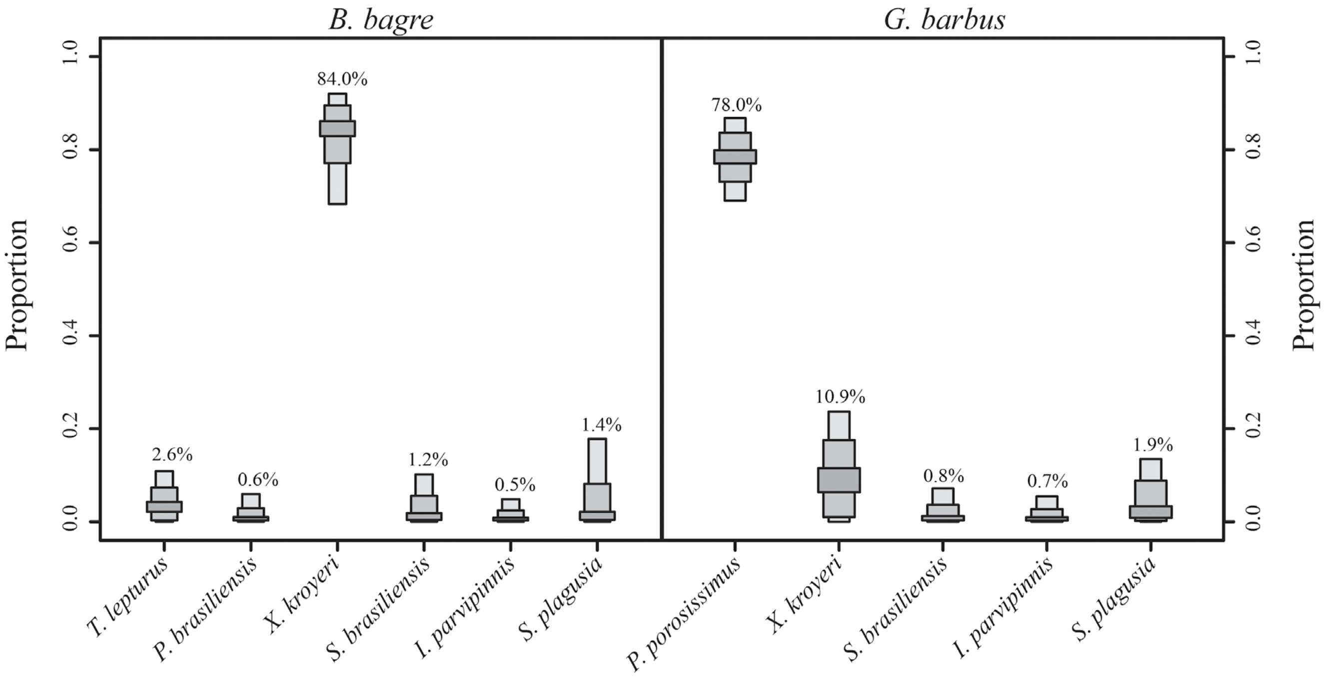

Figure 3. Results of SIAR (Stable Isotope Analysis in R) showing 95, 75 and 25% credibility intervals of prey species contributions to the diet of the catfishes *Bagre bagre* and *Genidens barbus* in northern Rio de Janeiro State, south-eastern Brazil. The mean contribution values (%) for each prey species are showed above the credibility intervals.

Table 1. Quantitative niche metrics of the catfishes *Bagre bagre* and *Genidens barbus* in northern Rio de Janeiro State, south-eastern Brazil. NR= δ^{15}N range, CR= δ^{13}C, CD= distance to centroid, SDNND= standard deviation of nearest neighbour distances, SEA= standard ellipse area, LQ = lower quartile and UQ = upper quartile.

	NR	CR	CD	SDNND	SEA (‰^{2})		
					LQ	Median	UQ
Bagre bagre	1.3	0.6	0.36	0.07	0.15	0.17	0.19
Genidens barbus	1.1	0.7	0.42	0.02	0.22	0.26	0.31

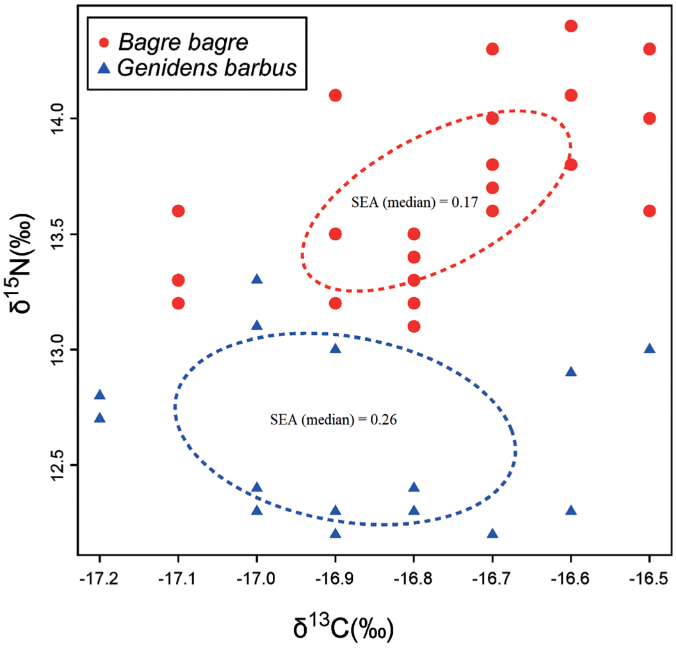

Figure 4. Stable isotope values of the catfishes *Bagre bagre* and *Genidens barbus* in northern Rio de Janeiro State, south-eastern Brazil. Lines depict the standard ellipse (the 40% confidence interval) for the isotopic niches.

of consumers' isotopic values without taking into account other food chain or web components can lead to misunderstandings about the species trophic role and/or their relationships in an ecosystem.

The second assumption that the isotopic niche of the consumers reflects their feeding preferences and assimilation was partially confirmed. The quantitative niche metrics indicated that specific differences are negligible regarding NR, CR and CD; however, SDNND and SEA were different between the species. The NR (δ^{15}N range) pointed to comparable carnivore levels between the two species and the CR (δ^{13}C range) indicated no difference in basal resources, with food web maintained mainly by bottom-associated resources. The metric CD (trophic diversity) revealed that these species are similar in terms of trophic function in the ecosystem (Layman et al. 2007). Although the SDNND values were low for *B. bagre* and *G. barbus*, indicating population trophic evenness for both species, the comparison showed a more even distribution of *G. barbus* in the trophic niche space. The trophic evenness reveals a more homogeneous feeding strategy among individuals from a population, which might minimise the interspecific competition. In turn, the possible increase of intraspecific competition in a homogeneous feeding strategy is compensated by ontogenetic differences in the diet, as widely reported in the literature for marine catfishes (*e.g.*, Yanez-Arancibia & Lara-Dominguez 1988, Mendonza-Carranza 2003, Mendonza-Carranza & Vieira 2009, Denadai et al. 2012). The larger SEA for *G. barbus* indicates a greater niche width in comparison with *B. bagre*, probably reflecting a greater use of the available food resources.

The comparison between the isotopic niche width of the adult specimens of *B. bagre* and *G. barbus* showed to what extent they are sharing or segregating feeding resources in a marine coastal area. The sampling included only adult specimens captured by coastal fisheries practised in the same space-time period, revealing fish specimens in the same ontogenetic phase sharing the habitat locally. The absence of niche overlaps together with the populations trophic evenness point to a reduced feeding overlap between these catfish species. Further analysis that include spatial, seasonal and ontogenetic differences regarding the feeding habits of *B. bagre* and *G. barbus* should be done for a more comprehensive understanding about their trophic relationships in northern Rio de Janeiro State, south-eastern Brazil.

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Author Contributions

Ana Paula Madeira Di Beneditto: contributed in the concept and design of the study, data analysis and interpretation and in the manuscript preparation.

Maria Thereza Manhães Tavares: contributed to data collection and in the early stage of data analysis and interpretation.

Leandro Rabello Monteiro: contributed in data analysis and interpretation.

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

Effects of habitat fragmentation on the persistence of medium and large mammal species in the Brazilian Savanna of Goiás State

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Abstract: Habitat loss and fragmentation are the main threats to the conservation of Cerrado biodiversity. The objective of this study was to evaluate the implications of habitat loss on the persistence of medium and large mammal species, considering the spatial and temporal changes (years 1985, 2000 and 2014) to the evaluated fragments. The study was carried out in 14 fragments (10.5 – 618 ha), located in Southeastern Goiás, Brazil. Records for 24 mammal species were obtained and revealed the two sites with the largest habitat amount in the landscape contained higher species richness than the remaining sites. The three mammal groups based on body mass (weight < 5 kg; weight between 5 and 20 kg; and weight > 20 kg) analyzed in this study showed different responses regarding landscape changes. For larger mammals (between 5 - 20 kg and > 20 kg), there was significant association between current species richness and the amount of habitat in 2014, while the species richness of smaller mammals did not significantly correlate with any of the variables assessed for any of the years. Therefore, the amount of habitat present within the current landscape was the most important variable regarding mammal species richness, especially for the larger species. The time lag was not evident at the time scale evaluated, and this delay in response may have occurred in a relatively short time (< 15 years). For the remaining fragments in the studied landscapes, most are too small to support populations of some larger mammal species and may also leave individuals more vulnerable to anthropogenic actions (e.g. hunting), whose effects may accelerate local extinctions.

Keywords: *Biodiversity conservation, Landscape ecology, Mammal fauna, Species richness.*

Efeitos da fragmentação de habitat na persistência de espécies de mamíferos de médio e grande porte no Cerrado em Goiás

Resumo: A perda e a fragmentação de habitats são as principais ameaças à conservação da biodiversidade no bioma Cerrado. O presente estudo objetivou avaliar as implicações da perda de habitat na persistência de espécies de mamíferos de médio e grande porte, levando em consideração as alterações espaciais e temporais dos fragmentos avaliados. O estudo foi realizado em 14 fragmentos (10,5 – 618 ha), localizados na região sudeste de Goiás, Brasil. Foram obtidos registros de 24 espécies de mamíferos, sendo que os dois locais com as maiores quantidades de habitat na paisagem apresentaram maior riqueza de espécies que as demais áreas. Os três grupos de mamíferos baseados na massa corporal criados neste estudo (peso < 5 kg; peso entre 5 e 20 kg; e peso > 20 kg) apresentaram respostas diferentes em relação às mudanças na paisagem. Para os mamíferos de maior porte, houve significativa associação entre a riqueza atual de espécies e a quantidade de habitat na paisagem de 2014, mas a riqueza de espécies de mamíferos de menor porte não apresentou relação significativa com nenhuma das variáveis das paisagens analisadas. Portanto, a quantidade de habitat presente na paisagem atual foi a variável mais importante para a riqueza de espécies de mamíferos, principalmente para as espécies de maior porte. O tempo de latência não ficou evidente na escala temporal avaliada, sendo que esse atraso na resposta pode ter ocorrido em tempo relativamente curto (< 15 anos), pois os fragmentos remanescentes nas paisagens estudadas em sua maioria são pequenos para suportar populações de mamíferos de maior porte e também podem deixar os indivíduos mais vulneráveis às ações antrópicas (e.g. caça), cujos efeitos podem acelerar as extinções locais.

Palavras-chave: *Conservação da biodiversidade, Ecologia de paisagem, Mastofauna, Riqueza de espécies.*

Introduction

Natural preserved areas have decreased due to human activities and become restricted in tropical regions (Laurance et al. 2014). Expanding agriculture, pasture and increasing human population density are some of the causes responsible for the reduction of natural areas. The region of Central Brazil is subject to intensive agricultural activities which contribute to natural habitat loss. The Brazilian savanna (nationally known as the Cerrado biome) originally occupied approximately 2 million km^2 of Central Brazil (approximately 23% of the country's territory), with vegetation physiognomy that includes forests, savannas and grasslands (Ribeiro & Walter 1998). This biome was included as one of the planet's 34 hotspots due to its highly endemic biodiversity and threatened status (Myers et al. 2000, Mittermeier et al. 2005). Over the past five decades, the Brazilian savanna has experienced a rapid reduction in original vegetation cover due to the expansion of the agricultural frontier in central Brazil.

Estimates indicate that approximately half of the original Brazilian savanna coverage has been transformed into planted pastures, annual crops and other types of land use (Ratter et al. 1997, Klink & Machado 2005). This marked expansion of socio-economic activity has contributed to the large-scale landscape change of the Brazilian savanna, resulting in many highly fragmented areas (Sano et al. 2007, Carvalho et al. 2009). These processes of habitat loss and fragmentation have been identified as the main threats to biodiversity (Ahumada et al. 2011, Gibson et al. 2011).

Habitat fragmentation can be defined as a process through which a continuous habitat is transformed into "small habitat pieces", with the disconnection of a previously continuous area (Pires et al. 2006, Carvalho et al. 2009). Habitat fragmentation processes include landscape changes such as habitat loss, reduction in habitat patch size, connectivity alteration, increased edge effects and increased matrix area (Uezu et al. 2005, Michalski & Peres 2007, Norris & Peres 2008). The quantification of landscape variables has assumed a preeminent role in landscape ecology (MacGarigal & Ene 2013), providing a measure of fragmentation (Carvalho et al. 2009). The alterations in these landscape variables cause changes in local biodiversity at both the population level – such as changes in the number, distribution, reproduction, survival and recruitment of individuals (Wolff et al. 1997, Fahrig 2003), and at the community level, with changes in species composition and richness (Chiarello 1999, Santos-Filho et al. 2012). Fahrig (2003) points out that negative fragmentation effects arise from two main causes: 1) habitat fragmentation products are smaller fragments than the original; these small fragments generally do not possess sufficient habitat area to support most of the species, or even for one individual. In this way, the persistence of most species, especially those sensitive to matrix, are confined to fragments; 2) the level of fragmentation in a landscape is positively related to the amount of edge effects in that landscape. This may increase the probability of the most sensitive and specialized species leaving the habitat fragment in search of more suitable areas, which can lead to increased mortality rates and reduced reproductive rates of the population if environments with favorable conditions are not found (Fahrig 2003). Additionally, the introduction of new forms of land use in place of the original vegetation can cause negative alterations in environmental heterogeneity and consequently, in the resource supply for the species (Lion et al. 2016), because it leads to greater homogeneity.

Several changes in biodiversity can be observed within a short period of time following changes in landscape structure, however some species decline and disappear only after prolonged periods of time (Kuussaari et al. 2009). The number of species expected to eventually become extinct as the community reaches a new equilibrium after environmental disturbance, also called extinction debt, is an important factor to be considered in biodiversity conservation (Kuussaari et al. 2009, Krauss et al. 2010). Extinction debt can be assumed when the past characteristics of a particular landscape explain the current species richness and composition better than current landscape characteristics (Kuussaari et al. 2009, Krauss et al. 2010). The likelihood and magnitude of extinction debt depend on the species' life history (*e.g.* dispersion capacity, reproductive rates, habitat demands), the spatial and temporal configuration of the habitat fragment, the time since the habitat was changed and the nature of the change (Kuussaari et al. 2009). The local group of specialist species finds a new balance point after the disturbance, which may take some time for those with a long life cycle (Krauss et al. 2010). Therefore, many of the management and conservation strategies adopted today may not be effective, as the delay in response to landscape alterations is not taken into consideration.

Different mammal taxa should be affected in different ways as a consequence of environmental change. As a result, the different mammal groups (based on body mass) should exhibit different responses to habitat fragmentation (Keinath et al. 2018). Mammals with large home ranges and longer life cycles are usually more sensitive than those with smaller home ranges and shorter life cycles (Morris et al. 2008). They need more resources and energy than smaller mammals to complete their life cycles and live in low densities and are exploited by humans (Cardilho et al. 2005). Therefore, conservation strategies consider mammals with a larger body mass as umbrella species, as protecting these species provides the indirect conservation of other species within the area.

Delayed response is still largely unexplored in researches investigating the effects of natural habitat change on species, especially in Brazil. Researches on extinction debt carried out so far have primarily evaluated plants and birds, whereas mammals have rarely been the object of study (Semper-Pascual et al. 2018). Thus, the objective of this study was to assess the implications of habitat loss on the persistence of medium and large mammal species, considering the spatial and temporal changes in the evaluated landscapes. We tested the hypothesis that fragmentation processes occurring within landscapes negatively affect mammalian species richness, with those effects felt for long periods of time, leading to the local extinction of some species, particularly larger mammals. Such information may help reduce the knowledge gap on the long-term effects of fragmentation on medium and large mammal species richness.

Materials and Methods

1. *Mammal study areas and data*

The areas studied are located in southeastern Goiás (Brazil), comprised of Brazilian savanna and include some places considered Atlantic forest enclaves (Felfili 2003). The climate is classified as Aw (tropical seasonal) with annual rainfall of approximately 1600 mm and is characterized by two distinct seasons, a dry winter and rainy summer, with temperatures averaging around 23° C (Alvares et al. 2014).

Data collection was conducted in two different methods: 1) secondary data surveys; and 2) primary data surveys in the field. By combining these methods, information was obtained from 14 savanna fragments located in southeastern Goiás (Figure 1). Recent studies carried out in the southeast of Goiás were used as secondary data sources on medium and large mammals, namely the mammal inventory of Parque Estadual Mata Atlântica - PEMA, Água Limpa municipality (Rocha et al. 2015). The secondary data was incorporated as it was derived from one of the largest protected areas in the region (PEMA), and could serve as a control for species richness. In addition, the data was collected (n = 16 days of sampling effort) during 2012 and 2013, the same period in which sampling took place at other locations.Thirteen Savanna fragments of different shapes and sizes were selected for field data collection (Appendix I). These habitat patches were situated in private properties containing native vegetation, with sampling sites located at areas of permanent protection along riverbanks and adjacent legal reserves. Fragments were chosen with the aid of satellite imagery, and areas were selected in the municipalities of Ipameri (n = 10), Catalão (n = 2) and Urutaí (n = 1).

Collection of field data was carried out between May 2011 and October 2014, with the 13 chosen fragments sampled four times each through direct (visual and vocal) and indirect (tracks, burrows and other signs) methods for recording mammal species. This sampling effort totalled 52 days of field data collection. Each fragment was randomly inspected. Roads, previously existing trails and riverbanks were searched for traces of mammal species. In addition, a camera trap was installed to complement the species inventory, with a total sampling effort of 72 traps*night.

Only mammal species that depend on the forest and dense savanna environment as an important habitat resource were included in this study, as alterations in this kind of habitat present in the landscape were evaluated. We used Reis et al. (2006) for this classification. Therefore, although they were recorded during field surveys, the species that prefer open habitats [*e.g.* hoary fox *Lycalopex vetulus* (Lund, 1842) and maned-wolf *Chrysocyon brachyurus* (Illiger, 1815)] and semi-aquatic habitat species [water opossum *Chironectes minimus* (Zimmermann, 1780), otter *Lontra longicaudis* (Olfers, 1818) and *Hydrochoerus hydrochaeris* (Linnaeus, 1766)] were not included. Furthermore, species groups based on body mass were created in order to assess whether effect of fragmentation change between mammals with different sizes classes. Average animal weights from Paglia et al. (2012) were used. Mammals were divided into three groups: 1 – less than 5 kg (n = 9 species); 2 – between 5 and 20 kg (n = 9 species); and 3 – greater than 20 kg (n = 7 species).

2. *Landscape data*

Analysis of the landscape was carried out using land use maps obtained from visual satellite imagery classification. Landsat 5 satellite images from 1985, 2000 and 2014 provided by the National Institute for Space Research (INPE) were used. Two land use classes were visually created in the mapping: 1) habitat – forests and/or non-open savanna areas that were at least two pixels wide (60 m); 2) non-habitat – matrix with altered original vegetation, pastures, lakes, rivers and open savanna areas (open shrubland and grassland).

To analyze the structure of the landscape, buffers were created with a 2-km radius from the center of each sampled area (Krauss et al. 2010). In a study that tested various scales, Lyra-Jorge et al. (2009) found a greater association between landscape and carnivore species at the highest scale assessed (buffer with 2-km radius). Furthermore, the chosen scale did not allow buffer overlapping, which avoided spatial autocorrelation. Based on the classified maps, landscape variables were generated [habitat amount (HA), number of habitat patches (NP), total edge (TE), largest patch index (LPI), landscape shape index (LSI), mean patch area (AREA_MN), contiguity index (CONTIG), total core area (TCA), mean Euclidean distance of nearest neighbors (ENN_MN), clumpiness index (CLUMPY) and splitting index (SPLIT)] for each year evaluated (1985, 2000 and 2014) using Fragstats 4.2 software (MacGarigal & Ene 2013). These variables, which are associated with all patches of habitat present in the landscape and measure the quantity and the spatial configuration of each patch, were used as measures of fragmentation (Carvalho et al. 2009, MacGarigal & Ene 2013).

3. *Data analysis*

Landscape variables generated for the years 1985, 2000 and 2014 were subjected to Principal Component Analysis (PCA) in order to reduce data dimensionality and indicate the selection of those which were less correlated. The data was standardized for analyses, with a covariance matrix then used. Broken Stick criterion was used to select axes. From the PCA results, three variables were selected as the main landscape characteristics: the habitat amount (HA), number of habitat patches within the landscape (NP), and average Euclidean distance of nearest neighbors in the landscape (ENN_MN). A Permutational Multivariate Analysis of Variance – PERMANOVA (Anderson 2001), using Euclidian Distance as a dissimilarity measure, and a *post-hoc* Dunn's test was applied to test if there was a difference in landscape structure between analyzed years. Association between mammal species richness and landscape characteristics, past and present, was tested by way of multiple regression analysis with a selection model (forward stepwise) used to select the best models. Statistical analyses were done in R program (R Development Core Team 2017), using the 'vegan' package (Oksanen et al. 2017).

Results

Landscape variables pointed to a significant change in landscape structure between 1985 and 2014 (Pseudo F = 2.70; p = 0.03). The average size of habitat patch (HA) within buffers was gradually reduced [year 1985 (mean = 477 ha, range = 144 – 943); year 2000 (mean = 397 ha, range = 165 – 741); year 2014 (mean = 318 ha, range = 97 – 815)], while the average number of habitat patches (NP) increased from 2000 to 2014 [year 1985 (mean = 11.9, range = 3 – 23); year 2000 (mean = 10.4, range = 4 – 18); year 2014 (mean = 18.8, range = 5 – 30)], and average Euclidean Distance of the nearest neighbors within the landscape (ENN_MN) increased from 1985 to 2000 and then has small reduction [year 1985 (mean = 156 m, range = 75 – 275); year 2000 (mean = 192 m, range = 114 – 293); year 2014 (mean = 173 m, range = 78 – 368)] (Figures 2 and 3). The changes in these variables reveal the progress of fragmentation during this period, with the average percentage of habitat area within buffers reduced from approximately 38% in 1985 to 32% in 2000 and 25% in 2014 (Figure 3).

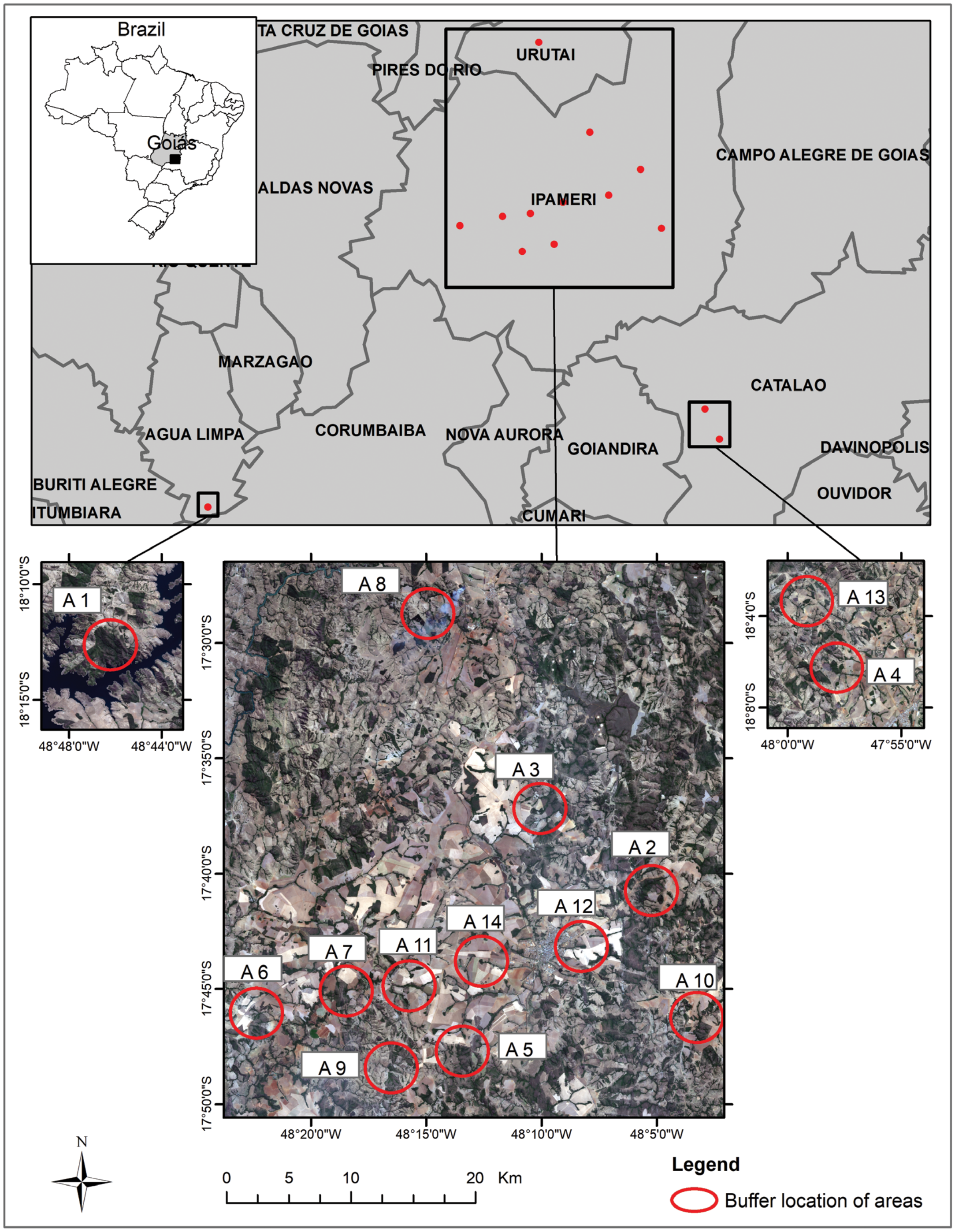

Figure 1. Location of the 14 fragments sampled in southeastern Goiás. Red circles represent the landscapes delimited with a radius of 2 km from the center of each fragment studied.

Records were obtained for 24 mammal species from eight orders that use the forest environment: Didelphimorphia (one species), Pilosa (two species), Cingulata (four species), Perissodactyla (one species) Artiodactyla (three species), Primates (three species), Carnivora (eight species) and Rodentia (two species) (Appendix II). Species were identified primarily by their tracks, but also by sightings, burrows, feces and camera trap. Among the recorded species, five are classified as endangered in Brazil (MMA 2014): Giant Anteater *Myrmecophaga tridactyla* (Linnaeus, 1758), Giant Armadillo *Priodontes maximus* (Kerr, 1792), Tapir *Tapirus terrestris* (Linnaeus, 1758), Puma *Puma concolor* (Linnaeus, 1771), and Jaguarundi *Puma yagouaroundi* (É. Geoffroy, 1803). Under IUCN criteria (IUCN 2017), the Giant Anteater, Giant Armadillo and Tapir are classified as vulnerable; the South American Red Brocket *Mazama Americana* (Erxleben, 1977) and Azara's Agouti *Dasyprocta azarae* Lichtenstein, 1823 are classified as data deficient; and the remaining species are classified as least concern.

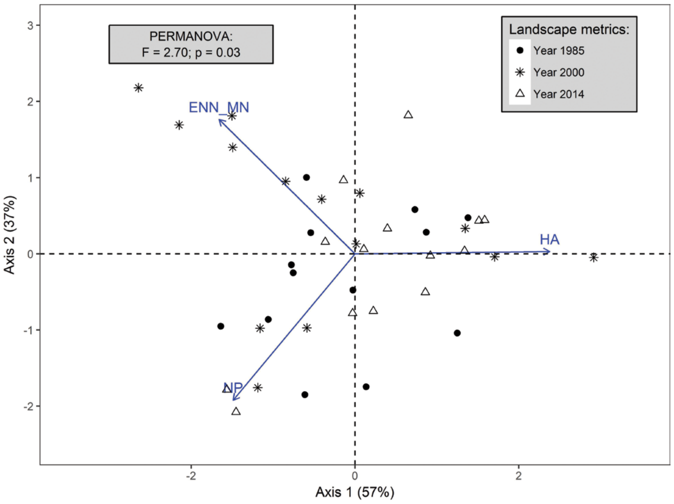

Figure 2. Principal Component Analysis (PCA) and Permutational Multivariate Analysis of Variance (PERMANOVA) of landscape variables (habitat amount (HA, in hectares); number of habitat patch (NP, without unity); and average Euclidean distance of nearest neighbors (ENN_MN, in meters), with data from the 14 fragments in southeastern Goiás.

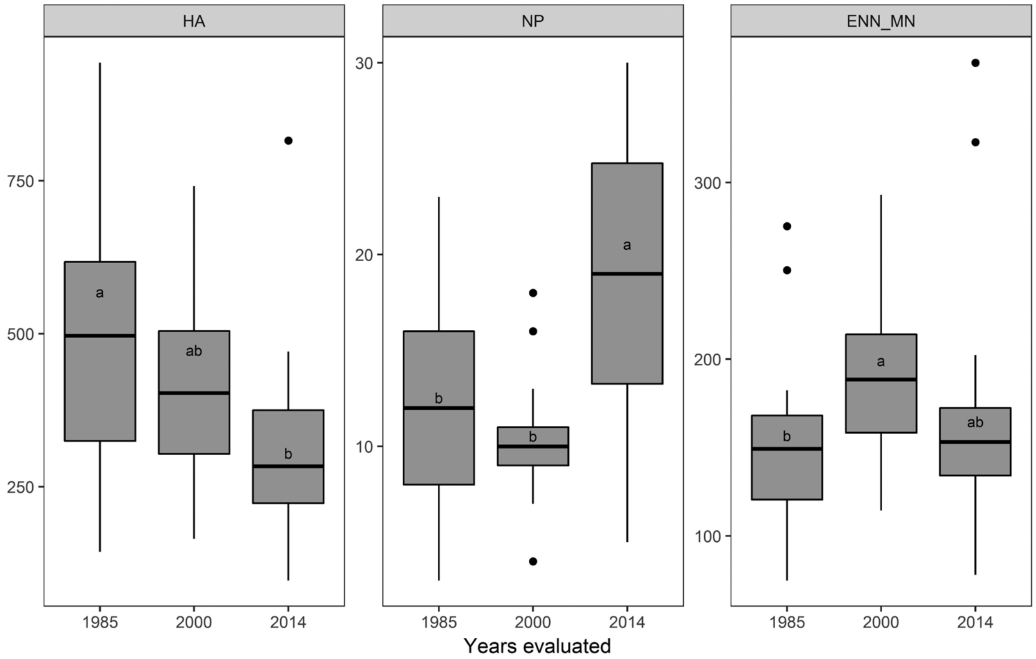

Figure 3. Box plot for the landscape variables analyzed [habitat amount (HA, in hectares); number of habitat patch (NP, without unity); and average Euclidean distance of nearest neighbors (ENN_MN, in meters)], with data from the 14 fragments in southeastern Goiás. For each variable, annual values accompanied by distinct letters differ by the Dunn's test ($p < 0.05$).

The multiple regression model adjusted for all recorded mammal species indicated that landscape characteristics affected the actual species richness ($F_{(3;\ 10)} = 11.62$; $p = 0.001$; adjusted $R^2 = 0.71$). The partial coefficients indicated that only total habitat area within the landscape (HA) for 2014 presented a significant effect ($p = 0.001$) on total species richness (Table 1), showing a positive relationship with the current mammal species richness.

The mammal groups created in this study based on body mass presented different responses to landscape change. Multiple regression models pointed to a significant effect of landscape characteristics on the species richness of mammals with body mass between 5 to 20 kg ($F_{(3;\ 10)} = 5.402$; $p = 0.018$; adjusted $R^2 = 0.504$) and species weighing over 20 kg ($F_{(3;\ 10)} = 5.400$; $p = 0.018$; adjusted $R^2 = 0.504$). For the species group with an intermediary body mass (5 to 20 kg), the variable HA for the year 2014 (positive effect) and the number of habitat patches within the landscape (NP) for the year 2000 (negative effect) exhibited a significant effect for partial coefficients ($p = 0.026$ and 0.047, respectively). For the species group with a larger body mass

Table 1. Coefficients of multiple regression evaluating the relationship between mammal species richness and landscape variables in 14 fragments in southeastern Goiás.

Variables	Beta	Std. Error of Beta	t value	p level
Total species richness ($F_{(5; 8)}$ = 6.921; p = 0.009; adjusted R^2 = 0.695)				
Intercept	5.558	5.448	1.020	0.337
HA year 2014	**0.017**	**0.005**	**3.510**	**0.008**
NP year 2000	- 0.385	0.188	- 2.049	0.075
NP year 1985	0.304	0.139	2.186	0.060
ENN_MN year 2014	- 0.019	0.015	- 1.219	0.258
ENN_MN year 2000	0.031	0.028	1.110	0.299
Species richness < 5 kg ($F_{(1; 12)}$ = 4.255; p = 0.061; adjusted R^2 = 0.200)				
Intercept	**2.158**	**0.738**	**2.926**	**0.012**
HA year 2014	0.004	0.002	2.063	0.062
Species richness 5 - 20 kg ($F_{(3; 10)}$ = 5.402; p = 0.018; adjusted R^2 = 0.504)				
Intercept	**5.025**	**1.621**	**3.101**	**0.011**
HA year 2014	**0.005**	**0.002**	**2.622**	**0.026**
NP year 2000	**- 0.213**	**0.094**	**- 2.262**	**0.047**
NP year 1985	0.102	0.063	1.614	0.138
Species richness > 20 kg ($F_{(3; 10)}$ = 5.400; p = 0.018; adjusted R^2 = 0.504)				
Intercept	- 0.240	1.755	- 0.137	0.894
HA year 2014	**0.006**	**0.002**	**3.303**	**0.008**
HA year 1985	0.002	0.002	1.296	0.224
NP year 1985	0.102	0.077	1.316	0.217

(> 20 kg), only the variable HA for the year 2014 indicated a positive and significant effect for partial coefficients (p = 0.008) and correlation analysis (r = 0.56; p = 0.037) (Table 1).

Discussion

The landscape alterations analyzed in this study highlighted an increase in fragmentation and a reduction of native vegetation in this region of the Brazilian savanna during the period 1985 to 2014 (Ratter et al. 1997, Carvalho et al. 2009). The species richness of medium and large mammals recorded in the studied fragments shows the importance of Parque Estadual Mata Atlântica and the fragments on private property in Southeast Goiás for the region's *in situ* conservation of mammals. These areas are situated within a highly fragmented landscape where there are very few large areas of native vegetation that can serve as a safeguard for wildlife. By comparison with nearby areas: Alves et al. (2014) recorded 18 species of mammals in three fragments in Uberlândia, state of Minas Gerais; Estrela et al. (2015) recorded 25 species of mammals in a fragment in the municipality of Urutaí, state of Goiás.

Overall, the areas with the largest habitat amount (HA) within the landscape showed greater mammal species richness than areas with less habitat amount, which shows the importance of areas with greater habitat extent in the conservation of this fauna group. This result supports the habitat amount hypothesis presented by Fahrig (2013), who argues that total habitat in the landscape (HA) is the variable with the greatest influence on species richness. The trend of increasing species richness in parallel with increasing forest fragment size was found in studies with medium and large-sized mammals in Atlantic Forest areas in Espírito Santo State (Chiarello 1999) and Southern Brazilian Amazonia (Michalski & Peres 2007). Furthermore, while studying small non-flying mammals in 23 fragments of the Southern Amazon, Santos-Filho et al. (2012) also observed a positive correlation between fragment size and species richness. Among the measurable landscape variables in habitat fragmentation, habitat reduction has the greatest effect on biodiversity (Fahrig 2003, 2013), with this effect mostly in a negative form.

The importance of landscape variables on mammal species richness varied according to the size class considered. In our study it was important only for classes above five kilograms, particularly HA for the year 2014, which stood out above the other variables. To understand the reasons behind different responses between mammal groups, it is necessary to take into account the landscape variables as well as the life history of the animals that make up each group (Kuussaari et al. 2009). In general, smaller mammals have less mobility and need less habitat when compared to larger mammals. Furthermore, they tend to have shorter life cycles and possibly a more rapid response to environmental changes (Morris et al. 2008). A response delay was not detected in the evaluated time scale. Similarly, Metzger et al. (2009) did not found extinction debt for small mammals in the Atlântic Forest. In this sense, studies with short-life-cycle animals (such as butterflies) have shown that the current landscape explains species richness better than the former landscape, indicating short time response. Lindborg (2007) observed that short-life-cycle plants were positively correlated with the current characteristics of the landscape, while long-life-cycle plants were more associated with historical landscape characteristics. Some studies have identified extinction debt in plants occurring between 40 and 160 years after environmental perturbation, depending on the degree of fragmentation and connectivity (Cousins & Vanhoenacker 2011).

For larger mammals in this study (> 5 kg), there are indications that extinction debt is occurring in short period (< 15 years after alterations to the landscape), given the association between recent landscape characteristics and current species richness, especially regarding the habitat amount within the landscape (HA) in 2014. In a study carried out in Argentine Dry Chaco, Semper-Pacual et al. (2018) found relaxation time (the time needed to reach a new equilibrium) ranging from 10 to 25 years for medium and large-bodied mammals. Detecting extinction debt may not be easy, as the length of time for extinction occurrence depends on the local ecosystem, the species group studied – due to species-specific time-lags that are affected by generation time and reproductive rate, the extent and pattern of fragmentation (Claudino et al. 2015, Kolk & Naaf 2015) and on the evaluated spatial scale (Cousins & Vanhoenacker 2011). Furthermore, the effects of hunting on mammals can influence local species extinction, particularly larger animals and those in smaller fragments (Chiarello 1999, Peres 2000). This effect is detected and shown to be important in other studies (Cullen Jr. et al. 2000, Peres 2000). Isolated populations are often more accessible to both natural and human predators and these factors tend to alter the resilience of species to hunting and amplify the impact of hunting in small and isolated fragments (Cullen Jr. et al. 2000).

Local mammal extinctions can be seen in this study when noticing that in any of the sampled areas records were obtained of some species that are present in large areas of Brazilian savanna in Goiás state, such as the Jaguar *Panthera onca* (Linnaeus, 1758) and the Peccary *Tayassu peccary* (Link, 1795) recorded in Emas National Park (Rodrigues et al. 2002). In this sense, landscapes with less habitat (HA) are not sufficient to support populations of large predators (e.g. big cats) and, in the absence of these apex predators, species richness may also decrease due to the strong competition between their prey and the increase in mesopredator abundance (Crooks & Soulé 1999, Prugh et al. 2009). The presence of Tapir *Tapirus terrestris* (Linnaeus, 1758) in only one area (A1) and South American Red Brocket *Mazama americana* (Erxleben, 1777) in another (A2), which are currently the two sites with the largest habitat amount (HA) within buffers, indicates that these species are already extinct in places with smaller areas of forest habitat. In one of the studied fragments (A14), old Giant Armadillo *Priodontes maximus* (Kerr, 1792) burrows were found, however no recent records of this species have been obtained, suggesting the local and relatively recent extinction in this area. By comparison, in a study carried out in Atlantic forest fragments in the state of Espírito Santo, Brazil, Chiarello (1999) did not obtain any records of big cats, peccaries, Giant Armadillos or Anteaters *Myrmecophaga tridactyla* Linnaeus, 1758 in small fragments (*e.g.* less than 200 ha).

Our results allow us to substantiate the hypothesis that fragmentation processes in Southeast Goiás negatively affect mammal species richness, mainly for those with a larger body mass. Habitat amount (HA) of the current landscape affecting the richness of mammalian species more strongly than the other variables tested (Table 1). Although these results have not shown the exact amount of time to local disappearance of medium and large mammals due to habitat loss, they confirm that the amount of habitat is crucial for the persistence of mammals, especially for larger species.

It should be noted that the strategies for management and conservation of mammal species in fragmented locations based solely on the current habitat situation may not be effective for all mammal species. In our study, the time lag was not evident at the time scale evaluated, and this delay in response may have occurred in a relatively short time (< 15 years). For the remaining habitat patches in the studied landscapes, most are too small to support populations of some larger mammal species and may also leave individuals more vulnerable to anthropogenic actions (e.g. hunting), whose effects may accelerate local extinctions.

Supplementary material

Appendix I - Information on the 14 sampled fragments in the state of Goiás, Brazil.

Appendix II - Mammal species recorded in 14 fragments in southeastern Goiás. See more details on sampled areas in Appendix I.

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Author Contributions

Ednaldo Cândido Rocha: concept and design of the study, performed collection of field data, and carried out the data analysis and wrote the paper.

Daniel Brito: contribution in the concept and design of the study, critical revision, and adding intellectual content.

Paulo Machado e Silva and Jhefferson Silva: performed collection of field data, and the data analysis.

Paulo Vitor dos Santos Bernardo and Leandro Juen: contribution in the data analysis, critical revision, and 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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