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Article

Mammals of Cajuru State Forest and surroundings: a neglected but important Protected Area for the Cerrado conservation in the São Paulo state, Brazil

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Abstract: The Cerrado has been severely impacted by anthropogenic disturbances, with a tiny proportion of its original extent remaining in its southern portions. In the state of São Paulo, only 7% of this vegetation remains and relatively little is known about the biodiversity of these fragments. To fill this knowledge gap, we inventoried medium and large-sized terrestrial mammals of a neglected region, including a sustainable use protected area, Cajuru State Forest (CSF), adjacent native vegetation remnants protected by the Native Vegetation Protection Law and owned by a paper and cellulose company (Dois Córregos Farm; DCF), and their surroundings. We recorded 20 native mammal species, five of which are endangered with extinction, including the giant anteater, puma, and maned wolf. We found no significant differences in species richness between CSF and DCF but we found higher estimated species richness for the surrounding areas. Besides encompassing a larger and more heterogeneous area, the surrounding area still has a relatively high proportion (>30%) of native vegetation, providing habitat and resources for many species. The estimated mammal species richness for the entire study area was similar to that found in the largest protected area of the Cerrado in São Paulo State, the Jataí Ecological Station and its surroundings. We conclude that our study area still harbors a relatively rich community of large mammals and is important for the conservation of endangered species. This finding is particularly timing since the current State administration is considering to transfer the economic exploitation of CSF to the private sector. We therefore endorse an existing proposal to uplist this protected area, which still lacks a management plan, to a more restricted management category. Besides actions regarding this governmental PA, we argue that it is also important to involve the private sector in a conservation plan for the region.

Keywords: *Brazilian Native Vegetation Protection Law; buffer zone; Cajuru State Forest, camera trap; species richness.*

Mamíferos da Floresta Estadual de Cajuru e arredores: uma Área Protegida negligenciada mas importante para a conservação do Cerrado no estado de São Paulo, Brasil

Resumo: O Cerrado tem sido severamente impactado por distúrbios antrópicos e, especialmente na sua porção sul, poucos são os fragmentos remanescentes desse bioma. No estado de São Paulo, resta aproximadamente 7% da cobertura original de Cerrado e relativamente pouco se sabe sobre a biodiversidade desses remanescentes. Para preencher essa lacuna, inventariamos mamíferos terrestres de médio e grande porte de uma região pouco estudada que inclui uma Unidade de Conservação (UC) de uso sustentável (Floresta Estadual de Cajuru; CSF), áreas de vegetação nativa protegidos pela Lei de Proteção da Vegetação Nativa e pertencentes a uma empresa de papel e celulose (Fazenda Dois Córregos; DCF), e seus entornos. Registramos 20 espécies de mamíferos nativos, cinco dos quais ameaçados de extinção, incluindo o tamanduá-bandeira, a onça parda e o lobo-guará. Não encontramos diferenças significativas na riqueza de espécies entre CSF e DCF, mas encontramos maior riqueza estimada de espécies para o entorno. Além de abranger uma área maior e mais heterogênea, essa área ainda apresenta uma proporção relativamente grande (> 30%) de vegetação nativa, fornecendo habitat e recursos para muitas espécies. A estimativa da riqueza de espécies para toda a nossa área de estudo foi semelhante à encontrada na maior UC de Cerrado no Estado de São Paulo, a Estação Ecológica de Jataí e entorno. Concluímos que a área de estudo abriga uma comunidade rica de grandes mamíferos, sendo importante para a conservação de várias espécies ameaçadas de extinção. Essa descoberta é particularmente oportuna, uma vez que o governo estadual está considerando transferir a exploração econômica da CSF para o setor privado. Endossamos, desta forma, uma proposta existente para elevar esta UC, que ainda carece de um plano de manejo, para uma categoria de manejo mais restritiva. Além disso, argumentamos que também é importante envolver a iniciativa privada em um plano de conservação para a região.

Palavras-chave: *Armadilha fotográfica; Código Florestal; Floresta Estadual de Cajuru; Lei de Proteção a Vegetação Nativa; riqueza de espécies; zona de amortecimento.*

Introduction

In a world increasingly modified by human action, the establishment of governmental protected areas (PAs) is one of the most efficient strategies for biodiversity conservation (Bruner et al. 2001). PAs are part of the territory where land use is restricted, aiming to conserve species, populations, ecosystems, and natural processes. The main function of these areas is to protect threatened elements of biodiversity from processes that compromise their long-term conservation (Margules & Pressey 2000). However, only 14.8% of the Earth's surface is covered by PAs (UNEP-WCMC & IUCN 2016), a value below the goal of 17% proposed by the Aichi Biodiversity Targets for 2020 (CBO 2013). This level of protection is often lower in areas considered hotspots for biodiversity conservation (i.e., with high endemism and high number of threatened species), such as the Atlantic Forest and the Cerrado biomes (Myers et al. 2000, Strassburg et al. 2017, Rezende et al. 2018). Therefore, the conservation afforded by the current network of protected areas has proven to be not sufficient to stop the global biodiversity decline, especially in the highly biodiverse tropical areas (Laurance et al. 2012, Geldmann et al. 2013).

In Brazil, another instrument for the conservation of biodiversity is the Native Vegetation Protection Law (NVPL) (Brancalion et al. 2016), which determines what features and how much of the private lands needs to be set aside for conservation purposes, either as Legal Reserves (LRs) or Areas of Permanent Protection (APPs) (Soares-Filho et al. 2014). The NVPL is an important conservation tool since 53% of the country's remaining native vegetation is inside private properties. On the other hand, there is still a deficit of more than 11 million hectares that need to be implemented in the form of LRs and many rural owners do not comply with this legislation (Freitas et al. 2017). Nevertheless, the establishment of private protected areas as required by law is fundamental for Brazilian biodiversity conservation and can complement or enhance the benefits promoted by the PAs (Brancalion et al. 2016).

However, both strategies – the creation and management of PAs and the preservation of native vegetation inside private properties – have been insufficient to guarantee the conservation of the second-largest Brazilian domain, the Cerrado (Françoso et al. 2015, Strassburg et al. 2017). This biome contains 4,800 species of endemic plants and vertebrates, supplies three of the largest hydrographic basins in South America, and contributes to 43% of Brazil's surface waters outside the Amazon (Strassburg et al. 2017). However, only 8% of the Cerrado is under public governmental protection (Françoso et al. 2015, Strassburg et al. 2017) and just 20% of any rural property within the Cerrado (outside the Legal Amazon) is required to be protected under LRs - whereas 80% is required in forested areas in the Amazon (Brancalion et al. 2016). The Cerrado has already lost about half of its original natural cover and it could lose up to 31-34% of its remaining vegetation by 2050, leading to the extinction of many endemic species and compromising the functionality of this ecosystem (Strassburg et al. 2017). This scenario is particularly worrying in the São Paulo state, where the Cerrado is reduced to only 7% of its original coverage (Kronka et al. 2005). Cerrado's remnants in São Paulo state are surrounded by sugar cane, pastures, perennial crops, and urban areas (Durigan et al. 2007), only 6.5% is protected as PAs and non-compliance with environmental legislation is frequent among local rural owners (Metzger & Rodrigues 2008, Soares-Filho et al. 2014).

The Cerrado remnants formed by the Cajuru State Forest (CSF), a public governmental PA, and the Dois Córregos Farm (DCF), a private rural property containing APPs and an LR adjacent to CSF, are exceptions to this reality. These areas are located in the northeast region of the São Paulo state and together preserve almost 3,500 ha of native vegetation, mainly composed by open Cerrado formations (savanna and grasslands). The presence of these open formations makes those areas even more relevant for the Cerrado conservation since the closed-canopy vegetation (i.e., "cerradão") has become the predominant native cover of Cerrado remnants in São Paulo state (Durigan & Ratter 2006). Nevertheless, there is not much information available on these areas, the CSF still lacks a management plan, and inventories assessing the diversity of important taxonomic groups, such as medium and large-sized mammals, have never been carried out there.

Mammals includes species with different sizes, habitat requirements, and occupied niches, playing several ecological roles and providing relevant ecosystem services (Lacher et al. 2019). Many ecological processes mediated by medium and large-sized mammals have significant effects on the community structure of plants and can alter communities of different trophic levels (Estes et al. 2011, Pérez-Méndez et al. 2016, Lacher et al. 2019). Despite their importance, medium and large-sized mammals are especially affected by habitat loss and fragmentation (Ceballos & Ehrlich 2012, Magioli et al. 2021), hunting (Cardillo et al. 2005), and illegal trade (Rosen & Smith 2010), being among the most vulnerable animal groups to the impacts of the Anthropocene (Dirzo et al. 2014, Tucker et al. 2018).

Hence, we inventoried medium and large-sized terrestrial mammals of the Cajuru State Forest, the Dois Córregos Farm and their surroundings. To better frame the conservation value of these areas, we compared our inventory with a study that used the same sampling approach but was conducted in the largest protected area of the Cerrado in the São Paulo State (the Jataí Ecological Station). Our data is useful for the assessment of the biodiversity of this region and for the development of management strategies for the Cerrado conservation.

Material and Methods

1. Study area

This study was carried out in the Cajuru State Forest (CSF; 2081.4 ha) and in the Dois Córregos Farm (DCF; 2017.2 ha), located in the northeast region of São Paulo state (Figure 1). The predominant climate of the region is defined as equatorial savanna with dry winter (Aw), with precipitation of the driest month below 60 mm and a monthly mean temperature of the coldest months higher than 18°C (Kottek et al. 2006, Beck et al. 2018). The relief of the region is defined as 'Cuestas Balasticas', with low hills (Martinelli 2009, Instituto Florestal 2021).

CSF is a public state-owned protected area of sustainable use, located between the municipalities of Altinópolis and Cajuru (21°06'00" to 21°12'00" S and 47°26'00" to 47°22'00" W, datum WGS84). This area is composed mainly of native vegetation (68,7%), with the predominance of open Cerrado formations, mostly grasslands (i.e., patches of "campo limpo", "campo sujo" and "campo cerrado", often surrounded by non-native invasive grasses) but also savannas (including flooded wetlands and "cerrado sensu stricto"; Figure 1, Figure 2, Table 1). CSF is also considerably covered by managed forests (31%), mostly of *Pinus* spp. plantations (Figure 1, Figure 2, Table 1). This PA does not have a management plan (Durigan et al. 2014, Secretaria de Meio Ambiente, 2020), despite its importance as a groundwater recharge area for the Guarani Aquifer System and one of the last remnants of open Cerrado vegetation of the São Paulo state (Secretaria de Meio Ambiente, 2020). The DCF (Floresta de Alto Valor de Conservação Dois Córregos) is a private rural property owned by Sylvamo do Brasil LTDA. company, encompassing important remnants of native vegetation, protected either as LRs or APPs. DCF is mainly composed of native vegetation (99.3%), with the predominance of open and closed Cerrado formations (Figure 1, Figure 2, Table 1). Both CSF and DCF are surrounded (5 km buffer) by an agro-silvicultural matrix composed of managed forests (27.3%), which are mainly *Eucalyptus* spp. plantations, sugar cane (23.3%), pastures (6.7%) and other less abundant crops, such as orange plantations (Figure 1, Table 1). Summarizing, sampled landscape is composed of a region formed by CSF and DCF where native vegetation predominates (hereafter "interior") and a 5 km buffer with a higher predominance of anthropogenic land cover types (hereafter "buffer") (Figure 1, Figure 2 and Table 1).

2. Sampling design and data collection

We sampled medium and large-sized terrestrial mammals with passive infrared cameras trap (Reconyx, model HC 500), during the dry season (April to September) of 2014. We restricted the sampling to the dry season to have better field conditions and easier access to all sampling sites. This restriction also reduces the interference of different weather conditions (e.g., heat and humidity) on cameras trap performance. We sampled 50 points in total, 24 within CSF (n=12) and DCF (n=12) and 26 within the 5 km buffer (Figure 1). Each sampling point is defined as one camera trap kept in operation 24h/day for approximately 30 days. Cameras trap were fixed to tree trunks at 40-50 cm above the ground. To distribute our sampling points, we overlaid a regular grid of square cells (1.4 km x 1.4 km, 200 ha each) over our study landscape. The center of each square cell was defined as a potential sampling point. The 200 ha size of the grid was chosen to ensure statistical independence between points, following the mammals' inventory protocol recommended by the Tropical Ecology Assessment and Monitoring Network (TEAM Network, 2011). Then, a similar number of sampling points were randomly allocated within the interior (CSF + DCF) and the 5 km buffer of our study areas, to equally distribute our sampling effort. In this way, our design enabled a thorough sampling of the study area, maintaining spatial independence among sampling points. Whenever a sampling point fell into sugar cane plantations or pastures, we relocated it to the nearest point with native vegetation, orienting the cameras towards the originally chosen cover class (either sugar cane or pasture). In 24 sampling points, cameras trap were installed in trails or dirt roads. Our total sampling effort was 1593.59 camera.days, with a mean of 31.87 ± 2.95 days sampled at each sampling point. To complement camera trapping, we searched for tracks, footprints, and other vestiges (e.g., feces, burrows, and carcasses) of mammals in 200 m-long "transects", i.e., the nearest dirt road/farm track from each camera trap, during the set-up and removal of cameras trap. In total, we walked 20 km throughout all transects. All vestiges were photographed for further identification with the aid of specific guides (Becker & Dalponte 1999, Borges & Tomás 2004, Carvalho Jr. & Luz 2008, Mamede & Alho 2008, Moro-Rios et al. 2008, Miranda et al. 2009).

3. Data analysis and species information

We analyzed randomized curves of observed species richness (Sest), estimated asymptotic species richness (Chao2) and uniques (i.e., species recorded in only one sampling point) to evaluate sampling sufficiency and compare species richness between: a) the interior (CSF + DCF), the 5 km buffer and the total area (CSF + DCF + 5 km buffer), and b) CSF and DCF separately. We used incidence data (presence/absence) of native species recorded by cameras trap and performed this analysis in the EstimateS software, version 9.1.0 (Colwell 2019). We did not use abundance data because photographic evidence that allow reliably individual distinctions between animals are rare (Choo et al., 2020).

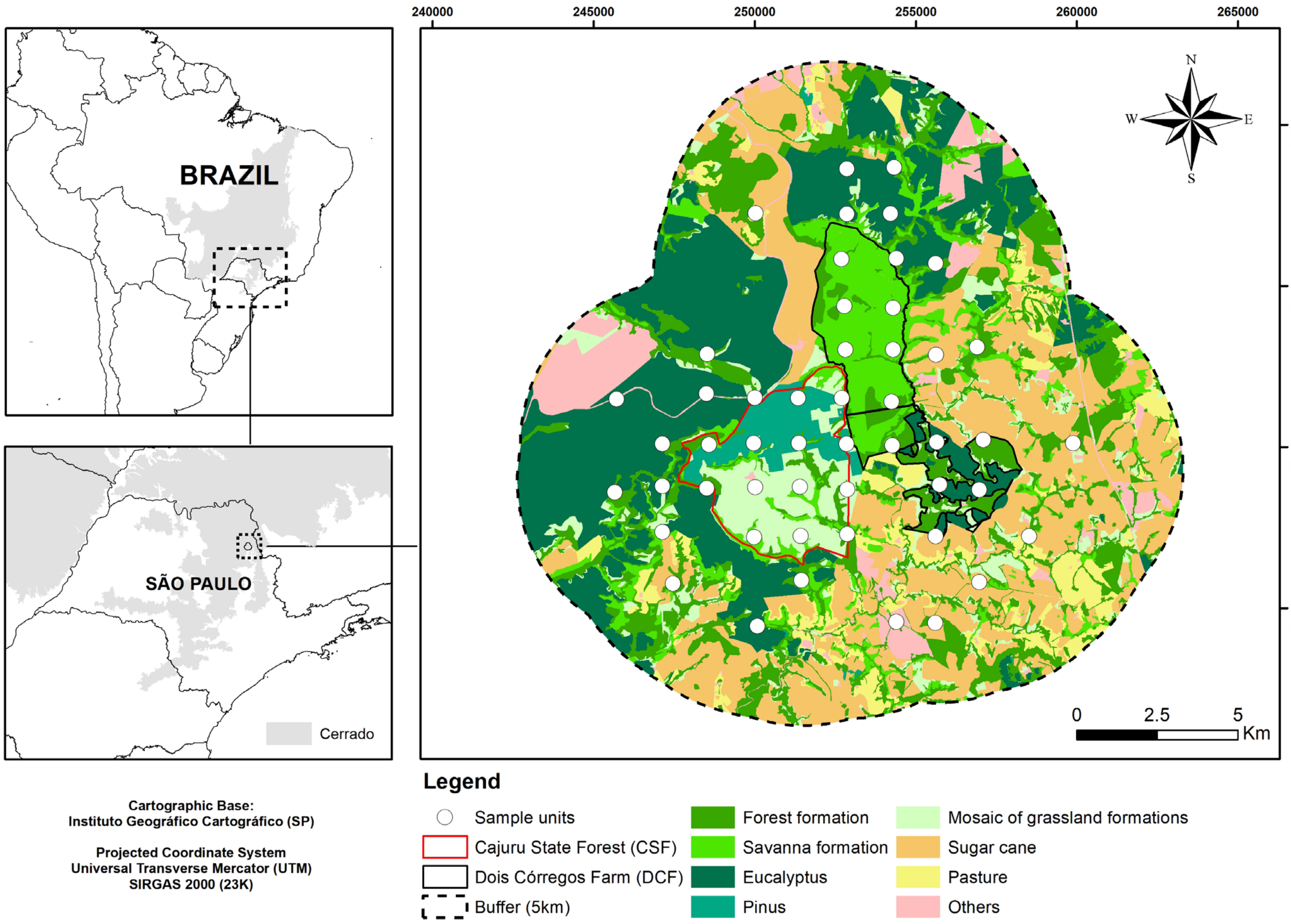

Figure 1. Land use and cover map of Cajuru State Forest (CSF), Dois Córregos Farm (DCF) and a 5 km buffer around these two areas, in the state of São Paulo, Brazil.

Table 1. Land use and cover of Cajuru State Forest (CSF), Dois Córregos Farm (DCF) and its surrounding (5 km buffer), in the state of São Paulo, Brazil.

Land use and cover	**CSF**		**DCF**		**Buffer**		**Total**	
	ha	**%**	**ha**	**%**	**ha**	**%**	**ha**	**%**
Forest formation	226.5	10.9	711.0	35.2	4949.9	18.9	5887.4	19.5
Savanna formation	216.0	10.4	1202.1	59.6	1611.4	6.2	3029.5	10.0
Mosaic of grassland formations	985.7	47.4	90.6	4.5	1405.4	5.4	2481.7	8.2
Managed forest (*Eucalyptus*)	4.9	0.2	9.4	0.5	7503.6	28.7	7517.9	24.9
Managed forest (*Pinus*)	642.1	30.8	0.0	0.0	76.5	0.3	718.6	2.4
Sugar cane	0.0	0.0	0.0	0.0	7054.1	27.0	7054.1	23.3
Pasture	0.1	0.0	1.0	0.0	1743.4	6.7	1744.5	5.8
Others	6.2	0.3	3.2	0.2	1789.0	6.8	1798.4	5.9
Total	2081.5	100	2017.3	100	26133.3	100	30232.1	100

Figure 2. Pictures depicting the study area located in the state of São Paulo, Brazil, characterized by different vegetation types and land uses: deciduous forest (a), cerrado under natural regeneration (b), exotic pine plantation (c), cerrado *stricto sensu* (d), lacustrine vegetation (e), wooded cerrado or cerradão (f), cropland-planted forest-riverine vegetation transition (g) and sugar cane plantation (h). All photos by A. G. Chiarello.

We compiled the conservation status of each recorded species based upon international (IUCN, 2020), national (Brazil Red Book of Threatened Species of Fauna; ICMBio, 2018) and state red lists (Decree N ° 63.853; Assembleia Legislativa do Estado de São Paulo, 2020). We also compared the native species recorded and the richness estimates of this study with the results obtained from similar research conducted in the largest protected Cerrado remnant of the São Paulo state. This area is characterized by a large fragment of native vegetation (including the Jataí Ecological Station - JES, 9013 ha; and the adjacent Luiz Antônio Experimental Station - LAES, 2009 ha), and the surrounding 5 km buffer (hereafter JES + LAES + buffer = JES landscape). JES and LAES are public governmental PAs and are located less than 50 km away from our study area (Paolino et al. 2016). The sampling design and data collection in our study and Paolino and collaborators' research followed the same protocol (as described in items 2 and 3) and were conducted by the same team of researchers. Paolino and collaborators (2016) sampled 105 points in total, 52 in the interior of the native fragment and 53 within the 5 km buffer (3150 camera.days) during April-September of 2013. We chose to make a formal comparison only with Paolino and collaborators' research because it was carried out with the same approach used in our study.

Results

We recorded 20 native species of medium and large-sized terrestrial mammals (seven orders and 14 families), with 15 of them being recorded in the interior (14 in CSF, 10 in DCF) and 19 in the buffer (Table 2, Figure 3). Only one species, the collared-peccary (*Dicotyles tajacu*), was detected solely by track surveys. Four species found in our study area are threatened with extinction at some level (global, national or state). Among these, the giant anteater (*Myrmecophaga tridactyla*), puma *(Puma concolor*), and maned wolf (*Chrysocyon brachyurus*), are listed as Vulnerable (VU) both at the national and state level (Figure 3). The naked tailed armadillo (*Cabassous* sp.) was detected by the cameras trap at CSF, but it was not possible to identify it to the species level from the photos. However, one individual of this genus was sighted crossing an internal unpaved road of DCF during a preliminary visit to this area by one of the authors (A. Chiarello). After a quick pursue, this individual was captured, photographed and returned to the same spot minutes later (Figure 3). The shape and size of the ears and the scute patterns in the head of this captured individual clearly indicate it belongs to *C. squamicaudis*, according to the recent review by Feijó & Anacleto (2021). Thus, we attest to the presence of this species in the sampled area, but we cannot ascertain that it is the only species of the genus present in our study area. We also recorded five domestic or exotic invasive species, namely: cattle (*Bos taurus*), European hare (*Lepus europaeus*), domestic dog (*Canis familiaris*), domestic cat (*Felis catus*), and horse (*Equus caballus*). They were recorded in the buffer, but three of them – cattle, European hare and domestic dog – were also recorded in the interior.

The rarefaction curves of the interior, buffer and total area did not reach asymptotes (Figure 4A), indicating that some additional species remained undetected. Since the 95% Confidence Intervals (hereafter, CIs) of these three rarefaction curves overlapped, we did not find strong evidence of differences in the observed species richness (Sest) between these areas (Figure 4A). The estimated asymptotic species richness (Chao2) for the interior (12.24, 95% CI = 12.01 to 16.65) was, however, lower than that estimated for the 5 km buffer (22.09, 95% CI = 17.6 to 47.41), as their 95% CIs did not overlap at the common number of samples (Figure 4C). Correspondingly, the curve of uniques show a clearly decreasing trend and a lower number of uniques for the interior, while uniques in the buffer did not start to decrease (Figure 4B), indicating a less complete sample for the later region. Analyzing the Sest, uniques, and Chao2 curves for CSF and DCF separately, we observe overlaps between their values, suggesting no significant difference in observed and estimated species richness and a comparable sampling completeness between these two protected areas (Figure 5). Estimated species richness (Chao2) for the CSF was 11.83 (95% CI = 10.23 to 24.87) and 13.83 for DCF (95% CI = 12.23 to 26.87).

The estimated species richness (Chao2) from camera trap records for our entire study area (CSF + DCF + 5 km buffer) was very similar (20.18; 95% CI = 19.14 to 29.20 species) to that found for the JES landscape using the same protocol (21.72, 95% CI = 19.80 to 35.84 species; Paolino et al. 2016), comparing the areas at the common number of samples. When comparing the list of recorded species resulting from camera traping and track surveys of our study area with that from JES landscape (Paolino et al. 2016), we noted that only four of 24 species of mammals were not detected in our study area, namely the tapir (*Tapirus terrestris*), the marsh deer (*Blastocerus dichotomus*), the capybara (*Hydrochoerus hydrochaeris*) and the Brazilian guinea pig (*Cavia aperea*) (Table 3).

Discussion

The study area harbors a rich community of mammals, with 20 species effectively detected, representing 47% of all medium and large-sized terrestrial mammal species known for the entire Cerrado (Marinho-Filho et al 2002). This number is probably higher, as our data did not reach the sample sufficiency, the inventory was conducted solely in the dry season, and the estimated richness is up to 29 species (95% CI = 19.14 to 29.20). This area seems to be especially relevant to the conservation of the large and vulnerable mammals' species that still survive in the Cerrado of São Paulo state (giant anteater, maned wolf, and puma), as these animals were widely detected. Species richness comparisons between our study area and the JES landscape, interior and buffer, and CSF and DCF lead us to specific considerations, but all results reinforce the ecological relevance of our study area and demonstrate the importance of adequate management measures for the region.

We found very similar values when we compare the estimated richness in our study area with the JES landscape, the largest protected area of the Cerrado of São Paulo state (Paolino et al. 2016). This equivalence might be a consequence of the similar proportion of native vegetation covering both areas (37.7% in JES landscape; 38.3% in our study area) since the large-sized mammal's richness in the Neotropics seems to be most affected by this parameter (Rios et al. 2021). Only four species detected in the JES landscape were not detected in the present study: tapir (*Tapirus terrestris*), marsh deer (*Blastocerus dichotomus*), capybara (*Hydrochoerus hydrochaeris)*, and Brazilian guinea pig (*Cavia aperea)* (Table 3). We advance three possibly explanations for the lack of detection of these species in our study area. First, the presence of some species could be related to the landscape configuration, apart from native vegetation proportion. Contrasting to the overall mammal species (Melo et al. 2017, Rios et al. 2021), the richness of medium and large-sized herbivores seems to be better predicted and negatively affected by the number of patches in the landscape (Rios et al., 2021), and native vegetation are much more fragmented in our study area (9.4 patches/100 ha) than in the JES landscape (2.1 patches/100 ha). This effect may be especially true for those species with a large body size such as the tapir, since our sampled fragments may not be large enough

Table 2. List of medium and large-sized mammals detected within Cajuru State Forest (CSF), Dois Córregos Farm (DCF) and within their surroundings (5 km buffer), in the state of São Paulo, Brazil. Conservation status according to the global (IUCN, International Union for Conservation of Nature), national (Br, Brazil Red Book of Threatened Species of Fauna) and state (SP, Decree N° 63.853) level. C = camera trap record; T = track survey record; NL = not listed; LC = least concern; VU = vulnerable. Taxonomic classification and nomenclature following Abreu et al. (2021).

ORDER/Family/Species	**Study area**			**Conservation Status**		
	CSF	**DCF**	**Buffer**	**IUCN**	**Br**	**SP**
DIDELPHIMORPHIA						
Didelphidae						
Didelphis albiventris (Lund, 1840)			C	LC	NL	NL
CINGULATA						
Dasypodidae						
Cabassous sp.	C		C			
Dasypus novemcinctus (Linnaeus, 1758)	C		C,T	LC	NL	NL
Chlamyphoridae						
Euphractus sexcinctus (Linnaeus, 1758)	C	C		LC	NL	NL
PILOSA						
Myrmecophagidae						
Tamandua tetradactyla (Linnaeus, 1758)	T	T	C,T	LC	NL	NL
Myrmecophaga tridactyla (Linnaeus, 1758)	C,T	C,T	C,T	VU	VU	VU
LAGOMORPHA						
Leporidae						
Sylvilagus minensis (Thomas, 1901)	T	C,T	C	NL	NL	NL
RODENTIA						
Dasyproctidae						
Dasyprocta azarae (Lichtenstein, 1823)		C,T	C,T	DD	NL	NL
Cuniculidae						
Cuniculus paca (Linnaeus, 1766)			C	LC	NL	NL
CARNIVORA						
Felidae						
Puma concolor (Linnaeus, 1771)	C,T	C,T	C,T	LC	VU	VU
Herpailurus yagouaroundi (Saint-Hilaire, 1803)	T		C	LC	VU	NL
Leopardus pardalis (Linnaeus, 1758)	C,T		CT	LC	NL	VU
Canidae						
Cerdocyon thous (Linnaeus, 1766)	C,T	C,T	C,T	LC	NL	NL
Chrysocyon brachyurus (Illiger, 1815)	C,T	C,T	C,T	NT	VU	VU
Procyonidae						
Nasua nasua (Linnaeus, 1766)			C	LC	NL	NL
Procyon cancrivorus (Cuvier, 1798)			C	LC	NL	NL
Mustelidae						
Eira barbara (Linnaeus, 1758)	T		C	LC	NL	NL
Mephitidae						
Conepatus semistriatus (Boddaert, 1785)	C,T	C,T	C,T	LC	NL	NL
ARTIODACTILA						
Cervidae						
Mazama gouazoubira (Fischer, 1814)	C,T	C,T	C,T	LC	NL	NL
Tayassuidae						
Dicotyles tajacu (Linnaeus, 1758)			T	LC	LC	NL
TOTAL	**14**	**10**	**19**			

Figure 3. Some species of medium and large-sized terrestrial mammals detected by cameras trap in our study region. a) *Cabassous squamicaudis* (Lund, 1845) captured and released by A. Chiarello during a preliminary visit in Cajuru State Forest in September 9, 2011. The animal was sighted when it crosses an unpaved road inside CSF at 12:59 h, being photographed and, minutes later, released in the same local inside this protected area. We thank Aurelio Fontes for the company and for holding this animal during the photography; b) *Leopardus pardalis* (Linnaeus, 1758); c) *Puma concolor* (Linnaeus, 1771); d) *Chrysocyon brachyurus* (Illiger, 1815) e) *Myrmecophaga tridactyla* (Linnaeus, 1758).

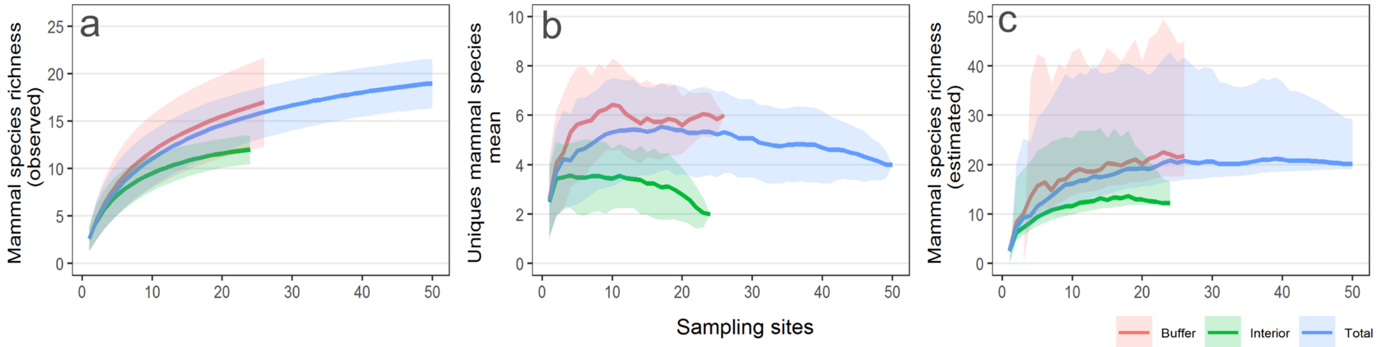

Figure 4. Rarefaction curves for observed richness (a), estimated number of uniques (b) and estimated richness by the Chao2 estimator (c) of native species recorded by camera trapping in the buffer (5 km), the interior (Cajuru State Forest and Dois Córregos Farm) and the total study area (interior+buffer). Shaded areas are 95% confidence intervals (a,c) or standard deviations (b).

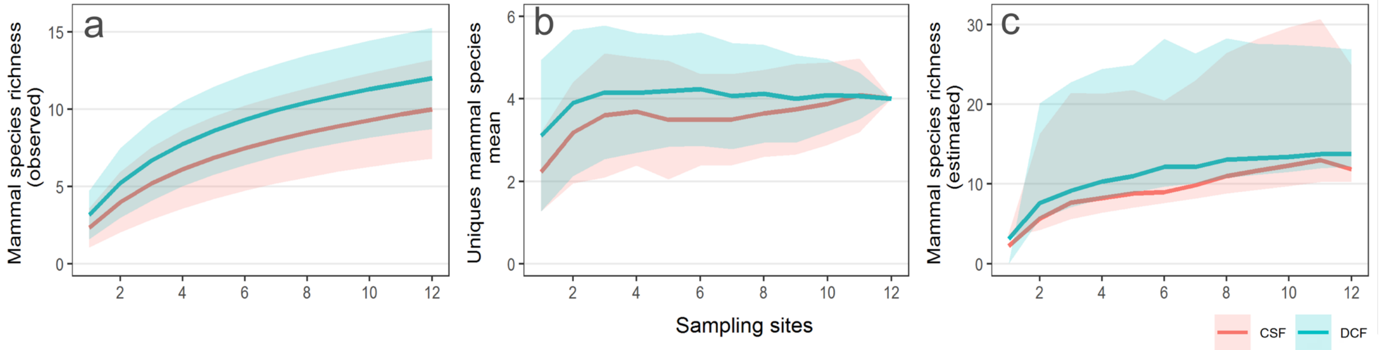

Figure 5. Rarefaction curves for observed richness (a), estimated number of uniques (b) and estimated species richness by the Chao2 estimator (c) of native mammals recorded by camera trapping in Cajuru State Forest (CSF) and Dois Corregos Farm (DCF). Shaded areas are 95% confidence intervals (a,c) or standard deviations (b).

to sustain a species with large spatial requirements for native vegetation (Medici et al. 2012), even though its current presence in JES could be represented by just one or a few individuals (Rodrigues et al. 2014). Second, the JES landscape has a greater amount of floodplains (2.3 times more than in our study area), which could explain the detection of species that are closely related to aquatic environments, such as the marsh deer - that was recently reintroduced in the JES landscape (Duarte et al. 2012) - and the capybara. Finally, since species as capybara and the Brazilian guinea pig are common and widespread throughout the state of São Paulo (Bressan et al. 2009, IUCN, 2020), a higher sampling effort with more detection methods could result in their detection in our study area. Indeed, other species found in the region could be detected if we had a higher effort, such as *Mazama americana, Pseudalopex vetulus, Galictis cuja* and *Dasypus septemcictus* (Fundação Florestal do Estado de São Paulo, 2009). On the whole, the similar species richness found between our study area and the largest remnant of Cerrado of São Paulo state reinforces the importance of CSF-DCF-buffer for the conservation of medium and large-sized mammals, an animal group severely threatened by human activities, but extremely relevant for biodiversity maintenance on a regional scale (Cardillo et al. 2005).

Given the ecological relevance of our study area, we advocate the need for the development of a management plan for Cajuru State Forest. We also endorse the proposal made by Durigan et al. (2014) to recategorize CSF to a more restricted management category, under the name of Cajuru Ecological Station and designating 70% of its area to scientific research and the conservation of natural ecosystems. The remaining 30% would be renamed Altinópolis State Forest, aiming for forestry production and the conservation of natural ecosystems. This proposed change is timely and relevant since CSF has been listed as one of the public protected areas of São Paulo state that could potentially be used and exploited by the private sector through concession. Currently, the state law that addresses this question (Nº 16.260/16, 29/06/2016) is under embargo by the Public Ministry of the State of São Paulo, which alleges unconstitutionality since there was no consultation either of the management plans or the traditional populations living inside public protected areas (Assembleia Legislativa do Estado de São Paulo, 2020). We argue that the public sector should consider more carefully the ecological relevance of this public-protected area, particularly concerning the recommended change of its current management category (Durigan et al. 2014), to which we fully endorse.

Considering the species richness comparison between interior and buffer, we could expect that the number of species would be greater in the interior, as it has the highest proportion of native vegetation and higher level of legal protection, however, estimated species richness was higher in the buffer (Figure 4C). We argue that this rather unexpected result might be explained by the species-area relationship, as the buffer is more than six times larger than the interior (Table 1) and can be more biodiverse simply because it encompasses a larger area (Arrhenius 1921, Holt et al. 1999). Accordingly, the sample sufficiency was lower for the buffer, which presented a higher number of unique species and no apparent decline in its curve (Figure 4B). In general, the number of singletons tends to decrease as sampling size increases (Coddington et al. 2009) and the same relationship might be expected for uniques. Apart from having a larger area, the buffer also encompasses a greater

Table 3. Comparison between native medium and large-sized mammal species detected with cameras trap and track surveys in different locations in the Cerrado of São Paulo State, Brazil. The data from our study correspond to sampling points in the interior of Cajuru State Forest (CSF) and Dois Córregos Farm (DCF), and within a 5 km buffer around these two areas. JES = Jataí Ecological Station; LAES = Luiz Antônio Experimental Station and a 5 km buffer, data from Paolino et al. (2016). Taxonomic classification and nomenclature following Abreu et al. (2021). Common names in the region according to Fundação Florestal do Estado de São Paulo (2009).

Species	Common names in the region	CSF+DCF+Buffer (30232 ha)	JES+LAES+Buffer (50744 ha)
Didelphis albiventris (Lund, 1840)	Gambá	X	X
Cabassous sp.	Tatu-do-rabo-mole	X	X
Dasypus novemcinctus (Linnaeus, 1758)	Tatu-galinha	X	X
Euphractus sexcinctus (Linnaeus, 1758)	Tatu-peba	X	X
Tamandua tetradactyla (Linnaeus, 1758)	Tamanduá mirim	X	X
Myrmecophaga tridactyla (Linnaeus, 1758)	Tamanduá bandeira	X	X
Sylvilagus minensis (Thomas, 1901)	Tapeti	X	X
Dasyprocta azarae (Lichtenstein, 1823)	Cutia	X	X
Hydrochoerus hydrochaeris (Linnaeus, 1766)	Capivara		X
Cuniculus paca (Linnaeus, 1766)	Paca	X	X
Cavia aperea (Erxleben, 1777)	Preá		X
Puma concolor (Linnaeus, 1771)	Onça parda	X	X
Herpailurus yagouaroundi (Saint-Hilaire, 1803)	Gato mourisco	X	X
Leopardus pardalis (Linnaeus, 1758)	Jaguatirica	X	X
Cerdocyon thous (Linnaeus, 1766)	Cachorro do mato	X	X
Chrysocyon brachyurus (Illiger, 1815)	Lobo-guará	X	X
Nasua nasua (Linnaeus, 1766)	Quati	X	X
Procyon cancrivorus (Cuvier, 1798)	Mão pelada	X	X
Eira barbara (Linnaeus, 1758)	Irara	X	X
Conepatus semistriatus (Boddaert, 1785)	Jaritataca	X	X
Tapirus terrestris (Linnaeus, 1758)	Anta		
Mazama gouazoubira (Fischer, 1814)	Veado-catingueiro	X	X
Blastocerus dichotomus (Illiger, 1815)	Cervo do pantanal		X
Dicotyles tajacu (Linnaeus, 1758)	Cateto	X	X
Total		**20**	**24**

compositional and configurational landscape heterogeneity, when compared to the interior (smaller and more homogeneous) (Figure 1, Table 1). Indeed, heterogeneous areas potentially harbor more species, as they provide greater diversity of resources easier to access (Fahrig et al. 2011). Also, the buffer area still has a relatively high proportion of native vegetation (>30%), preserved as APPs or LRs scattered across the agricultural matrix. The presence of interconnected patches of native vegetation in the landscape can facilitate animal dispersion and provide habitat and resources for many species, including large terrestrial mammals (Kremen & Merenlender 2018). As observed in previous studies, the presence of native vegetation areas is fundamental for large and vulnerable mammals' species living in agroecosystems, such as giant anteaters, maned wolves, and pumas (Coelho et al. 2008, Vynne et al. 2011, Magioli et al. 2014, Azevedo et al. 2020, Versiani et al. 2021). Thus, our result reinforces the importance of buffer zones around PAs and dialogues with other studies that indicate that these areas are used by mammals, especially the larger ones, which need extensive home ranges (Salafsky 1993, Vynne et al. 2011, Massara et al. 2012, Bamford et al. 2014). In this sense, our results also reinforce the high ecological value of heterogeneous agroecosystems that preserve 30% of the native vegetation and are connected to protected areas.

Regarding the comparison between CSF and DCF, we found both areas having a similar mammal richness. The fact that these areas have almost the same size and are adjacent to each other (Figure 5, Table 2) suggest a similar amount of resources (Arrhenius 1921, Holt et al. 1999) and high animal dispersion between them. This similarity reinforces the importance of private protected areas defined by the NVPL (as DCF) and shows that this type of area can be at least as rich as a PA of sustainable use (as CSF). But more than that, this result reveals that the strategy to connect government and private reserves in agroecosystems can help the landscape to harbor a considerable number of species.

However, compliance with the environmental legislation is generally compromised elsewhere, especially in sugar cane monocultures, the main agricultural crop in the region (Soares-Filho et al. 2014, Brancalion et al. 2016). Due to the association between public and private reserves, our study area has 38% of native vegetation cover, a percentage close to the minimum threshold (40%) suggested as necessary for the maintenance of native species in tropical agroecosystems (Arroyo-Rodríguez et al. 2020), but much higher than the typical pattern of the region, characterized by 19% of native vegetation (Ronquim 2017). The Cerrado of the state of São Paulo stands out nationally as having one of the highest LRs debt and, therefore, a high necessity for vegetation restoration (Freitas et al. 2017, Mello et al. 2021). Although these private native vegetation areas provide ecosystem services that are key for maintaining biodiversity, human well-being, and agricultural production as well, their restoration still generates conflicts with a parcel of rural owners, who do not agree to bear the restoration costs (Metzger et al. 2019, Strassburg et al. 2019). However, a systematic restoration plan can minimize costs and maximize the environmental benefits (Strassburg et al. 2019). By simply adhering to the law, sugar cane producers could increase the provision of ecosystem services, such as carbon sequestration and water purification, while making their products suited for the growing demands of the international market for more sustainable products (Kennedy et al. 2016, Kehoe et al. 2019).

The conservation of the Cerrado in São Paulo state would benefit from a better articulation and dialogue with the private sector. In the absence of natural areas for the establishment of new governmental PAs (Metzger & Rodrigues 2008), the conservation of the regional biodiversity depends on compliance with environmental legislation and restoration of native vegetation by farmers. Our results demonstrate that we can have a scenario of biodiversity preservation similar to that encompassed by larger and strictly protected nature reserves when APPs and LRs are implemented and connected with existing PAs. Thus, our study highlighted the need to develop a management plan that associates governmental and private reserves while changing rural producers' roles, from the villain of biodiversity to the protagonist of the conservation and restoration of the region.

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Conflicts of Interest

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

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Short Communication

Moving far from water: unusual dispersal movement of the water opossum (*Chironectes minimus:* Didelphimorphia, Didelphidae) in central Brazil

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Abstract: Unusual movements of an animal can potentially represent a dispersal event. A higher frequency of young males dispersing is a pattern observed for most part of polygynous or promiscuous mammals with these dispersion events occurring, mainly, before or at the beginning of the breeding season. The water opossum (*Chironectes minimus*), the only marsupial adapted for semi-aquatic life, occurs from Mexico to Argentina and it has been mostly described as a sensitive species to river and riparian vegetation degradation, occurring exclusively near water curses. Here we describe the first record of long-distance movement of a water opossum not associated with riverine vegetation through dry land. We captured a healthy adult male of *C. minimus* in July 2012 beside of a highway (DF-001) located 1,100 m from nearest gallery forest in the Federal District. The region is characterized by urban and suburban residential, small fragments of typical savanna and degraded gallery forests. Our unique record could be an event of dispersion through degraded dry lands as observed for other semi-aquatic mammals and also suggests that this species is more resistant to anthropogenic disturbances than previously described. Also, information about dispersal patterns of water opossum is scarce and may contributes to a deeper understanding of ecological requirements of this species.

Keywords: *Yapok; Cerrado; neotropical savanna; dryland movement; small mammals.*

Afastando-se da água: movimento de dispersão incomum da cuica d'água (*Chironectes minimus:* Didelphimorphia, Didelphidae) no Brasil central

Resumo: Deslocamentos incomuns realizados por um animal podem representar potencialmente um evento de dispersão. Grande parte dos mamíferos poligínicos ou promíscuos, apresentam uma maior frequência de machos jovens dispersando, e esses eventos ocorrem, principalmente, antes ou no início da estação reprodutiva. A cuíca d'água (*Chironectes minimus*), único marsupial adaptado à vida semiaquática, ocorre do México à Argentina. Tem sido descrito principalmente como uma espécie sensível à degradação de cursos d'água e matas ciliares, ocorrendo exclusivamente próximo aos cursos d'água. Desta forma, descrevemos aqui o primeiro registro do deslocamento de longa distância de uma cuíca d'água não associado à vegetação ribeirinha através de um ambiente seco. Capturamos um macho adulto saudável de *C. minimus* em julho de 2012 à margem de uma rodovia (DF-001) localizada a 1.100 m da mata de galeria mais próxima no Distrito Federal. A região é caracterizada por residências urbanas e suburbanas, pequenos fragmentos de cerrado sentido restrito e matas de galeria degradadas. Nosso registro singular pode ser um evento de dispersão através de ambientes secos antropizados, conforme observado para outros mamíferos semiaquáticos, o que, também, sugere que esta espécie é mais resistente a distúrbios antropogênicos do que descrito anteriormente. Além disso, as informações sobre os padrões de dispersão da cuíca d'água são escassas e podem contribuir para um entendimento mais profundo dos requisitos ecológicos desta espécie.

Palavras-chave: *Yapok; Cerrado; deslocamento em ambientes secos; pequenos mamíferos.*

Introduction

Dispersal events, which may be defined as the movement and subsequent breeding of individuals from one area to another, play a crucial role on the population dynamics (Ramakrishnan 2008). The understanding of dispersal patterns, especially related to long-distance dispersal, is crucial for species conservation specially with the current global scenario of land use change (Trakhtenbrot et al. 2005). Adequate information about dispersal of elusive species however, is not easily obtained. This is the case of the water opossum or yapok (*Chironectes minimus*, Zimmerman 1790) (Didelphimorphia, Didelphidae), an uncommon nocturnal small mammal (Galliez et al. 2009), which is the only known marsupial, fossil or living, adapted for semi-aquatic life (Brandão et al. 2015, Damasceno & Astúa 2016).

Like other semi-aquatic mammals, *C. minimus* displays numerous adaptations associated to this lifestyle as streamlined body shape, slightly flattened tail, large webbed hind feet, enlarged pisiform in the manus, dense and water-resistant pelage, and a well-developed pouch in both sexes. The water opossum feeds on fishes, crustaceans, insects, and occasionally on frogs and bats (Santori et al. 2006, Breviglieri & Pedro 2010). It is nocturnal and solitary (Galliez et al. 2009), with the breeding season beginning in June and extending to February (Fernandez et al. 2015).

The distribution of this marsupial ranges from southern Mexico to northeastern Argentina (Marshall 1978). In South America, recent records in southern Amazonia and central Brazil suggest that this species is distributed continuously from Amazonia through riparian forests in the Cerrado (Brazilian savanna) to the southern portion of Atlantic forest (Brandão et al. 2015). Most knowledge on about spatial patterns and population dynamics of water opossums, however, comes from studies conducted in southeastern Atlantic Forest, encompassing only a small part of this species' geographic range.

Although *C. minimus* is classified as a Least Concern (LC) species according to IUCN, some populations may be threatened by the intense degradation of freshwater ecosystems (Torremorell et al. 2021) and by climate change that could lead to a 22% reduction in the area of potential distribution of the water opossum (Freitas-Oliveira et al. 2021). At local spatial scale, the water opossum is potentially threatened by deforestation of riverine vegetation, contamination and deterioration of freshwater ecosystems (Pérez-Hernandez et al. 2016). In Atlantic Forest, *C. minimus* has been described as highly sensitive to the degradation of riparian forest and riverine vegetation, preferring well preserved habitats such as streams and rivers with stony substrate, clear and fast-running water associated with a highly preserved riparian forest (Handley 1976, Galliez et al. 2009, Palmeirim et al. 2014, Leite et al. 2016). However, the water opossum also seems to be able to occur in degraded habitats, with few studies showing that this marsupial is found in degraded riverine forests. Brandão et al. (2015) captured an individual in a narrow gallery forest surrounded by monoculture in central Brazil, Prist *et al.* (2020) registered the water opossum in culverts under a highway near a degraded riverine forest in Atlantic Forest biome, and Arias-Alzate *et al.* (2021) described the activity patterns of the water opossum in peri-urban areas in Colombia. A rapid decline in the potential distribution of the water opossum, however, has been observed, mainly caused by habitat loss and fragmentation (Prieto-Torres & Pinilla-Buitrago 2017).

Herein, we describe the first record of long-distance movement of a water opossum not associated with riverine forest through dry lands in central Brazil, in the core region of the Cerrado, a neotropical savanna. We also discuss potential implications of this record for the understanding of use of space and ecological requirements of the water opossum in the Cerrado.

Material and Methods

We hand-caught a water opossum in a fortuitous event at 11:30 PM on July 6th, 2012, alongside a highway (DF-001 or *Estrada Parque Contorno*) at the kilometer 22, near the entrance of a suburban residential (*Estância Quintas da Alvorada*) localized 10 km east of Brasília city, Federal District, Brazil (15º49'21.10" S 47º47'40.48" W), at an elevation of 1,120 m a.s.l, between Antas stream (Paranoá river sub-basin, upper Paraná river basin) located 1,100 m from the capture site and Taboquinha stream (São Bartolomeu river sub-basin, upper Paraná river basin) located 1,250 m from the capture site (Figure 1). The region is characterized by urban and suburban residential areas; small fragments (up to 38 ha) of typical savanna (cerrado *sensu stricto*); and degraded gallery forests. The nearest gallery forest, which occurred along the Antas stream was located inside of the Copaibas District Park (*Parque Distrital das Copaíbas*) (Figure 1). The climate of the region is tropical and highly seasonal (Köppen 1948), with only 10% of the annual rainfall occurring between April and September (Miranda et al. 1993). The average annual rainfall recorded over 25 years (from 1980 to 2004) is 1440 mm, with an average temperature of 22.1 °C (data obtained from the meteorological station RECOR/IBGE).

Results and Discussion

The captured individual was an adult but non-reproductive male, apparently in good body condition, with 640g of body mass; 281mm of head and body length; 345 mm of tail length; 30 mm of ear length; 63mm hind foot length; and complete dentition (i 5/4, c 1/1, p 3/3, m 4/4) (Figure 2). It was deposited (skin and skull) in the Mammal Collection of the University of Brasília with number CMUnB 3736.

Our observation is unique for water opossums and raises some scientific questions and hypotheses about use of space and environmental requirements of this marsupial. One of these is why was this individual caught so far from the nearest riverine vegetation (i.e., gallery forests). Stoddart (1970) defined long-distance movements as dispersal movements in with the individual moves away from the original area occupied by the local population. In this context, the capture of the non-reproductive male water opossum outside of riverine forests in July (dry season), in the beginning of the breeding season (Fernandez et al. 2015), possibly was an event of dispersion through dry lands. This kind of event has been observed for other semi-aquatic mammals, such as otters (Jancke & Giere 2011) and water voles (Stoddart 1970). Moreover, the month of the capture is in agreement with the indication that dispersal events of mammals usually occur before or at the beginning of the breeding season (Wolff 1994).

Our assumption that the male water opossum was captured during a long-distance dispersal event is supported by some pieces of evidence. The dispersal of polygynous or promiscuous small mammals (as is the case of *C. minimus*) is sex-biased, with the predominance of young males (Li & Kokko 2019, Wolff 1994, Quaglietta et al. 2013, Liberg & von Schantz 1985). Specifically for the water opossum, unlike most mammals, a male-biased sex ratio has been described (Mondolfi & Padilha 1958, Galliez et al. 2009). Thus, a particularly high competition among males (both for resources and for females) would cause an even higher dispersal of water opossum young males in comparison to other mammals.

Other relevant point that must be evaluated is the real sensitivity of the water opossum to the process of degradation and isolation of

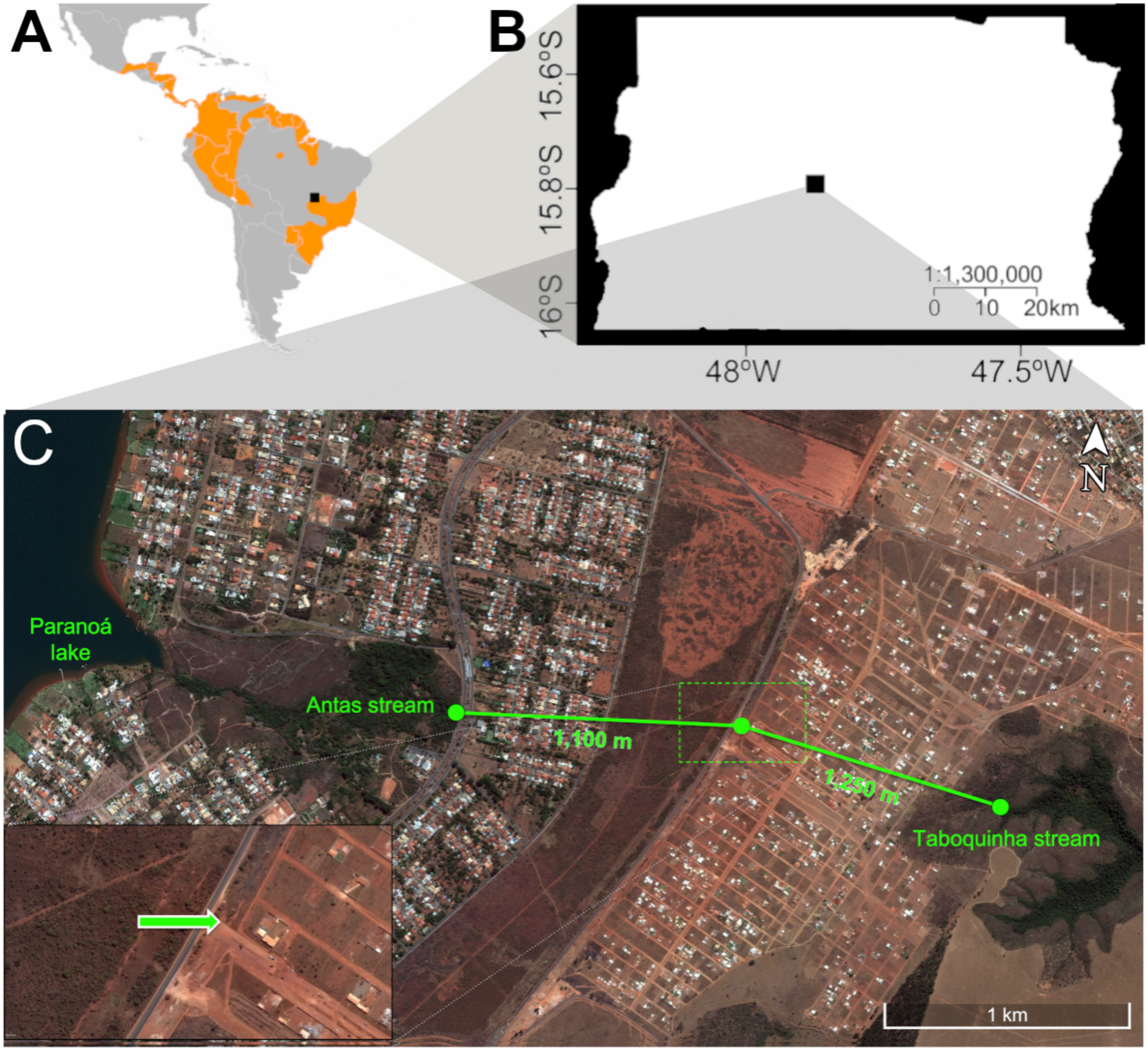

Figure 1. Capture location of *Chironectes minimus* in Federal District in central Brazil; (A) geographic distribution of *C. minimus*; (B) capture location in Federal District, Brazil; (C) the linear distance to nearest gallery forests from capture site; and green arrow indicate capture site.

riparian forests inserted in an anthropized matrix. Historically, the water opossum is described as a very sensitive species to the degradation of watercourses and riparian vegetation, occurring mainly in well-preserved streams with stony substrate and clear, fast-flowing water (Galliez et al. 2009, Prieto-Torres & Pinilla-Buitrago 2017, Handley 1976). Thus, Galliez *et al.* (2009) suggest that degradation of riparian areas may prevent dispersal to adjacent sub-basin. Furthermore, even the very few studies that registered water opossums in degraded habitats indicated that they were still associated with riverine vegetation (Arias-Alzate et al. 2021, Prist et al. 2020, Brandão et al. 2015). Our record indicates, however, that there are exceptions to this strong association. However, this sensitivity of *C. minimus* to degradation and isolation of riparian forests may vary between biomes. In open biomes such as tropical savannas, gallery forests have a restricted distribution along rivers and streams inserted in a savanna matrix (Oliveira-Filho & Ratter 2002), making the ability to move through dry land an potential factor in the maintenance of water opossum populations. Freitas-Oliveira et al. (2021) estimated that climate change will potentially lead to a 22% reduction in the geographic distribution of this marsupial, with most of this loss occurring in central Brazil due loss of riverine forests.

If the observed long-distance movement of the water opossum through dry lands was indeed a dispersal event, studies aiming to model the potential occurrence of this species should consider the scenarios in which this species may occur in anthropized environments. In addition, these studies should also consider the possible dispersal of this species between river basins, including the potential threats to the

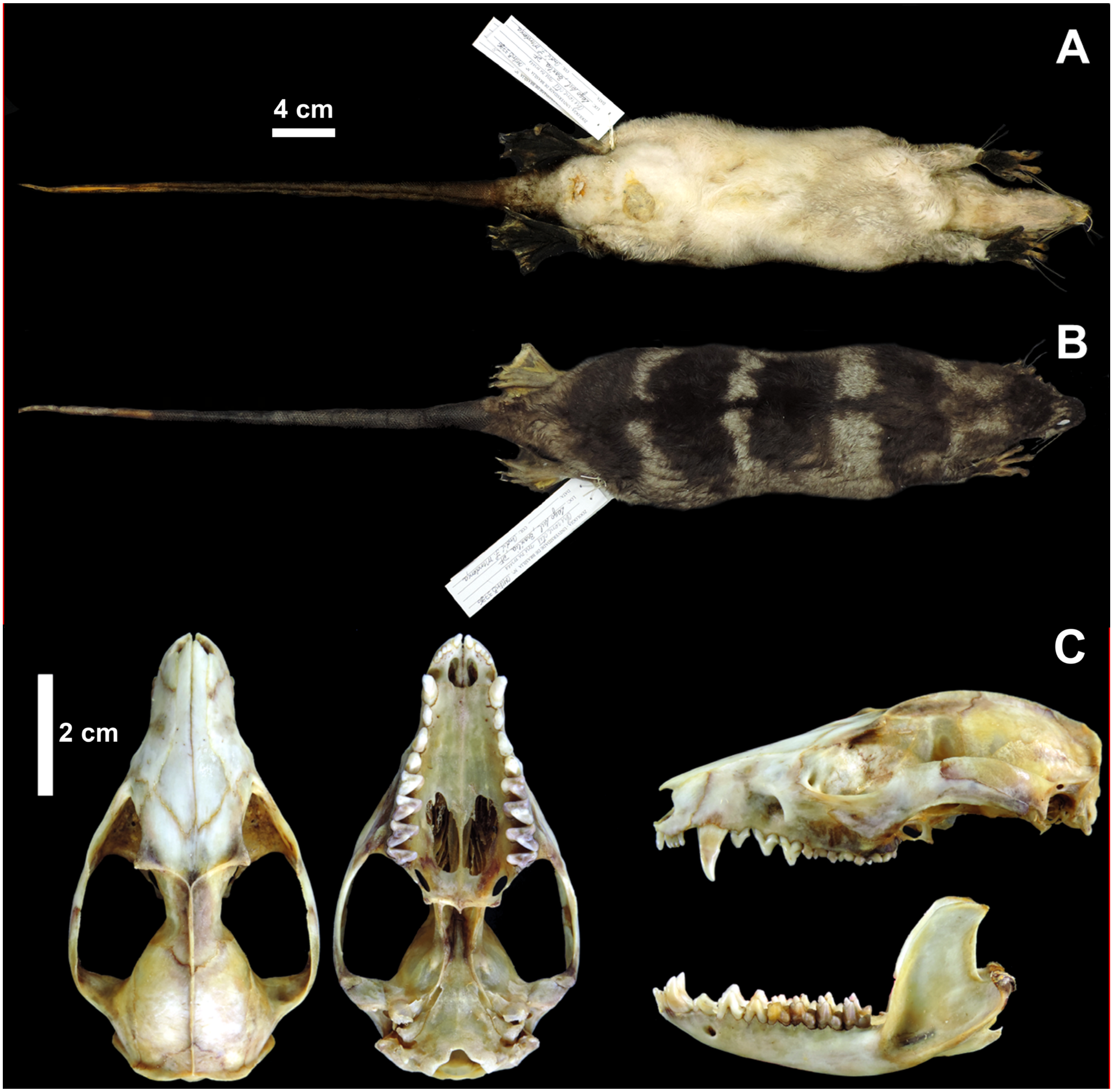

Figure 2. Ventral and dorsal views of skin (A and B, respectively); and dorsal, ventral, and lateral views of skull and lateral view of mandible (C) of *Chironectes minimus* (adult male, CMUnB 3736). External measurements (head-body length: 281mm; length of tail: 345 mm; height of ear: 30 mm; hindfoot length with claws: 63 mm; weight: 640 g; and tooth formula [i 5/4, c 1/1, p 3/3, m 4/4]).

individuals while dispersing (e.g., road crossing, lack of suitable paths). This possible dispersal event that we detected was not associated to a possible flooding and increase of suitable paths for the species, since the individual was captured in July, in the middle of the Cerrado dry season. Our assumption of long-distance dispersal event needs to be supported, however, by long-term studies focused on the evaluation of movements and space use by the water opossum mainly just before and in the beginning of the breeding season. Despite that, we believe that the evidence reported here will contribute to a deeper understanding of the use of space, long-distance dispersal, and ecological requirements of the water opossum in the Cerrado, the largest and most threatened tropical savannah in the world (Klink & Machado 2005).We expect that our previously unknown record will encourage further studies on the species, with potential for improving actions and policies for the conservation of this unique mammal.

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Author Contributions

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

Emerson Monteiro Vieira: 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.

Ethics

The individual was captured with permission from the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) (permanent licence nº 15424-1 granted to EMV).

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Inventory

Multi-taxon inventory and landscape characterization in an agrosystem of the Brazilian Midwest targeted for payment for environmental services

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Abstract: The replacement of natural landscapes by anthropic environments has led to habitat loss and consequently, to the decline and extinction of many species populations. Several strategies, such as the creation of protected areas (PAs) and payment for ecosystem services have been adopted to mitigate the consequences of this biodiversity crisis. In Rio Verde, Goiás, Brazil, a Water Producer Program (WPP) was established, which provides payment for ecosystem services for the maintenance of riparian vegetation in the region of the Ribeirão Abóbora, a river which supplies the city and metropolitan area. In the absence of environmental diagnostic studies in the region, this present study contributes to reducing some knowledge gaps, through the: (i) characterization of the landscape in the WPP area, (ii) characterization of species of fauna (amphibians, birds, fish, and medium and large terrestrial mammals) and woody flora that occur in this area, and (iii) comparison of species diversity (fauna and flora) found in our study area with the diversity observed in the Cerrado protected area network. Our results reveal a low native vegetation cover (~21% of total area), but we recorded 300 total species: 20 species of anuran amphibians, 100 birds, 10 fish, 16 terrestrial mammals (medium and large), and 154 woody plants. Our sample comprises species considered endemic to the Cerrado (e.g., Anuran - *Barycholos terntezi* and Mammal - *Callithrix penicillata*), threatened species (e.g., Mammals - *Herpailurus yagouaroundi, Myrmecophaga tridactyla* and *Tapirus terrestris*), and exotic invasive species (Mammal - *Sus scrofa*). The results presented in this study are relevant and may help in the proposal of management actions within the scope of this important program of payment for environmental services.
Keywords: *Anurans; birds; mammals; fishes; woody plants; exotic invasive species; threatened species.*

Inventário multi-táxon e caracterização da paisagem em um agrossistema no Centro-Oeste brasileiro alvo de pagamento por serviços ambientais

Resumo: A substituição das paisagens naturais por ambientes antropizados tem levado à perda de habitat e, consequentemente, ao declínio populacional e extinção de muitas espécies. Várias estratégias como a criação de Unidades de Conservação (UCs) e o pagamento de serviços ecossistêmicos têm sido adotadas para mitigar as consequências da crise de biodiversidade. Em Rio Verde, estado de Goiás, foi estabelecido um Programa Produtores de Água (PPA) que prevê o pagamento de serviços ecossistêmicos pela manutenção da vegetação ripária na região do ribeirão Abobora que abastece a cidade. Diante da ausência de estudos de diagnóstico ambiental da região, o presente estudo contribui para reduzir algumas lacunas de conhecimento, através da: i) caracterização da paisagem na área deste PPA, ii) caracterização das espécies da fauna (anfíbios, aves, peixes e mamíferos terrestres de médio e grande porte) e flora lenhosa que ocorrem neste local, iii) comparação da diversidade de espécies (fauna e flora) encontrada na área de estudo com aquela observada na rede de áreas protegidas do Cerrado. Nossos resultados revelam baixo índice de cobertura vegetal nativa (~21% da área total), mas por outro lado, registramos 300 espécies, sendo 20 de anfíbios anuros, 100 aves, 10 peixes, 16 mamíferos terrestres (médio e grande porte) e 154 plantas lenhosas. A nossa amostragem compreende espécies que são consideradas endêmicas do Cerrado (p.ex. Anfíbio - *Barycholos terntezi* e Mamífero - *Callithrix penicillata*), ameaçadas de extinção (p.ex. Mamíferos - *Herpailurus yagouaroundi, Myrmecophaga tridactyla* e *Tapirus terrestris*) ou exótica invasora (Mamífero - *Sus scrofa*). Os resultados apresentados no presente estudo são relevantes e podem auxiliar na proposição de ações de manejo no âmbito deste importante programa de pagamento por serviços ambientais.

Palavras-chave: *Anuros; aves; mamíferos; peixes; plantas lenhosas; espécies exóticas invasoras; espécies ameaçadas.*

Introduction

The rapid population decline and the consequent extinction of many species around the world have alarmed scientists and decision-makers (Pimm et al. 2014, Maxwell et al. 2016). To contain the consequences of this crisis, conservation actions must be urgently implemented and under this scenario, the creation and management of protected areas represent the main conservation strategy used worldwide (Rodrigues et al. 2004, Watson et al. 2014). However, due to the scarcity of financial resources and/or little political interest, this strategy has faced some obstacles, such as the lack of adequate funding, physical infrastructure, qualified technical staff, and the land regularization of the areas involved.

Thus, the implementation of alternative strategies is necessary and, in this context, the payment for environmental services (PES) has been adopted as a way to mitigate threats to biodiversity (Wunder 2008). PESs can be defined as an economic instrument that enables recompense and also encourage those people who provide environmental services, improving the profitability of activities of protection and sustainable use of natural resources (Pagiola et al. 2013, Wunder 2005). Currently, PESs are categorized into different modalities (see Pagiola et al. 2013, Wunder 2005), with special emphasis on those actions focused on water supply, which are called Water Producer Programs (WPP) (Pagiola et al. 2013). This PES modality aims to preserve and/or restore riparian vegetation along important water courses with a view to providing water, both in quality and quantity, for public supply (Wunder 2005).

Over the past few years, Water Producer Programs which are certified and regulated by the National Water Agency (NWA), have been implemented in Brazil (Coelho et al. 2021). Most of these initiatives lack detailed environmental diagnoses (e.g., Ruggiero et al. 2019), since little is known about the species of fauna and flora that occur in the areas of these programs. When considering that some fauna and flora species serve as indicators of environmental quality (Burger 2006), by providing a list of species for a given region, one can properly assess the effectiveness of actions implemented in the programs of payment for environmental services, and this can assist in the planning of future management actions. In 2011, the municipality of Rio Verde, located in the southwest of the state of Goiás, in the Brazilian Midwest, implemented a program of water producers in an important local agrosystem. The municipal government is responsible for the coordination of this program and has been promoting good agricultural practices (e.g. containment of erosive processes), as well as promoting management and conservation actions (e.g. fencing and floristic recomposition of water springs located in rural properties of this agrosystem). Despite these efforts, this WPP has had a considerable knowledge gap, due to the lack of detailed environmental diagnoses.

Considering this scenario, we characterized the landscape composition in the area of this WPP for the present study, and also provided a species list of local fauna (amphibians, birds, fish and medium and large terrestrial mammals) and woody flora that occur in this location. Finally, based on an intensive bibliographic search, we compared the species diversity (fauna and flora) found in our study area with the diversity observed in the network of protected areas of the Cerrado.

Material and Methods

1. *Study area and landscape metrics*

The present study was carried out in the hydrographic microbasin of the Ribeirão Abóbora (abbr. HMRA) (Figure 1), municipality of Rio Verde, southwestern Goiás State, Brazil. The area has 4,992 hectares and it is completely located within the Cerrado domain. The climate of the region is sub-humid tropical, with two well-defined seasons: a dry season (April to September) and a rainy season (October to March). In this area, the remnant vegetation is mainly fragments of forest formations (e.g., gallery forest and dry forest) and savannas (e.g., *veredas*), which represent areas of permanent preservation (APPs) and legal reserves of the properties located inside the microbasin. The remaining area is represented by anthropic environments, such as agroindustries, livestock and monocultural crops (e.g. soy, sorghum and corn).

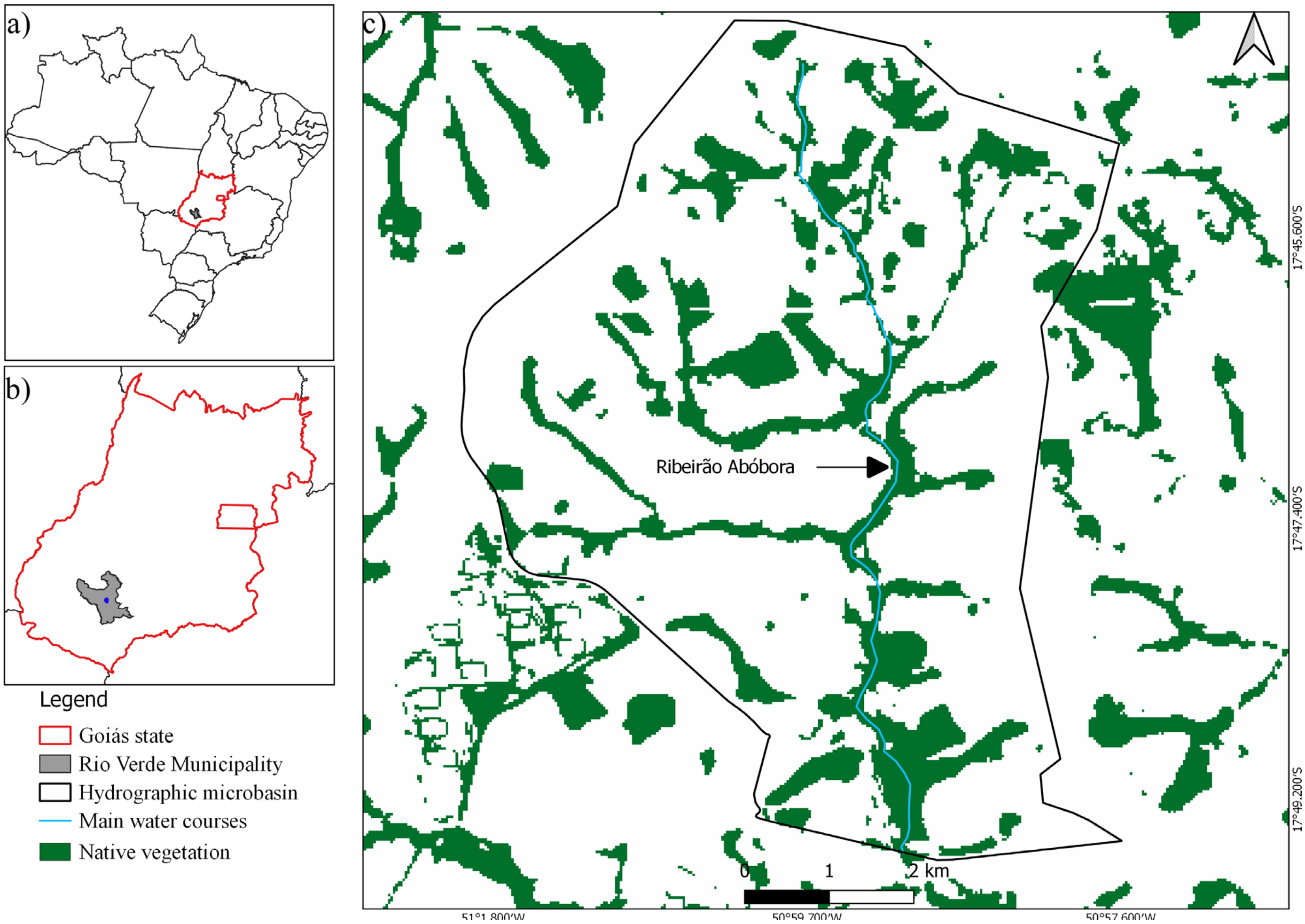

Figure 1. Study area located in the hydrographic microbasin of the Ribeirão Abóbora, municipality of Rio Verde, southwestern Goiás State, Brazil.

The HMRA is of socioeconomic importance, as a considerable portion of the city of Rio Verde's water supply comes from this area. Thus, the municipal government implemented an important mechanism of environmental management in this microbasin, known as the Water Producing Program - WPP ("*Programa Produtor de Águas*" or "*PPA*" in Portuguese) in partnership with the National Water Agency (NWA). Since the implementation of the WPP in 2011, the springs of the water courses located inside the area of the microbasin were cataloged, fenced, and went through a process of floristic restoration. In the long-term, these actions target the maintenance of the quantity and quality of the water appropriated for the public supply of the city of Rio Verde.

For landscape characterization of the HMRA, we used a classified image of Mapbiomas collection 5.0 with a resolution of ~5 m per pixel (Projeto Mapbiomas 2020), which we cropped for the study area and transformed into a binary image (native vegetation = 1; non-native vegetation = 0). We considered the following as native vegetation: forest, savanna, natural non-forest vegetation, wetlands and swampy areas, which could be present in the MHRA. Then, we used R software (R Core Team 2020) to calculate landscape metrics for the entire microbasin, using the functions *lsm_p_area* and *lsm_c_enn_mn* of "landscapemetrics" package (Hesselbarth et al. 2019), from which we extracted the following metrics: percentage of native vegetation in the microbasin (%NC), number of fragments and mean Euclidian distance for the nearest fragment.

2. *Species inventories*

The characterization of the biota in the study area considered the species richness of amphibian (only anurans), birds, fish, medium- and large-sized terrestrial mammals and woody plants. The field sampling occurred in the permanent preservation areas (PPAs) and legal reserves (LRs) of the rural properties located in the HMRA. It should be noted that the choice of the sampling sites (and the amount of sampling sites), as well as the sampling periods throughout the day, were determined according to the ecological specificities of each taxonomic group, as described below.

2.1. Anurans

The sampling of species of anuran amphibians was carried out between February and April 2018 (collecting permit number 47358-1/SISBIO), using complementary methodologies, such as active search in breeding sites, passive acoustic monitoring, and pitfall traps with drift fences. Suitable sites for reproduction of the anurans were actively sampled following the methodology proposed by Scott & Woodward (1994). In this case, active search occurred in five waterbodies, each site being sampled on two different nights, in the period from 19:00 to 24:00 h. These waterbodies were characterized as lentic sites, such as dams, ponds and/or swamps. During the search, some species had their advertisement calls recorded, using a Sennheiser ME 66 microphone coupled to a MARANTZ PMD 660 or TASCAN DR-40 recorder for later identification in the laboratory.

The passive acoustic monitoring technique was also used for the recording of species in the study area. We installed Tigrinus GT001. T1.0V audio-recorders (https://www.tigrinus.com.br/gravador-deudio) in other five water bodies, which remained for four consecutive nights. Each night, the automatic recorders were programmed to perform five minutes of recording every hour, in the period from 18:00 to 6:00 h. Records were obtained in WAV format, with 48 kHz and 24 bits of resolution. Therefore, we obtained 260 minutes of recordings in each sample point, totaling 1,300 minutes (~22 hours). In the laboratory, vocalizations were analyzed using the *Raven* 1.4 software (K. Lisa Yang Center for Conservation Bioacoustics 2011). The sound files obtained in this study were deposited in the Neotropical Amphibian Sound Archives Collection (CASAN, in Portuguese) of the Instituto Federal Goiano (IF Goiano – Campus Rio Verde).

Pitfall traps with drift fences, as described by Cechin & Martins (2000), were used to sample litter amphibians. Six sets of traps were installed in three fragments of the forest formation (e.g., gallery forest and dry forest). For this purpose, we selected the most representative fragments in terms of area. Each set of traps consisted of four 60-liter buckets buried in the soil, arranged in a line and interconnected by a screen of plastic netting, 60 cm high. The buckets were 10 m apart from each other. The traps remained open for 10 consecutive days and were reviewed every 24 hours, always in the morning. The nomenclature for amphibian species is in accordance with Frost (2021).

2.2. Birds

The sampling of avifauna occurred during the months of March and April 2018, totaling a sampling effort of ten days in the field. To characterize the avian species in the region, the following methods were used: 1) interviews with people living in the study area; 2) linear transects along the forest fragments present in the area, according to the methodology proposed by Burnham et al. (1980, 1981, 1985); and 3) techniques of automatic acoustic monitoring.

The linear transects were according to the shape and size of the 10 fragments. The transects were slowly coursed on foot, recording the species of birds visually and/or by their specific vocalizations. The transects were initiated shortly after sunrise, inside the fragment or at the fragment border, extending for up to four hours. For the visual records of bird species, we used a CANON EOS Rebel T6 EF-S 18-55 f/3.5-5.6 III camera and a CANON EF 75-300mm f/4-5.6 III lens. For the survey of nocturnal birds, we used the technique of passive acoustic monitoring, just as described in the sampling of amphibian species. The analyzed recordings occurred between 18:00 and 06:00 h, totaling a sample effort of 1,300 minutes (~22 hours) of recordings.

Identification of bird species was based on field guides (Sick 1997, Sigrist 2009, Develey & Endrigo 2004) and followed the most recent edition of the List of Birds of Brazil (Pacheco et al. 2021). Vocalizations heard *in situ* and recorded by means of automatic monitoring systems were compared with the archives deposited at the Fonoteca Neotropical Jacques Vielliard (FNJV) of the Museum of Zoology at Universidade Estadual de Campinas (UNICAMP).

2.3. Terrestrial medium- and large- sized mammals

The survey of terrestrial mammal species (medium- and large-sized) was carried out in March and April 2018, through active search, occasional records, and camera traps. The active search was conducted in 10 forest fragments present in the study area, where we searched for evidence or vestiges of the occurrence of species, as described by Becker & Dalponte (1991). As evidence of occurrence, we considered: (i) visualization of the individual, (ii) footprints, (iii) burrows (in the case of armadillos), (iv) feces, and (v) sounds registered during the active search (in the case of primates). Occasional records made during car travel inside the microbasin were also considered. Finally, we installed the camera traps (Bushnell model 119436) in five forest fragments, considering the ones most representative in terms of area. We installed one camera trap per fragment, at approximately 50 cm above the ground, which was placed in the vicinity of trails possibly explored by the animals. The traps remained installed for ten consecutive nights in each one of the sampled fragments and were programmed to make 10-second videos after being activated.

2.4. Fishes

The sampling of ichthyofauna was conducted in the streams (first order) and lakes located in the HMRA (tributary of the São Tomaz River located at the Bois River basin) in April 2018. The sampling occurred in the morning and afternoon periods for 10 days (collecting permit number 34479-1/SISBIO). Specimens were sampled through the Rapid Assessment Program (RAP) with some modifications, such as: 1) interviews with fishermen and local residents; 2) visualization of fish with the naked eye at the edge of water courses; and 3) active fishing with hand nets (mesh size = 2 mm), sieves and covo traps, intended to increase the sampling effort in the study area. Active fishing occurred in eight water courses under the influence of the study area, which comprise first-order streams and ponds. Easily identifiable specimens were photographed and released in the same location, following the guidelines of the IBAMA normative instruction nº 179, of June 25, 2008.

2.5. Woody plants

The records of woody plant species occurred between March and April 2018, through floristic surveys carried out during the course of transects and parcels. The transects occurred in areas of permanent preservation and legal reserves of the rural properties located in the HMRA. In these areas, the sampled fragments were classified as forest (gallery forest and semi-deciduous dry forest, *sensu* Ribeiro & Walter 2008) or savanna formations (Cerrado *Strictu Sensu* and *Veredas, sensu* Ribeiro & Walter 2008). The transects occurred both in the interior and at the edge of the fragments, allowing for records of species with different ecological requirements. To increase our sampling effort, we selected one fragment of semi-deciduous dry forest with sufficient size and randomly allocated 20 plots of 10 x 10 m area (Felfili et al. 2011). In the plots, we identified only those individuals with circumference at breast height (CBH) greater than or equal to 15 cm. The identification of most specimens occurred in the field, based on their vegetative and reproductive characteristics. However, individuals with difficult identification had samples taken. These samples were packed in plastic bags of 50 liters for later identification in the laboratory with the aid of bibliographic material. We used the Angiosperm Phylogeny Group III classification system (APG 2009).

2.6. Scientometrics search

We compared the species richness of amphibians, birds, mammals, fish and woody plants found in the HMRA with those observed in protected areas of the Cerrado domain. We searched for studies carried out in the Cerrado protected area network and considered only those studies that provided lists of species for each one of the taxonomic groups mentioned above as inclusion criterion (Supplementary Material 1). For this, we accessed the Scopus (https://www.scopus.com), Scielo (https://www.scielo.br/) and Web of Science (https://www.webofscience.com) databases and used a combination of the following keywords: Anuran OR Bird OR Fish OR Mammal OR Plant*, Species List, AND Inventory AND Cerrado* AND Protected Area* OR Conservation Unit OR Park OR Biological Reserve OR Ecological Station OR Extractive Reserve OR Sustainable Development Reserve OR Wildlife Reserve OR Natural Monument OR Forest OR Environmental Protection Area OR Area of Relevant Ecological Interest and Private Reserve of Natural Heritage. We compiled the species richness in the protected areas of the Cerrado domain for all of the studied taxonomic groups. All of the studies in redundancy were removed and papers published after November 2021 were not included. We did not consider gray literature (e.g. abstracts presented in congresses, theses and dissertations) in our database.

Results

1. Landscape characterization

In 2018, only 21.32% (1,064.73 ha) of the total mapped area was represented by native vegetation cover (forest, Cerrado *Strictu Sensu* and *veredas*), while 78.68% (3,928.26 ha) was covered by anthropic areas (e.g., plantations and agro-industries). We observed that the remaining native vegetation was distributed in 80 fragments, with sizes varying from 0.05 to 224.23 ha (mean = 13.30±37.5; n = 80 fragments; Figure 2). Of the 80 fragments, 64 (80%) had less than 10 ha, while only three fragments covered more than 100 ha. The average Euclidean distance between the fragments was 108.78 m.

2. Species diversity and comparison with protected areas of the Cerrado domain

We recorded 300 species distributed in the five studied taxonomic groups (Table 1, Figure 3 and 4, Supplementary Material 2). Our sampling includes 20 amphibian species (only anurans), 100 birds, 10 fishes, 16 medium- and large-sized mammals and 154 woody plants. Only 4% (12 spp.) of the 300 species found in the HMRA are endemic to the Cerrado domain, including five amphibian species (*Barycholos terntezi*, *Dendropsophus cruzi*, *D. jimi*, *Pseudopaludicola saltica* and *Scinax constrictus*); one medium- and large-sized mammal (*Callithrix penicillata*) and six plants species (*Aspidosperma tomentosum*, *Byrsonima basiloba*, *Dalbergia miscolobium*, *Miconia burchellii*, *Ocotea spixiana* and *Ouratea spectabilis*).

Only three medium- and large-sized mammals (*Herpailurus yagouaroundi, Myrmecophaga tridactyla* and *Tapirus terrestris*) and one woody plant (*Cedrela odorata*) are classified as vulnerable according to the National Red List of Threatened Species (Flora do Brasil 2020, MMA 2014). On the other hand, *Sus scrofa* (a medium- and large-sized mammal) is considered an alien species (Brasil 2017). Species richness in protected areas is greater than that

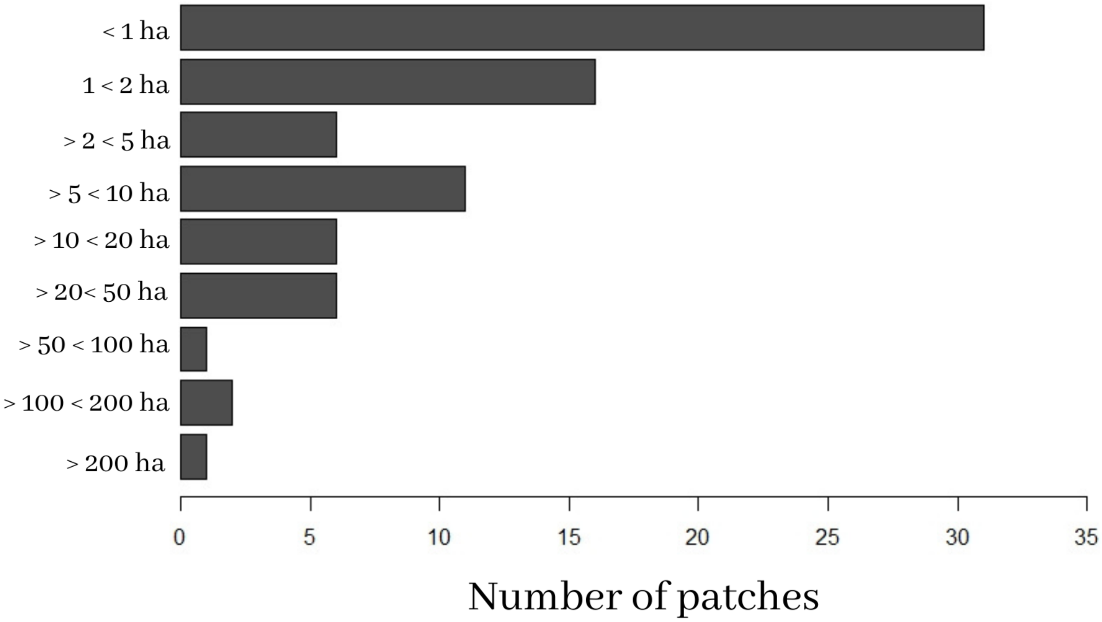

Figure 2. Size in hectares (ha) of native vegetation fragments (patches) in the hydrographic microbasin area of the Ribeirão Abobora, Rio Verde, Goiás.

Table 1. Number of species belonging to each taxonomic group sampled in the hydrographic microbasin area of the Ribeirão Abobora.

Taxonomic Group	Total number of species	Number of endemic species of the Cerrado domain	Number of threatened species
Amphibian	20	5	0
Bird	100	0	0
Fish	10	0	0
Mammals (Medium- and large sized)	16	1	3
Plant	154	6	1
Total	300	12	4

Figure 3. Anuran and bird species registered in the hydrographic microbasin of the Ribeirão Abóbora, municipality of Rio Verde, southwestern Goiás State, Brazil. A) *Dendropsophus cruzi*, B) *Pseudis bolbodactyla*, C) *Leptodactylus mystacinus*, D) *Leptodactylus labyrinthicus*, E) *Scinax constrictus*, F) *Boana albopunctata*, G) *Rupornis magnirostris*, H) *Monasa nigrifrons*, I) *Syrigma sibilatrix*, J) *Colaptes campestris*, K) *Sicalis flaveola*, L) *Pteroglossus castanotis*.

found in the HMRA (Table 2). In addition, we also observed that the species richness of amphibians, birds, fish, medium- and large-sized mammals, and woody plants represent, respectively, 64.77%, 45.23%, 39.78%, 65.98% and 68.94% of the average number of species found in protected areas of the Cerrado domain (Table 2).

Discussion

In this study, we characterized the landscape attributes and also the species richness of vertebrates and woody plants present in the HMRA, in Rio Verde, GO. We observed a low index of native vegetation cover (~21%) in this area, but we recorded an expressive number of species of fauna and woody flora, including some species that are considered endemic to the Cerrado, threatened with extinction or by exotic invasive species. This is a relevant result, since local biodiversity can

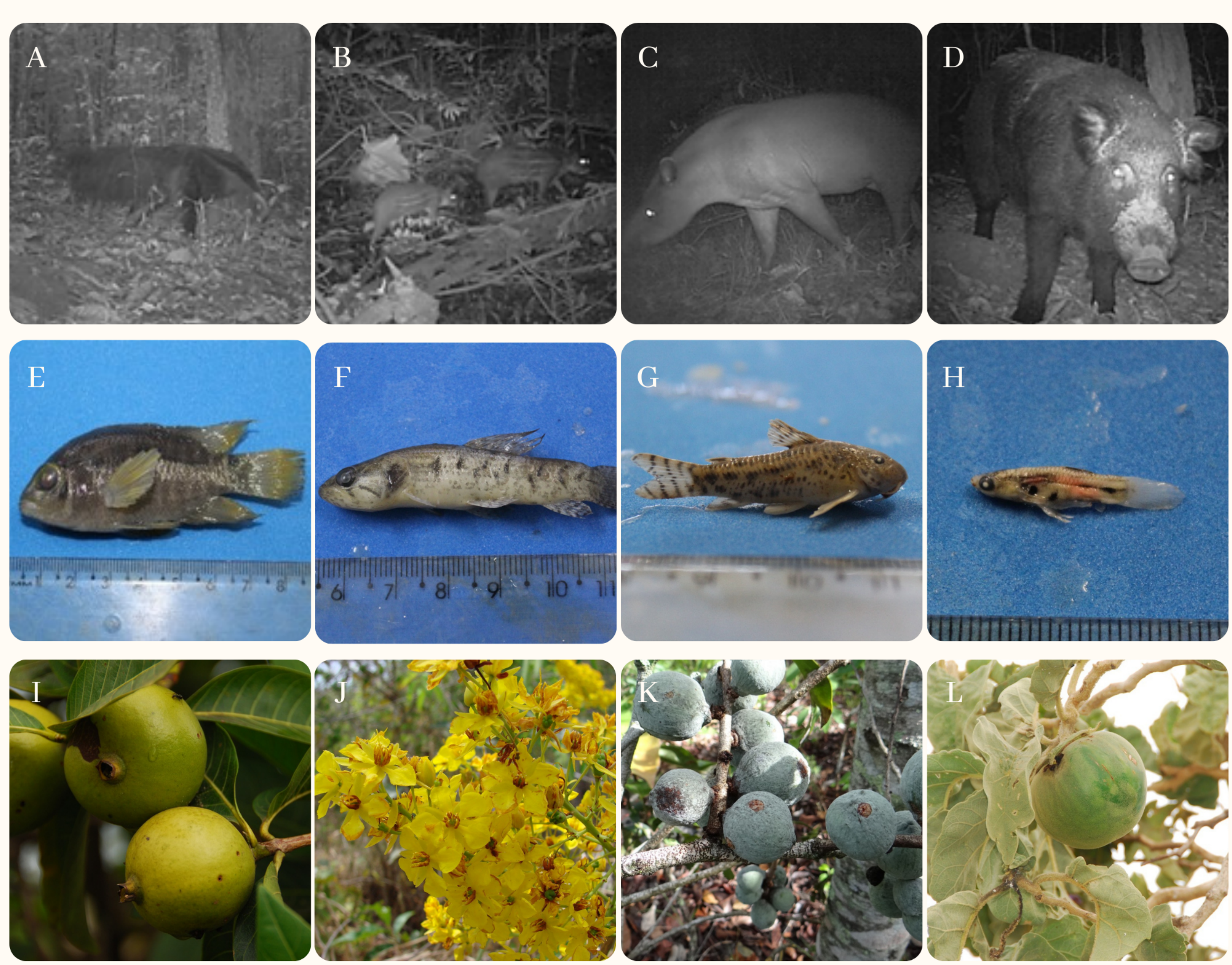

Figure 4. Mammal, fish and woody plant species registered in the hydrographic microbasin of the Ribeirão Abóbora, municipality of Rio Verde, southwestern Goiás State, Brazil. A) *Myrmecophaga tridactyla*, B) *Cuniculus paca*, C) *Tapirus terrestris*, D) *Sus scrofa*, E) *Cichlasoma paranaense*, F) *Hoplias Malabaricus*, G) *Aspidoras fuscoguttatus*, H) *Poecilia reticulata*, I) *Alibertia edulis*, J) *Ouratea hexasperma*, K) *Salacia crassifolia*, L) *Solanum lycocarpum*.

play a key role in the maintenance of important ecosystem services in a given location (e.g., Hooper et al. 2005, Lefcheck et al. 2015). Located in a region of intense agricultural activity, the HMRA consists of rural properties that are beneficiaries of an important program of payment for environmental services, known as the WPP. Since its implementation, this program has promoted actions (the implementation of soil management techniques, fencing of water springs, floristic recomposition of degraded springs and others) that target the maintenance of the springs (PPAs) of water courses located in this agrosystem and that contribute to the public supply of water to the city of Rio Verde. Despite the socioeconomic importance of the HMRA and also the actions already implemented under the WPP, there has been a knowledge gap about the species of fauna and flora that occur in this area. Thus, the present characterization, in addition to filling this gap, has the potential to contribute to future management actions.

The municipality of Rio Verde, a national pillar of grain production, has experienced an intense process of anthropization in recent decades, resulting in the conversion of many natural habitats to agricultural environments (Siqueira & Faria 2019). This transformation has been due to the expansion of agricultural activities, which currently occupy about 77% of this municipality's territory (Siqueira & Faria 2019). This pattern is repeated at the local scale, as our results showed a predominance of anthropic environments in the HMRA. Only 21.32% of our study area is represented by native vegetation remnants, which corresponds to the permanent preservation areas (PPAs) and legal reserves (LRs) of the rural properties located in the HMRA. In Brazil, the Native Vegetation Protection Law (No. 12651/2012) provides that deforested PPAs occupied with agroforestry activities through July 2008 are considered to be consolidated and, therefore, the owners of such areas are not required to carry out their full restoration. Considering this scenario, it is noteworthy that part of the PPAs inserted in the HMRA and participants of the WPP would be in this consolidated situation, which would release them from the obligation of their full recovery. Despite this possibility, many of these PPAs have been targeted by WPP interventions since 2011, which has ultimately contributed to their restoration.

Arroyo-Rodríguez et al. (2020) state that, in terms of biodiversity conservation and maintenance of ecosystem services, an optimal landscape should contain about 40% of vegetation cover. These authors also argue that in these landscapes, 10% of native vegetation should be concentrated in one or a few unique large fragments, and the remaining 30% should be distributed in a number of small fragments throughout the landscape. In the HMRA, we observed that the remnants of native vegetation are distributed in dozens of fragments (or patches) of varying sizes and within approximately 100 meters of each other. This is important information, based on the theoretical framework proposed by Arroyo-Rodríguez et al. (2020), since understanding the distribution pattern of native vegetation within the HMRA may contribute to the process of decision-making. This enables the proposition of more refined management actions focused on specific areas within the microbasin and this may intensify the returns obtained through the WPP.

Knowing the species that occur in a given area is essential (Bruner et al. 2001), as this allows for more elaborated ecological studies and also for the proposal of more specific management actions. When considering different taxonomic groups, we recorded a considerable number of species of fauna and flora occurring in the HMRA, some of which are endemic to the Cerrado, threatened by extinction, exotic invasive species, or relevant ecological and economic potential. For instance, management actions based on the national action plans (PANs) can be implemented so that the population of these threatened species can be viable in the long term. This implies that educating the local population can help avoid overexploitation of these species and maintain natural habitats suitable for their persistence. On the other hand, management actions must be taken to reduce or remove populations of exotic invasive species (boars - *Sus scrofa*) observed in the HMRA.

In the Cerrado, protected areas have been shown to be effective in maintaining a high diversity of species when compared to sites which are not legally protected (e.g., Oliveira et al. 2019). This pattern was observed in the present study, since the species diversity of fauna and flora found in the HMRA is lower than the average number of species found in the protected areas of the Cerrado. One explanation for this pattern is that, when compared to the HMRA, the Cerrado protected areas have a greater amount of native vegetation, especially those of integral protection (Françoso et al. 2015), which enables the long-term persistence of native species. Unlike the protected areas, the region in which the HMRA is inserted has experienced a long history of habitat changes, which can decrease the viability of native species populations. Nevertheless, the occurrence of species with specific ecological requirements (e.g. tapir - *Tapirus terrestris*) in the HMRA can be indicative that interventions carried out under the WPP have positively impacted the local biodiversity.

To our knowledge, this is the first multi-taxon inventory carried out on an agrosystem that is a beneficiary of an important payment program for ecosystem services of water supply. These results represent the first step for conservation and management actions to be properly implemented in this area. In this sense, focusing on future planning, as well as assessing the impacts of WPP, we suggest the establishment of a long-term monitoring program that considers the landscape dynamics and also the species of fauna and flora present in the HMRA.

Supplementary Material

The following online material is available for this article:

Supplementary Material 1 - List of studies found through the scientometrics search.

Supplementary Material 2 - Species list (Amphibians, Birds, Mammals, Fishes, Woody Plants) registered in the hydrographic microbasin of Ribeirão Abóbora, municipality of Rio Verde, southwestern of Goiás State, Brazil.

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Associate Editor

Carlos Joly

Author Contributions

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

Roniel Freitas-Oliveira: Contribution to data collection, contribution to data analysis and interpretation, contribution to manuscript preparation and contribution to critical revision, adding intellectual content.

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Conflicts of Interest

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

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Inventory

Fishes of the Mitú Region: middle basin of the río Vaupés, Colombian Amazon

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Abstract: The Amazon River basin hosts the most diverse freshwater ichthyofauna in the world, and yet huge areas of the basin remain unexplored. This is the case for the upper tributaries of the rio Negro, especially those draining the Colombian territory. Here we present a list of 224 species derived from the examination of specimens collected in the Mitú region (Vaupés Department, Colombia), the middle basin of the río Vaupés. Of the species identified in our study, 10 species are recorded from Colombia for the first time, and 26 species are newly recorded from the Colombian Amazon. The number of species we present here comprise almost one-third of the known species diversity of the Colombian Amazon and nearly a tenth of the total number of those known across the entirety of the Amazon basin. The most diverse orders were Characiformes (120 species) and Siluriformes (65 species), and the remaining six orders comprised less than 20% of total species. The study area comprised blackwater systems, which are considered to be nutrient-poor environments. We discuss some ecological aspects that might explain how this highly diverse ichthyofauna originates and is maintain in less productive systems. The list presented here adds an important number of new records and complements the information derived from previous studies, carried out thus far with regards to the fish fauna of the Colombian Amazon.

Keywords: *Distribution; Neotropical ichthyology; species diversity; upper rio Negro basin.*

Peces de la región de Mitú: cuenca media del río Vaupés, Amazonia colombiana

Resumo: La cuenca del río Amazonas alberga la ictiofauna dulceacuícola más diversa del mundo, sin embargo, grandes áreas de la cuenca permanecen inexploradas. Este es el caso de los afluentes de la parte alta del río Negro, especialmente los sistemas que drenan el territorio colombiano. A continuación, presentamos un listado de 224 especies derivadas del análisis de especímenes recolectados en la región de Mitú, cuenca media del río Vaupés (Departamento de Vaupés, Colombia). De las especies identificadas, 10 especies se registran en Colombia por primera vez y 26 especies para la Amazonía colombiana. El número de especies que presentamos aquí comprende casi un tercio de las especies conocidas para la Amazonía colombiana y casi una décima parte del total de las conocidas para la gran cuenca del Amazonas. Los órdenes más diversos fueron Characiformes (120 especies) y Siluriformes (65 especies), y los seis órdenes restantes comprendieron menos del 20% del total de especies. El área de estudio comprende sistemas de aguas negras que se consideran ambientes poco productivos por sus bajos contenidos de nutrientes. Discutimos aquí algunos aspectos ecológicos que podrían explicar cómo esta ictiofauna tan diversa tiene su origen y es mantenida en estos sistemas poco productivos. La información derivada del presente estudio adiciona nuevos registros de especies de peces para Colombia, y complementa la información derivada de los estudios realizados a la fecha en la Amazonía colombiana.

Palavras-chave: *Distribución de especies; diversidad de especies; cuenca alta del río Negroictiología Neotropical.*

Introduction

The río Vaupés is one of the main affluents of the río Negro in the Amazon Basin in Colombia (IDEAM 2004, Latrubesse & Franzinelli 2005). Its headwaters are located at the Department of Guaviare in the Vega of Caquetá. An area named by Hamilton Rice (1910), where he assumed as the commencement of the Amazon forest and where the río Guaviare and río Inírida also originate. Even though the upper reaches of the Vaupés system are located in the Vega de Caquetá, it is termed as the río Vaupés downstream of the confluence of the río Unilla and the río Itilla, at 300 MASL (IGAC 1999), NW of the Miraflores Municipality. The río Vaupés drains from west to east, through the southeastern territory of the Guaviare department and the central region of the Vaupés Department, extending to the confluence with the río Papurí, at the border between Colombia and Brazil, where it becomes the rio Uaupés (Figure 1).

The río Vaupés forms a meandric system with high sediment load at its upper reaches. Downstream, it becomes less meandric and water properties turn out to typically Amazonian blackwaters (Hamilton Rice 1910). Blackwater systems drain *terra firme* forests and savannas. Electrolyte sequestering by the root-mycorrhiza of the forest and the highly leached soils of the catchment area, account for the low turbidity and nutrient levels. Because these systems are typically free of sediment and the high contents of humic compounds, they are darkly stained (Sioli 1984a, Leenheer 1980).

As a typical Amazonian system, it exhibits a flood pulse, such as the adjacent forest remains flooded during high water periods – which in this case lasts up to five months of the year. This flood regime is regulated by the pluviosity conditions of the regional watershed, as well as local rainy events. The seasonal cycle consists of four well-recognized hydrological periods: high waters, low waters, and two transition periods of rising and falling waters (IGAC 1996, 1999).

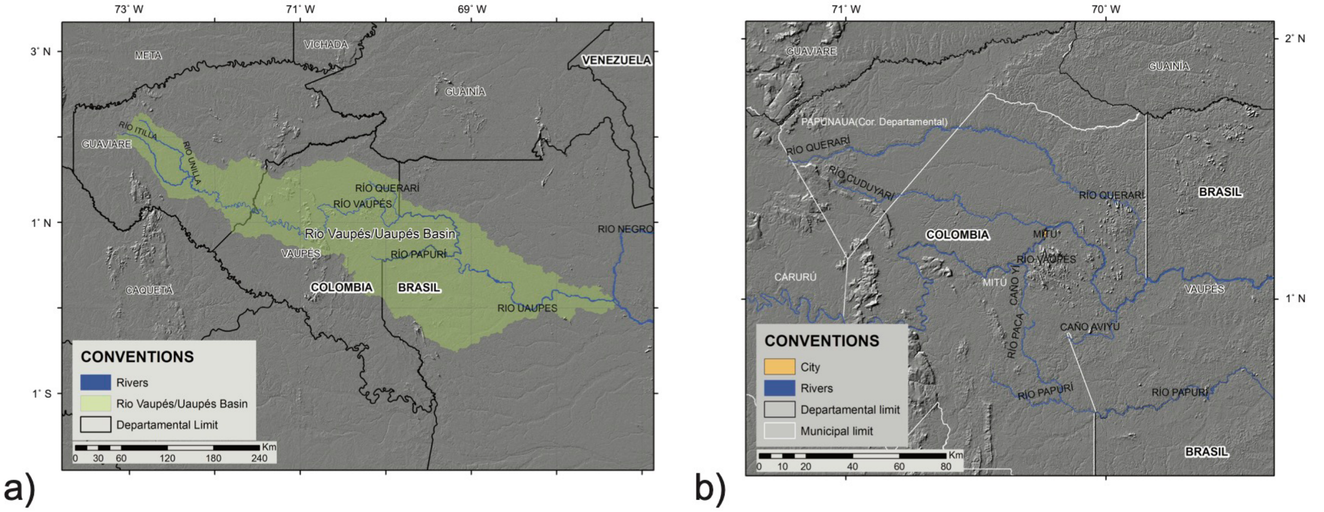

Figure 1. a) Río Vaupés/Uaupes Basin. Red square = study area. b) Study area: Mitú Region. Dots = collecting sites.

The area contains well-preserved forested areas, with 3 main floristic types: flooded forests, white-sand forests, and terra firme forests (Rudas Lleras 2009). Those three floristic types are found along the margin of the aquatic systems. The main river channel has a strong influence on its smaller tributaries, due to transportation of higher volumes of water, 8.200 m^3/s (IGAC 1999). Streams are characterized by a stained brown color, with sandy and rocky substrates, which may present considerable areas of beach during the low water season.

Studies have reported lower fish species richness in blackwater systems compared to nutrient rich environments (Henderson & Crampton 1997, Bogotá-Gregory, Lima, et al. 2020). Nevertheless, studies have shown that they support relatively diverse ichthyofaunas composed mainly of small fishes adapted to survive in less productive habitats (Goulding et al. 1988, Arbeláez, Duivenvoorden, Maldonado-Ocampo, et al. 2008, Arbelaez et al. 2004, Mojica et al. 2009, Machado-Allison et al. 2013, Antonio & Lasso 2003, Machado-Allison et al. 2003). Fish community comparisons (Saint-Paul et al. 2000) support the notion of "rich life in poor water" as originally described by Goulding et al. (1988), which maintains that the paradoxically high fish species richness of tropical oligotrophic waters is possible because the energy sustaining fish populations is derived primarily from allochthonous forest inputs, rather than *in situ* autochthonous productivity. Studies of terrestrial-aquatic trophic linkages in blackwater rivers have subsequently confirmed that allochthonous inputs from flooded forests are the dominant energy source for fish in blackwater rivers (Correa & Winemiller 2018).

Consistent with the rich life in poor water hypothesis (Goulding et al. 1988), we report high species diversity, with a list of 224 fish species from the Mitú Region, an area in which the freshwater fish diversity was previously underestimated. Previous fish reports are mostly based on reviews that have attempted to compile available information for the río Vaupes (e.g. Mojica 1999; Bogotá-Gregory and Maldonado-Ocampo 2006). These studies have reported fewer than 30 fish species for the whole río Vaupés basin in the Colombian territory. The relatively small number of reports from the basin only reflects the paucity of scientific research studies pertaining to fishes in the region. The most likely explanation for why more complete species inventories has yet to be undertaken in the region is due to its geographical location. Access to the area is logistically demanding for a number of reasons, and further limited for social reasons, given that a history of armed conflict has hampered biological surveys in general. Nevertheless, because of recent improvements in the social context of the region, it is now relatively accessible for biological surveys, and more complete species inventories in the region can be carried out.

Despite the recognition of the Amazon River basin as the region with the highest freshwater fish diversity, there are huge areas that remain unexplored. One such area is the hydrographic network that drains the soils of the Mitú Region, an Amazonian system that was previously poorly explored and may be characterized by specialized ichthyofaunas. Our results are part of an inter-institutional initiative of the Instituto Amazónico de Investigaciones Científicas-SINCHI, the environmental corporation CDA, the Universidad del Tolima-UT, and WWF-Colombia, with the intention to fill major gaps in our knowledge of remote areas of the Amazon Basin. The aim of this initiative is to contribute to national species inventory (DoNascimiento et al. 2017, Maldonado-Ocampo et al. 2008) and strengthen the scientific reference collections with the aid of local communities.

Materials and Methods

The study area is located near to the Mitú municipality, department of Vaupés, Colombia, along the main channel of the río Vaupés, río Papuri, río Cuduyari, río Paca, and the Mituceño, and Yi streams (Figure 1). The area is a typical equatorial zone, where daily temperature range between and 18 – 34˚C. The area presents a unimodal precipitation regime, that is to say, the highest precipitation values are registered between June and July, followed by a transition period where rainfall decrease in August and September, and the period with the lowest precipitation values falls between December and March (IGAC 1996).

To document species composition in the study area and not for comparative purposes, a series of sampling events were conducted between 2012 and 2019, during both dry and rainy seasons. Fishes were collected with conventional fishing gear (*i.e.* gill-nets, beach seines, and dipnets). Once fishes were captured, they were euthanized with 600 mg L^{-1} eugenol, fixed with 10% formaldehyde solution and later preserved in 70% ethanol solution. Specimen vouchers are deposited in the ichthyological collections of the Universidad del Tolima (CZUT-IC) and SINCHI institute (CIACOL). The taxonomic list herein, follows the classification adopted in Fricke et al. (2020) for orders and families and genera and species are listed alphabetically. Validity of the species identified were confirmed also with Fricke et al. (2020). Usma et al. (2009) was used to determine species that present relatively long-distance migratory longitudinal movements.

Acronyms of fish collection where specimens are deposited are provided for future revisions. Data from CIACOL will be available at the Catalogue of Species of the Colombia Biodiversity Information System (http://www.biodiversidad.co/). Currently, CIACOL data is hosted in the online collection's catalogue (https://sinchi.org.co/ciacol) and those of CZUT-IC are available at: https://doi.org/10.15472/bhprvq

We classified species based on standard length (SL) into four size classes; miniature species, less than 2.6 cm of SL; small species, over 2.6 and less than 15 cm of SL; medium species, between 15 and 45; and large species, with over 45 cm of SL. This simple scheme for size classification is a unification of Weitzman and Vari (1988), Castro (1999), and Castro et al. (2005), which allowed us to calculate percentage of miniature and small body species that represent the overall species composition in our study area. Body sizes were obtained with Reis et al. (2003), original species descriptions, and direct measurements in the case of undetermined species. To calculate the proportion of species exclusive to blackwaters we defined species affiliations to water type (i.e. whitewater, clearwater, and blackwater) sensu Sioli (1984b). Designation of the species to a given water type was based on empirical evidence.

Results

A total of 224 species belonging to eight taxonomic orders, 37 families, and 123 genera (Table 1) were recognized for the Mitú Region. The orders with most families, genera, and species were Characiformes (17 families, 52 genera, and 120 species) and Siluriformes (10 families, 46 genera, and 65 species) (Figure 2a). The remaining five orders account less than 20% of the total genera and species identified. At the family level, Characidae and Cichlidae were the richest families, with 45 and 13 species, respectively (Figure 2b).

Of the species reported here (Table 1), 10 are new records for Colombia and 26 constitute new records for the Colombian Amazon River basin (DoNascimiento et al. 2017). Most of the species that are recorded for the Colombian Amazon for the first time (over the 10 % of total species reported herein), were previously recorded for other sections of the rio Negro in Brazil. None of the listed species are categorized as threatened (Mojica et al. 2012) or considered exotic or invasive (see natural distributions in Reis et al. 2003 and Fricke et al. 2020). 15 of the species are classified as migratory (Usma et al. 2009).We included in the list six undetermined species of *Odonthocharacidium*, *Tetragonopterus*, *Tyttocharax*, *Ituglanis, Myoglanis*, *Nemuroglanis*, and *Aequidens*. Unequivocal identification for these species could not be reached and further studies are required to review their specific identities. Moreover, we anticipate that some of these species may eventually be recognized as undescribed.

The percentage of species unique to blackwater systems greatly exceed those that are present in two types of water of the ones present in all three blackwater, clearwater, and whitewater systems (Figure 3a). According to the criterion defined herein for designation of size classes, the proportion of miniature and small body size species greatly exceeded that of medium and large size species (Figure 3b).

Discussion

Overall, the species composition found in the middle basin of the río Vaupés (Mitú region), with taxonomic dominance of Characiformes and Siluriformes, agrees with the general pattern documented for the ichthyofaunas of the basin in the Brazilian territory (Beltrão et al. 2019) and other basins of the Neotropical Region (Maldonado-ocampo et al. 2006, Ortega et al. 2006, Ortega-lara et al. 2012, Bogotá-Gregory, Lima, et al. 2020).

The list presented here adds 26 new species records for the Colombian Amazon. Among the new records, *Melanocharacidium dispilomma* Buckup 1993 was indeed reported in Bogotá-Gregory & Maldonado-Ocampo (2006). Nevertheless, this report was based on specimens collected in Brazil, near the border of Colombia [Buckup, 1993: MNRJ 5934, río Tiquié (trib. of río Vaupés)]. Therefore, this record was dismissed in DoNascimiento *et al*. (2017) by not coming from the Colombian Amazon. The specimens referenced herein are actually the first record of the species in Colombian territory. *Copella compta* (Myers 1927) was included in Galvis *et al*. (2007). Nevertheless, the specimens referenced in that paper are misidentifications and belong to a species with a more conspicuous lateral band [see comments and details on diagnostic characters in Zarske & Géry (2006)].

Table 1. List of species. New records: Col = Colombia, Amz = Amazon basin. System: cud = río Cuduyarí, gav = Caño Gavilán, mic = Caño Mico, mit = Caño Mituseño, pac = río Paca, pap = río Papurí, tuc = Tucundira, vau = río Vaupes, yi = Caño Yi. CZUT-IC = Colección Zoológica, Universidad del Tolima. CIACOL = Colección Ictiológica de la Amazonía Colombiana. Commercial species: orna = ornamental, cons = consumption. BW = balckwater, CW = clearwater, and WW = whitewater.

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	**CIACOL**		**BW**	**CW**	**WW**			**Col**	**Amz**	**Mojica 1991**	**Bogotá-Gregory & Maldonado 2006**	**Galvis et al. 2007**	**Bogotá-Gregory et al. 2020**
Order Characiformes															
Family Crenuchidae															
Characidium crandellii Steindachner 1915	pac, vau		708, 961, 962, 963, 983	small	x	x	x								x
Characidium longum Taphorn, Montaña & Buckup 2006	vau		706	small	x	x					x				
Characidium pellucidum Eigenmann 1909	cud, tuc	4424, 4474, 4530, 4867, 4872	707, 709, 3051, 3052, 4359	small	x	x			orna						
Characidium zebra Eigenmann 1909	pap	3607	958-960	small	x	x	x		orna						
Elachocharax pulcher Myers 1927	cud	4889		miniature	x	x								x	
Melanocharacidium dispilomma Buckup 1993	pap	3608		small	x	x			orna		x		x		
Melanocharacidium pectorale Buckup 1993	cud, pap	3593, 4908, 12284		small	x										
Odontocharacidium sp.	cud	4534, 4552		miniature	x										
Poecilocharax weitzmani Géry 1965	cud, vau	4123, 4838	2377, 3104, 3111, 3112, 3822, 3823-3825, 4342	small	x				orna						
Family Erythrinidae															
Erythrinus erythrinus (Bloch & Schneider 1801)	yi		732	medium	x	x									
Hoplerythrinus unitaeniatus (Spix & Agassiz 1829)	pap, vau, yi	3603	731, 829, 1167, 3018, 3752, 3753, 3754, 3788	medium	x	x	x		cons, orna			x	x	x	
Hoplias malabaricus (Bloch 1794)	cud, pac, pap, vau, yi	3587, 4521, 4904, 12296	726-730, 826, 975-982, 1155, 1302, 2338, 2360, 3017, 3794, 4340, 4349, 4353, 4360	large	x	x	x		cons, orna					x	
Family Cynodontidae															

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Hydrolycus wallacei Toledo-Piza, Menezes & Santos 1999	cud, mit		695, 696, 824, 828, 1169	medium	x			Yes	cons					x	
Family Serrasalmidae															
Metynnis hypsauchen (Müller & Troschel 1844)	cud	4856		small	x	x	x	Yes	orna					x	
Myloplus asterias (Müller & Troschel 1844)	vau		1173	medium	x	x									
Myloplus rubripinnis (Müller & Troschel 1844)	cud, vau	3495, 4942	1459	medium	x	x		Yes	cons, orna						
Serrasalmus gouldingi Fink & Machado-Alison 1992	pac		974	medium	x	x									
Serrasalmus manueli (Fernández-Yépez & Ramírez 1967)	pac		973	medium	x	x									
Serrasalmus rhombeus (Linnaeus 1766)	vau	4286		medium	x	x	x		cons						
Family Hemiodontidae															
Argonectes longiceps (Kner 1858)	cud		739	medium	x										
Bivibranchia fowleri (Steindachner 1908)	cud	3545, 4391		small	x	x			orna					x	
Hemiodus gracilis Günther 1864	vau		965, 967, 968, 2352, 4328	medium	x	x		Yes	orna						
Hemiodus immaculatus Kner 1858	cud	4498		medium	x				orna					x	
Hemiodus semitaeniatus Kner 1858	cud, mit, vau	4506, 4512, 4517, 4944	738, 741, 742	medium	x	x	x		orna					x	
Hemiodus thayeria Böhlke 1955	cud, mit, vau, yi	3501, 3537, 4381, 4511, 4555, 4911	964, 966, 1079, 1080, 2353, 2354	small	x									x	
Hemiodus unimaculatus (Bloch 1794)	cud	4401		medium	x	x	x		orna						
Family Anostomidae															
Anostomoides atrianalis Pellegrin 1909	vau		821	small	x	x	x								
Anostomus ternetzi Fernández-Yépez 1949	cud	4836		small	x		x		orna		x				
Gnathodolus bidens Myers 1927	vau	12278		small	x	x					x				

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Laemolyta garmani (Borodin 1931)	vau		954, 955, 1166	medium	x	x	x	Yes	orna						
Laemolyta taeniata (Kner 1858)	cud		743, 744, 745	medium	x	x	x		orna						
Leporinus agassizi Steindachner 1876	vau		1149	medium	x		x							x	
Leporinus aripuanaensis Garavello & Santos 1981	pap	3594		small	x	x									
Leporinus brunneus Myers 1950	cud, vau, yi	3493	746, 823	medium	x	x	x							x	
Leporinus fasciatus (Bloch 1794)	vau	3479, 3480, 4402, 12269	1156	medium	x	x	x	Yes	cons, orna						
Leporinus friderici (Bloch 1794)	cud, pap, vau, yi	3491, 3647, 4061	748, 749, 1154, 3789	medium	x	x	x	Yes	cons, orna					x	
Leporinus klausewitzi Géry 1960	cud, pac, vau	4440, 4489, 4493, 4851, 12289, 12292	956	medium	x		x							x	
Synaptolaemus latofasciatus (Steindachner 1910)	vau	4092, 12279		medium	x	x									
Family Chilodontidae															
Caenotropus mestomorgmatos Vari, Castro & Raredon 1995	vau	3492	724, 725	medium	x				orna						
Chilodus punctatus Müller & Troschel 1844	cud	4515, 4846		small	x	x	x		orna						
Family Curimatidae															
Curimatella immaculata (Fernández-Yépez 1948)	vau		659, 720, 1506, 1507, 1509	small	x	x	x								
Curimatopsis evelynae Géry 1964	cud	4913		small	x		x		orna		x			x	
Curimatopsis macrolepis (Steindachner 1876)	cud, mit	4477		small	x		x								
Cyphocharax festivus Vari 1992	cud, mit, vau	4861, 4870, 4917, 4945	711, 721, 1494	small	x	x	x								
Cyphocharax leucostictus (Eigenmann & Eigenmann 1889)	vau		948-950	small	x		x							x	

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Cyphocharax multilineatus (Myers 1927)	cud, pac, pap, mit, vau, yi	3562, 3598, 4412	714-718, 937-946, 1492, 3772	small	x				orna					x	
Cyphocharax spiluropsis (Eigenmann & Eigenmann 1889)	pap	3599	713, 951, 952, 1089	small	x		x								
Cyphocharax spilurus (Günther 1864)	cud, pac	4505, 4919	647	small	x	x	x		orna						
Steindachnerina guentheri (Eigenmann & Eigenmann 1889)	cud	4529		small	x		x								
Family Lebiasinidae															
Copella compta (Myers 1927)	pap	7881	2415, 3019-3024, 3779	small	x				orna	x				x	
Copella eigenmanni (Regan 1912)	cud, vau	4542, 4854, 4932		small	x	x	x		orna						
Copella nattereri (Steindachner 1876)	vau, yi		993-996, 1081, 3778, 3780, 3781	small	x		x		orna						
Nannostomus eques Steindachner 1876	pac		998	small	x	x			orna						
Nannostomus marginatus Eigenmann 1909	cud, pac, vau	3496, 3529, 3583, 4096, 4492, 4547	997, 4337	small	x	x			orna						
Nannostomus trifasciatus Steindachner 1876	vau		2339, 3025-3027, 3787	small	x	x									
Pyrrhulina laeta (Cope 1872)	pap	3588		small	x				orna						
Family Ctenuluciidae															
Boulengerella cuvieri (Spix & Agassiz 1829)	vau		755, 992, 1144, 2022	large	x	x	x								
Boulengerella lucius (Cuvier 1816)	vau		753	medium	x	x	x				x				
Boulengerella maculata (Valenciennes 1850)	cud, vau	4420, 4833, 4898	991	medium	x	x	x		orna						
Family Chalceidae															
Chalceus macrolepidotus Cuvier 1818	cud	4482	733, 734, 1151	medium	x	x		Yes	orna				x	x	
Family Triportheidae															
Triportheus albus Cope 1872	cud, vau		650, 735, 736, 830	small	x	x	x	Yes						x	
Family Gasteropelecidae															
Carnegiella marthae Myers 1927	vau	4076		small	x				orna						

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Carnegiella strigata (Günther 1864)	cud, vau, yi	3586	704, 705, 957, 4332	small	x	x			orna						
Family Bryconidae															
Brycon pesu Müller & Troschel 1845	cud, vau	3538, 4375, 4428, 4933	657, 2057	medium	x	x	x	Yes	orna					x	
Family Iguanodectidae															
Bryconops alburnoides Kner 1858.	vau	4119		small	x	x	x								
Bryconops caudomaculatus (Günther 1864)	pac, pap, vau, yi	12295, 12316	686, 1058, 1076, 3090-3093, 3795, 3827	small	x	x	x		orna						
Bryconops giacopinii (Fernández-Yépez 1950)	cud, pac, vau, yi	4899, 4914, 4915, 4837	681, 702, 1060, 1064, 1066-1071, 1085, 1333, 2021, 2026, 2345-2347, 2349, 2350, 3089, 3094, 3095, 3451, 3456, 3830, 3831, 3834	small	x	x			orna						
Bryconops humeralis Machado-Allison, Chernoff & Buckup 1996	cud, vau	3531, 3539, 3551, 3557, 4847	1062, 1063, 2056, 2340	small	x						x				
Bryconops inpai Knöppel, Junk & Géry 1968	yi		1061, 1072, 1078, 2031, 3796, 3828, 3835	small	x									x	
Iguanodectes purusii (Steindachner 1908)	cud, vau		653, 658, 1056	small	x	x									
Iguanodectes spilurus (Günther 1864)	cud	4538, 4855		small	x	x	x		orna						
Family Acestrorhynchidae															
Acestrorhynchus falcatus (Bloch 1794)	cud, pac, pap, vau	3613, 4322, 4484, 4496, 4844, 12293	661, 665, 666, 668, 987, 988, 989, 990, 1305, 4358	medium	x	x									
Acestrorhynchus falcirostris (Cuvier 1819)	cud, mit, vau	4941	667, 751, 752, 827, 1170	medium	x	x								x	
Acestrorhynchus microlepis (Jardine 1841)	pac	12294	984, 985, 986	medium	x	x	x		orna						
Acestrorhynchus nasutus Eigenmann 1912	cud		662	small	x						x				
Family Characidae															

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Acestrocephalus sardina (Fowler 1913)	vau	12313	697, 1039	small	x	x									
Astyanax anterior Eigenmann 1908	vau, yi	4415	699, 1019, 1021, 1026, 1073, 2369, 2799, 3829, 3832, 3833	small	x	x									
Bryconamericus orinocoensis Román-Valencia 2003	cud	4916, 4949		miniature	x	x									
Charax delimai Menezes & Lucena 2014	cud, vau, yi	4852	651, 700, 1035, 1036, 2375, 3797	small	x					x			x		
Charax pauciradiatus (Günther 1864)	mit		1037, 1413, 2059	small	x					x					
Creagrutus maxillaris (Myers 1927)	pap	3601		small	x	x									
Hemigrammus analis Durbin 1909	cud, vau	3523, 3528, 3543, 3552, 3559, 3566, 3574, 3582, 4175, 4383, 4959, 4920, 4959, 18076	675, 1049, 2378	small	x	x									
Hemigrammus bellottii (Steindachner 1882)	cud, vau	3526, 3534, 3573, 3581, 3650, 4361, 4439, 4843	673, 1011, 2365, 2465, 2469, 2470	miniature	x	x									
Hemigrammus luelingi Géry 1964	mit, pac, vau, yi	4207, 4308	671, 674, 1031, 1042, 1655, 2459	miniature	x	x									
Hemigrammus microstomus Durbin 1918	cud	3579, 3617, 4399, 4553, 4900	676, 2814, 2872, 3444	small	x	x	x								
Hemigrammus newboldi (Fernández-Yépez 1949)	vau		2785	small	x		x								
Hemigrammus ocellifer (Steindachner 1882)	vau	3536		small	x				orna						
Hemigrammus orthus Durbin 1909	tuc		672, 1047, 1048, 1050, 1053, 1657, 3099, 3100, 3105	small	x					x					

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Hemigrammus schmardae (Steindachner 1882)	cud, vau	3499, 4419, 4840, 4845, 4866, 4869, 4957, 12282	1045, 1046	small	x	x									
Hemigrammus vorderwinkleri Géry 1963	cud	4896		small	x	x			orna						
Hemigrammus yinyang Lima & Sousa 2009	cud	4323, 4533, 4857, 4903	2879	small	x										
Hyphessobrycon agulha Fowler 1913	vau	3618, 4540, 4848	2366, 2372, 3097	small	x	x			orna						
Hyphessobrycon bentosi Durbin 1908	cud	4427		small	x	x			orna						
Hyphessobrycon copelandi Durbin 1908	cud, vau	3535, 3576, 4432, 4510, 4859	710, 1040, 1088	small	x	x	x		orna						
Hyphessobrycon dorsalis Zarske, 2014	cud		2781	small	x						x				
Jupiaba abramoides (Eigenmann 1909)	mit		3050	small	x	x					x				
Jupiaba anteroides (Géry 1965)	pap	3596, 12314	698, 3455	small	x	x							x		
Jupiaba poekotero Zanata & Lima 2005	cud, pap, vau	3542, 3572, 3611	1057	small	x								x		
Jupiaba zonata (Eigenmann 1908)	vau	4405	1024, 1055, 2809	small	x	x									
Knodus heteresthes (Eigenmann 1908)	vau	4151, 4200, 12308		small	x	x							x		
Knodus tiquiensis Ferreira & Lima 2006	pac	12307	999, 1001, 1003, 1004, 1029	small	x	x									
Makunaima guianensis (Eigenmann 1909)	vau		1041, 3762-3765	small	x	x									
Microschemobrycon callops Böhlke 1953	pac		1000	small	x						x				
Microschemobrycon casiquiare Böhlke 1953	pac, vau	4404, 4936	1002, 2357	small	x	x								x	
Microschemobrycon geisleri Géry 1973	cud	3505, 4337, 4341, 4426, 4431, 4868		small	x	x									
Moenkhausia ceros Eigenmann 1908	cud, vau	4509, 4907		small	x	x					x				

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Moenkhausia collettii (Steindachner 1882)	cud, pac, pap, vau	3503, 3610, 4199, 4860, 4901, 4906, 18075	684, 1005-1010, 1030, 1083, 1276, 2367	small	x	x			orna						
Moenkhausia comma Eigenmann 1908	yi		693, 703, 2364, 3056	small	x	x			orna						
Moenkhausia cotinho Eigenmann 1908	cud, mit, vau	3554, 3564, 3648, 4194,, 18074	679, 680, 1012, 1013, 1681, 2040	small	x	x	x	Yes							
Moenkhausia diktyota Lima & Toledo-Piza 2001	vau, yi		1022, 1023, 1087, 2370, 2414, 3053, 3054, 3057	small	x										x
Moenkhausia grandisquamis (Müller & Troschel 1845)	vau		1028	small	x	x	x								
Moenkhausia lata Eigenmann 1908	mit		690	small	x	x	x								x
Moenkhausia lepidura (Kner 1858)	mit, pac, pap, vau	3568, 3605, 4961, 5383, 18078	685, 688, 689, 694, 1065, 1075, 1077, 1686, 3447, 3449, 3450	small	x	x	x	Yes	orna					x	
Moenkhausia mikia Marinho & Langeani 2010	cud, pap, vau	3498, 3555, 3584, 4910, 4918, 8069	1084, 2368	small	x										
Moenkhausia oligolepis (Günther 1864)	cud, pac, pap, vau	3500, 3502, 3504, 3561, 3595, 4386, 4409, 4514, 4539, 4865	663, 664, 678, 1014-1017, 2028, 2052, 2054, 2355, 2802, 2884, 3820, 3821	small	x	x	x		orna						
Petitella bleheri (Géry & Mahnert 1986)	vau	4174		small	x						x				
Phenacogaster pectinatus (Cope 1870)	cud, pap, vau	3506, 3532, 3602, 4481, 4548, 4864		small	x	x	x								
Tetragonopterus chalceus Spix & Agassiz 1829	vau, yi	12315	649, 660, 1018	small	x	x			orna				x		
Tetragonopterus sp.	vau	4177		small	x										
Tyttocharax sp.	mic		3114	small	x										
Order Gymnotiformes															

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Family Apteronotidae															
Apteronotus albifrons (Linnaeus 1766)	pac	12323	882, 1386	large	x	x	x								
Family Sternopygidae															
Distocyclus conirostris (Eigenmann & Allen 1942)	vau	12290		large	x	x	x								
Eigenmannia macrops (Boulenger 1897)	pac, vau		879-881	medium	x		x				x				
Eigenmannia sp.	pap	3635	773-777, 877	medium	x				orna						
Rhabdolichops eastwardi Lundberg & Mago-Leccia 1986	pap	3642, 3987		medium	x		x								
Sternopygus macrurus (Bloch & Schneider 1801)	cud, vau, yi	4550, 4948, 12288, 12324	781, 784, 883, 1146, 1384, 1385, 2669, 2671, 2672	large	x	x	x		cons, orna					x	
Sternopygus obtusirostris Steindachner 1881	vau		2670	large	x		x			x					
Family Gymnotidae															
Gymnotus anguillaris Hoedeman 1962	mit, vau		2473, 2520, 2521, 2538, 2576, 2578, 2580, 2581, 2583, 2590-2593, 2597	medium	x		x				x				
Gymnotus carapo Linnaeus 1758	cei, cud, mit, vau, yi	4141	782, 785, 884, 885, 2484, 2517, 2572, 2587, 2588, 2594	medium	x	x	x		orna				x	x	
Gymnotus coropinae Hoedeman 1962	vau		2499, 2501-2504, 2615	medium	x										
Gymnotus javari Albert, Crampton & Hagedorn 2003	pap	3636		medium	x		x		orna						
Gymnotus tiquie Maxime, Lima & Albert 2011	mit		2498	medium	x										x
Family Hypopomidae															
Brachyhypopomus batesi Crampton, de Santana, Waddell & Lovejoy 2016	gav, vau		2474-2477, 2486-2493, 2495, 2500, 2532, 2605, 2608, 2610, 2611, 2612, 2613, 2614	medium	x										

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	**CIACOL**		**BW**	**CW**	**WW**			**Col**	**Amz**	**Mojica 1991**	**Bogotá-Gregory & Maldonado 2006**	**Galvis et al. 2007**	**Bogotá-Gregory et al. 2020**
Brachyhypopomus beebei (Schultz 1944)	gav		2514, 2515, 2546, 2547, 2556-2558, 2567, 2569, 2571, 2574, 2579, 2584-2586, 2589, 2598, 2601-2604, 2606, 2607, 2609, 2652, 2653	medium	x	x	x								
Brachyhypopomus hamiltoni Crampton, de Santana, Waddell & Lovejoy 2017	gav, mit		2480, 2668	medium	x		x			x					
Microsternarchus aff. bilineatus Fernández-Yépez 1968	cei, mit		2485, 2505, 2508-2510, 2518, 2530, 2531, 2533, 2537, 2539, 2541, 2543, 2544, 2548, 2550, 2553, 2562-2566, 2655, 2656, 2880, 3761	small	x						x				
Family Rhamphichthyidae															
Gymnorhamphichthys hypostomus Ellis 1912	pap	3637		medium	x		x								
Gymnorhamphichthys rondoni (Miranda Ribeiro 1920)	mic, yi		779, 2551, 2552, 2595, 2596, 2599, 2600, 3816, 4329	medium	x	x	x								
Hypopygus lepturus Hoedeman 1962	cud, pac, vau	4147, 4429, 4513, 4946	875, 876, 2478, 2481-2483, 2494, 2516, 2524-2528, 2559, 2560, 2568, 2570, 2573, 2616-2651, 2654	small	x	x	x		orna						
Rhamphichthys rostratus (Linnaeus 1766)	vau		783, 2673-2676	large	x		x		orna						
Steatogenys elegans (Steindachner 1880)	yi		778	medium	x	x	x		orna						
Order Siluriformes															
Family Trichomycteridae															
Ituglanis sp.	mit		855	small	x										

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Ituglanis metae (Eigenmann 1917)	pap	3638		small	x						x				
Ochmacanthus reinhardtii (Steindachner 1882)	vau	4414		small	x		x								
Paracanthopoma parva Giltay 1935	vau		2029	small	x		x								
Stauroglanis gouldingi de Pinna 1989	mic		3116, 3624	small	x					x					
Family Callichthyidae															
Callichthys callichthys (Linnaeus 1758)	pap, mit, vau	3629, 4491	851, 4352	medium	x	x	x		orna					x	
Callichthys serralabium Lehmann A. & Reis 2004	yi		852	medium	x										x
Corydoras melanistius Regan 1912	cud	4389		small	x				orna		x				
Corydoras melini Lönnberg & Rendahl 1930	cud, pap	3597, 3622, 3623, 4873	810	small	x				orna				x		
Corydoras sp.	cud, yi		811, 833	small	x										
Hoplosternum littorale (Hancock 1828)	vau	4049		small	x	x	x		orna						
Megalechis picta (Müller & Troschel 1849)	cud		809, 853, 3609	medium	x	x	x								
Family Loricariidae															
Hemiancistrus subviridis Werneke, Sabaj Pérez, Lujan & Armbruster 2005	vau	4830, 4934		small	x				orna		x				
Hypancistrus inspector Armbruster 2002	vau		1599	small	x						x				
Hypostomus oculeus (Fowler 1943)	cud	4541		medium	x		x								
Loricaria cataphracta Linnaeus 1758	cud, vau	4519, 12301	3809	medium	x	x	x							x	
Rineloricaria daraha Rapp Py-Daniel & Fichberg 2008	pac		850	medium	x										
Rineloricaria formosa Isbrücker & Nijssen 1979	pac, yi		843-848, 3045-3047	small	x		x		orna		x				
Spatuloricaria caquetae (Fowler 1943)	vau	4421		medium	x		x		orna						
Family Cetopsidae															

Taxa/Taxon	**System**	**Collection**		**Size class**	**Water type**			**Migratory**	**Trade**	**New Records**		**Reported previously**			
		CZUT-IC/ IAvH-P	**CIACOL**		**BW**	**CW**	**WW**			**Col**	**Amz**	**Mojica 1991**	**Bogotá-Gregory & Maldonado 2006**	**Galvis et al. 2007**	**Bogotá-Gregory et al. 2020**
Cetopsis coecutiens (Lichtenstein 1819)	vau	12275	1650	medium	x	x	x								
Denticetopsis seducta Vari, Ferraris & de Pinna 2005	cei		857	small	x						x				
Helogenes marmoratus Günther 1863	pap, vau	3640	856, 2358, 3041, 3042, 4343, 4344	small	x				orna					x	
Family Aspredinidae															
Bunocephalus coracoideus (Cope 1874)	yi		820	small	x	x	x								
Bunocephalus knerii Steindachner 1882	cud		858	small	x		x								
Family Auchenipteridae															
Ageneiosus inermis (Linnaeus 1766)	vau	12274		large	x	x	x								
Ageneiosus polystictus Steindachner 1915	cud, vau		786, 1158	medium	x										x
Ageneiosus ucayalensis Castelnau 1855	vau	4834		medium	x	x	x								
Auchenipterichthys coracoideus (Eigenmann & Allen 1942)	cud, vau		792-795, 866, 1159	small	x	x	x								
Centromochlus heckelii (De Filippi 1853)	cud	4902, 12285		small	x	x	x								
Balroglanis macracanthus (Soares-Porto 2000)	pap	14318		small	x										
Tatia brunnea Mees 1974	pac, pap	3631	796, 867, 869, 870, 3607	small	x										x
Tatia gyrina (Eigenmann & Allen 1942)	mit		3606, 3608	small	x										
Tatia intermedia (Steindachner 1877)	cud	4472, 4495, 4526		small	x	x			orna						
Tatia nigra Sarmento-Soares & Martins-Pinheiro 2008	vau	4441, 4544		small	x										
Tatia strigata Soares-Porto 1995	vau	4527		small	x										
Trachelyopterus galeatus (Linnaeus 1766)	cud, pap, vau, yi	3490, 3494, 3630, 3633, 4897	787-791, 868, 1163	medium	x	x	x		orna					x	
Family Doradidae															
Acanthodoras cataphractus (Linnaeus 1758)	cud, vau		871, 3861	small	x							x			

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Acanthodoras spinosissimus (Eigenmann & Eigenmann 1888)	pap, vau	3624, 3625, 4288, 4363		small	x				orna						
Amblydoras affinis (Kner 1855)	cud, pap, vau, yi	3486, 3627, 4418, 4480, 4525, 4951	812, 813, 814, 872, 3605	small	x										
Anduzedoras oxyrhynchus (Valenciennes 1821)	vau	3487	873	medium	x				orna		x				
Hassar orestis (Steindachner 1875)	vau	4135, 4137		medium	x	x			orna						
Leptodoras copei (Fernández-Yépez 1968)	vau	12297		small	x		x			x					
Megalodoras uranoscopus (Eigenmann & Eigenmann 1888)	vau	4832		large	x				cons						
Rhinodoras boehlkei Glodek, Whitmire & Orcés V. 1976	vau	12272, 12318	1648	small	x		x								
Scorpiodoras heckelii (Kner 1855)	pap	3626		medium	x	x			orna						
Family Heptapteridae															
Gladioglanis conquistador Lundberg, Bornbusch & Mago-Leccia 1991	vau		3622	small	x										
Leptorhamdia sp.	vau	12320		small	x										
Mastiglanis asopos Bockmann 1994	cud, pac, yi	4384, 4871	819, 859, 860, 3043, 3044, 3616, 4334	small	x	x			orna					x	
Myoglanis sp.	vau	4953, 12273		small	x										
Nemuroglanis sp.	cud		2386	small	x										
Pariolius armillatus Cope 1872	vau		887, 888, 4348	small	x										
Pimelodella buckleyi (Boulenger 1887)	yi		816, 818, 2343	medium	x										
Pimelodella cristata (Müller & Troschel 1849)	vau	4376	862	medium	x	x	x								
Rhamdia laukidi Bleeker 1858	cud, yi		799, 800, 822, 863, 864	medium	x				cons		x				
Rhamdia muelleri (Günther 1864)	vau	12291		medium	x	x	x				x				
Rhamdia sp.	cud, pap, yi		797, 798, 865, 3615	medium	x				cons						
Family Pimelodidae															

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Megalonema platycephalum Eigenmann 1912	vau		1164	medium	x		x				x				
Pimelodus albofasciatus Mees 1974	vau	12299		medium	x	x									
Pimelodus blochii Valenciennes 1840	cud, vau	3482, 3483, 3484, 3485, 4422	801, 802, 1153	medium	x	x	x	Yes	cons, orna					x	
Pimelodus ornatus Kner 1858	vau	3481, 4136, 12298	3614	medium	x	x		Yes	cons, orna						
Pinirampus pirinampu (Spix & Agassiz 1829)	vau		1143	large	x	x	x								
Pseudoplatystoma tigrinum (Valenciennes 1840)	vau		1171	large	x		x								
Family Pseudopimelodidae															
Batrochoglanis raninus (Valenciennes 1840)	vau	4388		medium	x	x	x		orna						
Batrochoglanis villosus (Eigenmann 1912)	cud		815, 854	small	x	x	x						x		
Pseudopimelodus bufonius (Valenciennes 1840)	mit	12319		medium	x	x									
Order Synbranchiformes															
Family Synbranchidae															
Synbranchus marmoratus Bloch 1795	cud, pap, vau, yi	3639, 4494, 4874	889, 2341, 2359	large	x	x	x		orna			x	x		
Order Cichliformes															
Family Cichlidae															
Aequidens sp.	vau	4935	3029-3032, 3037, 3038	small	x										
Apistogramma regani Kullander 1980	cud	4362, 4523		small	x		x		orna	x					
Cichla orinocensis Humboldt 1821	mit		927, 1147	large	x			Yes	cons				x		

Taxa/Taxon	System	Collection		Size class	Water type			Migratory	Trade	New Records		Reported previously			
		CZUT-IC/ IAvH-P	CIACOL		BW	CW	WW			Col	Amz	Mojica 1991	Bogotá-Gregory & Maldonado 2006	Galvis et al. 2007	Bogotá-Gregory et al. 2020
Cichla temensis Humboldt 1821	cud, mit	4479, 12302	756, 1145	large	x				cons, orna					x	
Crenicichla anthurus Cope 1872	cud, pac, vau	4332, 4339, 4483, 4487	772, 919, 922, 923, 925, 926	medium	x				orna						
Crenicichla lenticulata Heckel 1840	cud	4360, 4895, 12276, 12303		medium	x				orna				x		
Crenicichla lugubris Heckel 1840	cud	3488		medium	x	x									
Crenicichla marmorata Pellegrin 1904	mit		928	medium	x										x
Crenicichla saxatilis -group	pap, yi		918, 921, 924, 3033	medium	x				orna						
Geophagus abalios López-Fernández & Taphorn 2004	vau		768, 1161	small	x	x			orna					x	
Geophagus winemilleri López-Fernández & Taphorn 2004	pac		935, 936	small	x				orna						
Heros efasciatus Heckel 1840	pac		916, 917	small	x				orna						
Satanoperca jurupari (Heckel 1840)	cud, vau	4955, 12305	760, 761, 767	medium	x	x	x		orna					x	
Order Cyprinodontiformes															
Family Rivulidae															
Anablepsoides ornatus (Garman 1895)	vau	4940	2380	small	x				orna						
Laimosemion amanapira (Costa 2004)	vau		890, 891	small	x					x					
Order Beloniformes															
Family Belonidae															
Potamorrhaphis guianensis (Jardine 1843)	cud, pac, vau		892, 893, 1389, 2657-2664, 3808	medium	x	x	x							x	
Incertae sedis in Eupercaria															
Family Sciaenidae															
Plagioscion squamosissimus (Heckel 1840)	vau		1168	large	x	x	x		cons						

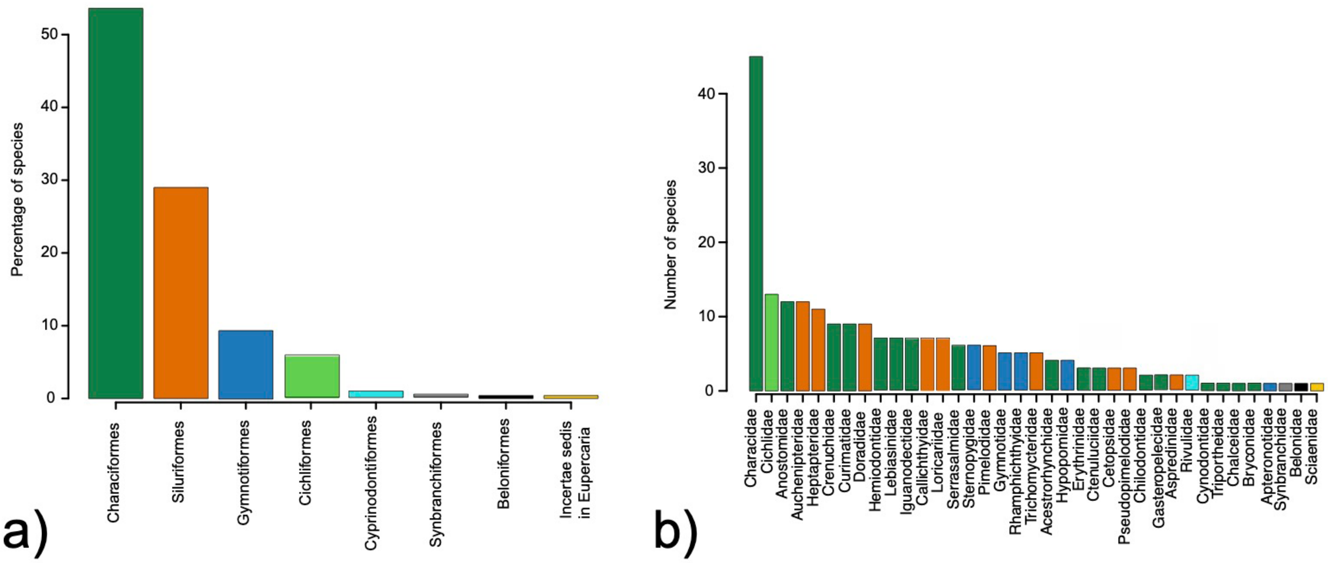

Figure 2. (a) Percentage of species by order and (b) number of species per family.

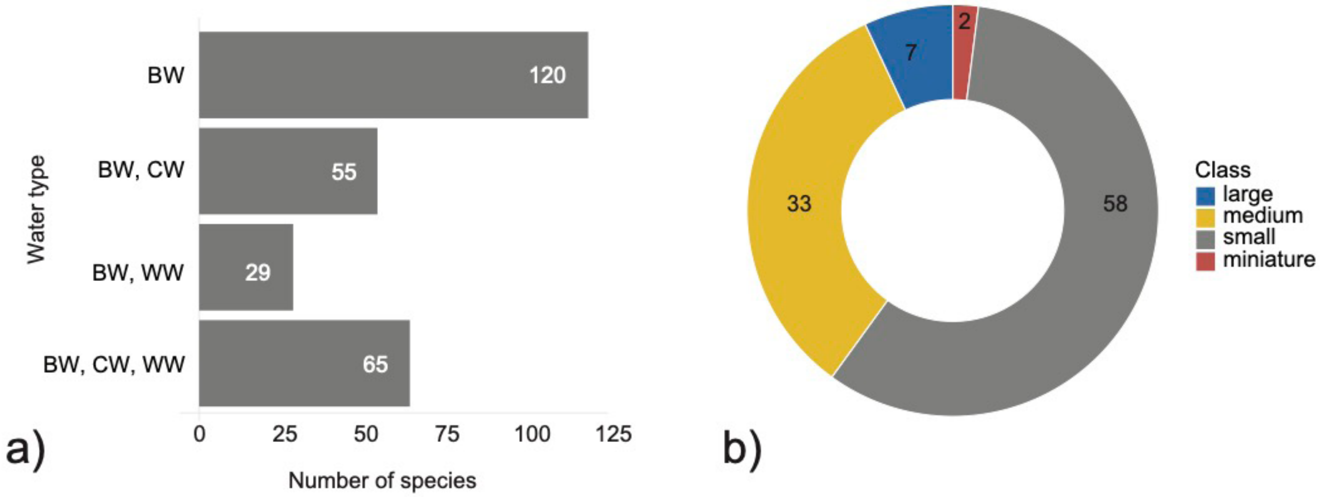

Figure 3. (a) number of species per water type: BW = blackwater, CW = clearwater, and WW = whitewater and (b) Percentage of species per size-class

In one of the first efforts to document the freshwater fishes from Colombia (Mojica 1999), only four species were listed across the entirety of the río Vaupés basin: *Hoplerythrinus unitaeniatus, Acanthodoras cataphractus, Platydoras hancockii*, and *Synbranchus marmoratus.* We confirmed the presence of these species, except *Platydoras hancockii*, which is the only species of the genus reported for the río Negro basin (Piorski et al. 2008). Because of this, its presence in the río Vaupés is expected to be documented when more sampling efforts can be conducted in the area. Bogotá-Gregory and Maldonado-Ocampo (2006) increased species number in the río Vaupés to 24 species, from which 14 were here recorded (see Table S1 for species not identified in our study, but which were previously reported). Galvis et al. (2007) in his study focused on fish species used in ornamental trade, also reported additional species for the río Vaupés basin. Lima and collaborators (2020) described *Hemigrammus xaveriellus*, from the río Itilla, at the headwaters of the río Vaupés basin. More recently, Bogotá-Gregory et al. (2020a) reported eight species in the Mitú Region that were not previously registered from Colombia and are included here. As can be verified from this brief account, studies regarding fish composition of the río Vaupés basin are scant. Additionally, the low species richness previously reported (Bogotá-Gregory & Maldonado-Ocampo 2006) was mainly the result of isolated sampling events, rather than the actual figure derived from extensive collection efforts in the implied region, as demonstrated in this work. The previous studies in the area are not exclusively based on specimens available in ichthyological collections, but also in literature records. This type of taxonomic lists may contain discrepancies in species identification, which are difficult to assess due to unavailability of reference specimens of the bibliographic records. Discrepancies can also arise from outdated taxonomic identifications. In our study, we counted with the collaboration of specialists in different taxonomic groups that ensures reliable taxonomic identifications.

A recent checklist for the entire portion of the rio Negro basin in Brazil, reported 1,165 species (Beltrão et al. 2019). However, there is little published information concerning the ichthyofauna from its upper section, above São Gabriel da Cachoeira, and particularly from the rio Uaupés basin. Lima et al. (2005) reviewed the available information for the area published at that time. The same authors presented a list for the species found in the upper portion of the rio Tiquié, an important tributary of the rio Uaupés, with headwaters within the Departamento of Vaupés, and reported 147 species for the area. Subsequent collecting in the middle portion of the rio Tiquié raised the number of species to 265 (F.C.T. Lima, unpubl. data). Several species were described in the last years, based on this fish survey (Lima & Sousa 2009, Marinho & Lima 2009, Carvalho et al. 2010, Maxime et al. 2011, Birindelli et al. 2012, Lima & Sazima 2017, Pablo Lehmann et al. 2018, Soares et al. 2019). However, other portions of the rio Uaupés basin in Brazil remain virtually unknown ichthyologically.

Despite recent efforts documenting fish migrations in Colombia (e.g. Usma et al. 2009), the río Vaupés and more generally, the Amazon basin are still incompletely studied, and movement patterns of fishes remain poorly documented. Therefore, species listed as migratory here might not be the only ones that perform large longitudinal migrations in the basin. Upstream migrations of some *Leporinus* species were reported by Lima et al. (2005) from the rio Tiquié.

Blackwaters have extremely low pH values, due to high content of acidic humic compounds, largely derived from incompletely decomposed organic matter, which gives the water its typical tea stained coloration (Goulding et al. 1988, Leenheer 1980). The extremely low pH exerts physiological constraints on the biota, which affects ionic balance and osmoregulation in freshwater fishes (McDonald 1982, Wilson et al. 1999, Matsuo & Val 2002). Physiological studies have provided experimental support for mechanisms to prevent acidification in some fish species of these kind of environments (Hirata et al. 2003). Thus, the fish communities that characterizes the blackwater systems, like those we sampled in our study area, are most likely represented by species especially adapted to extreme pH conditions (Val & de Almeida 1995). As we evidenced in our study, more than 50% of the species identified are unique to these low pH aquatic systems.

Blackwater systems are also known for their low productivity in terms of biomass (Goulding et al. 1988, Bogotá-Gregory, Lima, et al. 2020). This is partially reflected in the small number of blackwater-endemic fishes, only 16 species, collected commercially for human consumption (Lasso et al. 2011). In contrast, 85 species have commercial importance in the ornamental trade in Colombia (Galvis et al. 2007, AUNAP 2016, Nogueira et al. 2012, Landines et al. 2005) (Table 1). The majority of the species in this study have small body size, which agrees with similar findings in other recent studies that have shown that the relatively nutrient-poor systems can indeed maintain a relatively diverse ichthyofauna, yet mainly composed of small fishes that had acquired proper adaptations to thrive in less productive habitats (Arbeláez, Duivenvoorden & Maldonado-Ocampo 2008, Arbelaez et al. 2004, Mojica et al. 2009, Goulding et al. 1988, Machado-Allison et al. 2013, 2003, Lasso et al. 2006). On the contrary, the majority of medium to large size species are associated with large longitudinal migratory movements (Castro & Polaz 2020). And because of this they're mostly found in the main channels of more productive systems where they found better conditions as nursery areas (De Lima & Araujo-Lima 2004).

In less productive systems, like the blackwater rivers of the Amazon basin, most of the energy obtained by the aquatic fauna is of allochthonous origin. The adjacent forests provide food resources in the form of fruits, seeds, and arthropods (Goulding et al. 1988, Lowe-McConnell 1987). One of the strategies by which these highly diverse communities can be maintained could be by reducing their biomass. In this manner, blackwater systems can maintain highly diverse fish communities with relatively low abundances, as shown by Saint-Paul et al. (2000), in a comparison between blackwater and whitewater floodplain fish communities. Another of the most important adaptations observed thus far is miniaturization, which includes paedomorphic retention of juvenile characteristics and reductive morphological evolution. Miniaturization has evolved on multiple occasions in Neotropical fishes, as a way to occupy interstitial habitats of marginal vegetation and leaf litter of the substrate (Crampton 2011, Weitzman & Vari 1988).

The new records presented here for the Colombian Amazon reflects scarcity in the studies carried out thus far with regards to the fish fauna of the region. While most ichthyological surveys have been performed in the surroundings of populated localities, with emphasis on species of commercial importance (Bogotá-Gregory & Maldonado-Ocampo 2006), this study was carried out to survey previously little-explored areas. Over the last four decades, intensive field collections and improvements in taxonomy have yielded an improved understanding of how Amazonian fish species are distributed among the Amazon's aquatic ecosystems. However, there are still huge remote areas that remain unexplored, and we are not yet close to knowing the species diversity of these pristine areas.

Initiatives that encompass studies like ours, allow us to document the ichthyofauna of the most diverse region in the world. The information and analyses generated by this study will be of utility to a broad range of researchers interested – including from the fields of ecology, biogeography, and taxonomy. Aditionally, it constitute important baselines for its application in management and conservation plans. This is important in light of the habitat degradation that freshwater fish species are facing due to anthropogenic activities. Deforestation, mining, dam-construction, and overfishing threaten most of the Amazon region, and management and conservation plans are still lacking (Abell et al. 2008).

Supplementary Material

The following online material is available for this article:

Table S1 - Species not reported in our study but previously registered for the Vaupés basin.

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Associate Editor

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Conflicts of Interest

The authors declare that they have no conflict of interest.

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Article

Birds of Parque Estadual Ilha do Cardoso: ecology, conservation and natural history

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Abstract: The Parque Estadual Ilha do Cardoso (PEIC), located on extreme South of São Paulo's cost, in Brazil, holds an important Atlantic Forest remnant which still in a good state of conservation, but lack a deepen study about the avian community that habits the island. This study aimed to elaborate a census of avian species that occur in the park approaching richness, occurrence frequency, occurrence status and the structure of trophic guilds. A total of 25 field expedictions occurred between September 2015 and September 2017 during five days each, the field work included different ecosystems as mangrove, restinga, forest, sandbank, beach and marine. The census was made combine three different techniques used in ornithological studies: visual identification, auditory identification and catch by mist-nets (with five fixed sites in mangrove, restinga and forest). Were recorded 335 avian species, with 28 of them endemic from Brazil and 33 being threatened with extinction. Seventy-three species were recorded in all sampled months (FO 100%), while 46 were recorded in just one month (FO = 4%). About occurrence status, 55% of species are residents, 20% occasional visitors, 13% unusual residents, 6% migratory and 6% visitors. According the recorded species were recognized 25 trophic guilds based on food items, corporal size and strata that commonly forage, which of most representative in the community, the guild of "of insectivorous of medium-strata" (N = 55), "canopy omnivorous" (N = 33), "aquatic invertebrates consummers" and "piscivorous" (N = 31). Finally, with the current study, we aimed through a significative field effort bring a better knowledge about avifauna of PEIC, which could be a good base when is necessary take actions that aim to park management and the Conservation Unities around it.

Keywords: *Avifauna; Trophic Guilds; Mangrove; PEIC; Restinga.*

Aves do Parque Estadual Ilha do Cardos: ecologia, conservação e história natural

Resumo: O Parque Estadual Ilha do Cardoso (PEIC), localizado no estremo sul do litoral paulista, reúne um importante remanescente de Floresta Atlântica em bom estado de conservação, carecendo, porém, de um estudo aprofundado sobre a comunidade de aves que habita a ilha. Diante disso, o presente estudo visou a elaborar um inventário das espécies de aves do parque abordando a riqueza, frequência de ocorrência, status de ocorrência e divisão das espécies em guildas. Foram realizadas entre setembro de 2015 e setembro de 2017, 25 expedições de campo com cinco dias de duração cada, incluindo os ecossistemas de manguezal, restinga, floresta, baixio, praia e marinho. O inventário foi realizado combinando três técnicas para estudos ornitológicos: identificação visual, identificação auditiva e captura com redes-de-neblina (cinco pontos fixos no manguezal, restinga e floresta). Foram registradas 335 espécies de aves, sendo 28 espécies consideradas endêmicas para o Brasil e 33 com algum grau de risco de extinção. Setenta e três espécies foram registradas em todos os meses amostrados (FO = 100%), enquanto que 46 apresentaram registros em apenas um mês (FO = 4%). No que se refere ao status de ocorrência, 55% das espécies são residentes, 20% visitantes ocasionais, 13% residentes incomuns, 6% migratórias e 6% visitantes. As espécies registradas permitiram o reconhecimento de 25 guildas com base nos itens alimentares, tamanho corporal e estrato em que comumente forrageiam, sendo as mais representativas na comunidade, as guildas de "Insetívoros de estrato médio" (N = 55), "Onívoros de copa" (N = 33), "Consumidores de Invertebrados Aquáticos" e "Piscívoros" (N = 31). Por fim, com o presente estudo, buscamos através de um significativo esforço em campo trazer um melhor conhecimento no que diz respeito a avifauna do PEIC, que pode vir a servir de base na hora de traçar ações visando ao manejo do parque bem como das unidades de conservação que se distribuem no seu entorno.

Palavras-chave: *Avifauna; Guildas tróficas; Manguezal; PEIC; Restinga.*

Introduction

Biological communities correspond a set of organism's populations that inhabits a certain area and interact among each other and with the abiotic environment (Ricklefs 2016). The structure of a biological community, on a large scale, is determined basically by the biogeographic history of the organisms which composes it (Reif et al. 2010) and by climatic factors (Hawkins et al. 2003). In local level, their structure is influenced mainly by ecological relations, like interactions between the organism (Martin et al. 2016).

In terms of fauna, decrease in abundance of individuals and community loss of biological diversity occurs mainly due to environment structure alterations that affect fundamental portions of niches occupied by species (Karr & Freemark 1983, Johns 1991). Such environment alterations, in the most cases, are associated with human activities, as habitats fragmentation, species introductions and the natural resources overexploitation (Primack & Rodrigues 2001).

One of the most critic cases occur in Atlantic Forest, which initially covered almost all Brazilian coast, with a total area of 1.3 million km^2, being currently restricted to less than 8.5% of its total (SOS Mata Atlântica 2015). In São Paulo's State, such biome originally covered 69% of its territory, however, as in another Brazilians states, had its area reduced, covering nowadays just 13.7% of this total and being restrict mainly to mountain and coastal regions remnants (SOS Mata Atlântica 2017).

Concerning biological diversity of Atlantic Forest, birds correspond of most representative vertebrate's group, with 927 species in southeastern Brazil, which 141 endemic species and 83 threatened with extintion (Ridgely et al. 2015). However, evaluating in a populational level, many of these species have a representative numeric decrease, being considered rare, because they live in restrict environment or for having a small geographical distribution (Goerck 1997). Thus, even though ecological and evolutional factors may justify the potential rarity of species, human actions seem to be the main aggravating factor in populational size decreasing (Develey 2004, Pereira et al. 2014, Morante-Filho et al. 2015).

Into this context, the Parque Estadual Ilha do Cardoso (PEIC), located on extreme South of São Paulo's coast, correspond to a good example of a landscape mosaic in a good state of conservation. However, just a few studies involving vertebrate's fauna, in community level, were made in the park, which were focused in herpetological fauna (Bertoluci et al. 2007, Rocha et al. 2008, Pinheiro 2009, Vilela et al. 2011, Prado 2012) and mammal fauna (Nakano-Oliveira 2006, Martins 2013, Silveira 2017). Concerning bird's communities, there is only a species list, that is in the management plan of the park (São Paulo 1998, 2002) and the Marsden et al. (2004) study which addresses the forest bird's community that inhabit the island. Therefore, based on assumption that there is a gap to be filled in the avifauna knowledge of PEIC, this study aims to elaborate a census of resident, migratory and occasional occurrence bird's species of the park and address aspects about richness, occurrence frequency, occurrence status and species division into guilds.

Material and Methods

1. Study's area

This study was developed in the Parque Estadual Ilha do Cardoso (PEIC), located on extreme South of São Paulo's coast (in 25°03'05" to 25°18'18"S and in 47°53'48" to 48°05'42"W; Figure 1). The park belongs to the Cananéia city and was officially created from decree nº 40.319 from 03/07/1962. Has an area from approximately 13.300 hectares, with 30 km of extension in the portion which borders the Atlantic Ocean and with a maximum width 10 km, showing an irregular shape which is larger in the North portion and becoming thin towards the South, where its ends with a slim portion of sand already on the Paraná's State border (Parque Nacional Superagui; Negreiros et al. 1974). The island's relief is very rugged, beginning on the sea level with modern sand ridges and reaching more than 800 m of elevation mountain range located on the island's central position, which its formation goes back to the Precambrian Period (Negreiros et al. 1974).

Inserted on Atlantic Forest biome, PEIC covers different ecosystems, as forest, restinga, mangrove, beach (considered here as sand portions without vegetations), sandbar (located over Ararapira's channel) and marine (channels which delimit the island on portions north and west as well in the east marine portion of the island). In this study, we considered as "restinga" areas starting from herbaceous and shrub vegetation located in high tide limit to the most arboreous portion featured to still present sandy soil with a thin layer of litterfall. In addition, we considered "forest" as the formations present starting from ecotone limit with restinga, encompassing flatland forest and hillside forest (or from Serra do Mar).

The region's weather, which include both areas, is featured as hot and humid, with an average thermal amplitude from 17°C to 26°C and an average monthly rainfall of 157 mm (recorded from 2002 to 2016; CIIAGRO 2017). The region presents predominance of tropical air masses in summer and a discrete predominance of polar air masses in winter (Schaeffer-Novelli et al. 1990).

Specifically, in Ilha do Cardoso, the weather shows some particular features, especially with regard to variations in rainfall levels and temperature. According Negreiros et al. (1987) these variations are due to local factors, such as maritimity, rugged topography and vegetation characteristics.

2. Sample design

A total of 25 field expedictions occurred between September 2015 and September 2017 during five days each SISBIO license 39328-8 and COTEC 277/2016). The field started at 06:00 AM and extended until 18:00 PM, being carried out in ecosystems such as mangrove, restinga and forest, located in north east of island (Figure 1). Short nocturnal incursions were made on these ecosystems aim to register birds from Orders Strigiformes, Caprimulgiformes e Nyctibiiformes. The sandbar, beach and channel which separate Ilha de Cananéia from Ilha do Cardoso were sampled once a month by onboard incursions and by walk in sand strip on northern portion of the island (Pereirinha's beach). The species that were recorded only flying over any of the mentioned ecosystems were categorized as "flying", since it was impossible to determine whether the species was just moving or occupied the ecosystem in question.

The inventory was carried out by combining three ornithological studies techniques: visual identification, auditory identification as catch by mist-nets. The mist-nets were placed in fixed sites during two consecutive days in the main three ecosystems which compose the island on the northeast portion: mangrove (on the Perequê's river mouth), restinga (over a road and Morro das Almas trail) and forest

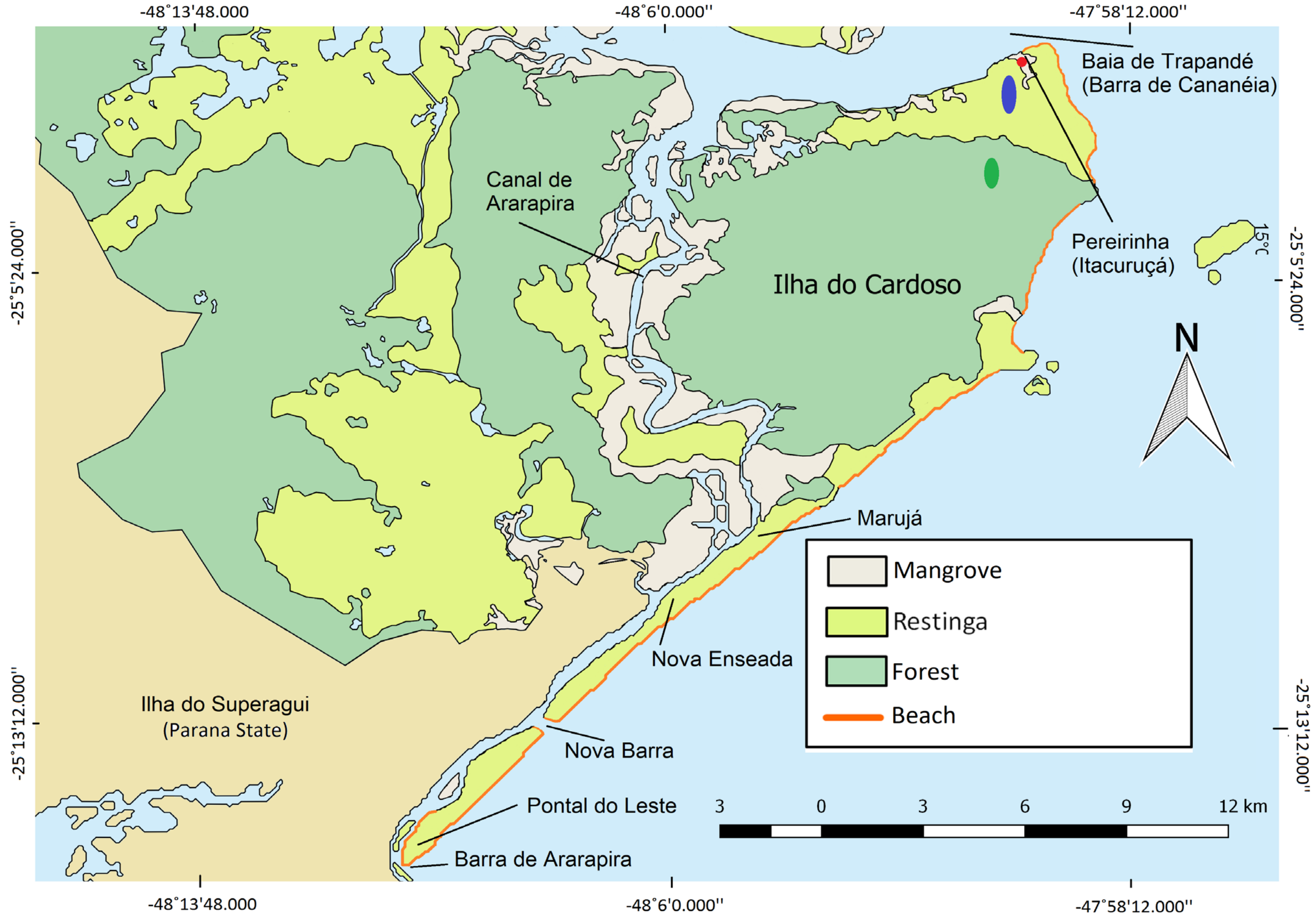

Figure 1. Sites localization's where was used mist-nets in Northeast portion of Ilha do Cardoso, South coast of São Paulo's State (● mangrove, ● restinga e ● forest).

(next to an old house which was used as electric power center). For this, six mist-nets were used in each ecosystem (Figure 1) (stitch of 19, 20, 25 and 30 mm), which were opened at 06:00 AM. and closed at 18:00 PM. Mist-nets were reviewed each 25 minutes aimed to take the specimen that were catched by it to identify and mark with rings. All captured birds were marked in the right tarsus with metal rings provided by CEMAVE (ringing authorization 3999).

Was also included species which were identified by picture records made by local residents and by Projeto Monitoramento de Praias – Bacia de Santos (PMP-BS) which monitors beaches of Ilha do Cardoso everyday. In addition, including species recorded in the period from September 2017 to December 2020 observed in field expeditions without a defined frequency. For the three situations were excluded of occurrence frequency calculation. The taxonomy classification used followed the proposal by the Brazilian Comitee of Ornithological Records (Pacheco et al. 2021).

3. *Data analysis*

The occurrence frequency was calculated by FO = A/n.100, where "A" means number of months that a specie was recorded and "n" is the number of total months sampled (N = 25). Occurrence status was defined in this study after analyzing the occurrence of the species over the sampled period, with the occurrence frequency results calculated and information available in the literature (Sick 2001, Pacheco et al. 2021). It was considered as **Residents** birds with FO above 20% or more; **Unusual Residents**, those which present FO above 10% but under 20%; **Occasional Visitors**, those species that besides doesn't show presence/absence patters defined, presented FO under 10%; **Migratory** those species with FO above or equal 20% and that show a clear similar pattern of presence/absence over the two sampled years. Species on migratory routes that used the area for a brief period and that is, in this study, showed FO under 10%, was classified as **Visitors from North Hemisphere** and **Visitors from South Hemisphere**, following what was purposed by Pacheco et al. (2021).

We used here the status "Unusual Resident" (FO over 10%), by the fact however a species has not been recorded on certain months, we cannot simply affirm that such specie is not occurring in that area at that time, once their "absence" could be just associated to their low conspicuity (Marterer 1996). The difference in "Unusual Resident", therefore, to refer to those less conspicuous in the area (or potentially rare). Concerning birds classified as "Migratory", was related to their behavior on Ilha do Cardoso, that could differ to the general pattern described in literature.

The species division in guilds was grounded in the concept proposed by Willis (1979) with some adequations aimed to encompass all community. For this was adopted some information available in Sick (2001) and Kissling et al. (2011), besides the observations made in field. The classification considered the predominant diet items in each specie, the body size and the main strata that they foraging: **LSF** (Large Soil Frugivores), **LTF** (Large Canopy Frugivores), **STF** (Small and Medium Canopy Frugivores), **MSF** (Medium Strata Frugivores), **AO** (Aquatic Omnivores), **MSO** (Medium-Strata Omnivores), **CO** (Canopy Omnivores), **SO** (Soil Omnivores), **UO** (Understory Omnivores), **P** (Piscivores), **DC** (Daytime Carnivores), **NC** (Nocturnal Carnivores), **AIC** (Aquatic Invertebrate Consumers), **TIC** (Terrestrial Invertebrates Consumers), **SI** (Soil Insectivores), **MSI** (Medium-Strata Insectivores), **UI** (Understory Insectivores), **CI** (Canopy Insectivores), **DAI** (Daytime Aerial Insectivores), **NAI** (Nocturnal Aerial Insectivores), **S** (Scavengers), **SG** (Soil Granivores), **UG** (Understory Granivores), **MSG** (Medium-Strata Granivores) and **N** (Nectarivores).

To test if there was a significative difference in species richness in ecosystems between years and seasons, was used a Permutational Multivariate Analysis of Variance (PERMANOVA), which factors (years and season) were considered as fixed effects and number of permutations used was 9.999. This analysis was made using software "Past" (Hammer et al. 2001), where similarity matrix by similarity coefficient of Jaccard was constructed with richness data. Seasons was grouped in: December, January and February (summer); March, April and May (fall); June, July and August (winter); September, October and November (spring) for better attending region's characteristics. An accumulation curve was made based in species richness data (presence/absence matrix) in function of the number of sampling, aimed to observe whether the community has already been sampled satisfactorily.

Results

In the end of the 25 sampled months, were totalized 1.500 hours in field, distributed in 125 days. Concerning captures, were 1.116 hours of open mist-nets, of which 588 hours were on mangrove, 576 on restinga and 576 hours on the forest. As result of strong rainfall, for a day on mangrove, two days on restinga and four days on forest the mist-nets were not opened.

Was recorded 335 bird species belonging to 25 orders and 75 families (Table 1). Of these, seven were identified through pictures made by island's locals: *Amazonetta brasiliensis* (Brazilian Teal), *Limosa haemastica* (Hudsonian Godwit), *Ramphastos dicolorus* (Red-breasted Toucan), *Sturnella superciliaris* (White-browed Meadowlark), *Himantopus melanurus* (White-backed Stilt), *Notharcus swainsoni* (Buff-bellied Puffbird) e *Schistochlamys ruficapillus* (Cinnamon Tanager). More seven species were included based on records made by Projeto Monitoramento de Praias – Bacia de Santos (PMP-BS) executed by Instituto de Pesquisas Cananéia (IPeC): *Spheniscus magellanicus* (Magellanic Penguin), *Thalassarche chlororhynchos* (Yellow-nosed Albatross), *Thalassarche melanophris* (Black-browed Albatross), *Macronectes giganteus* (Southern giant-Petrel), *Macronectes halli* (Northern giant-Petrel), *Procellaria aequinoctialis* (White-chinned Petrel) e *Calonectris borealis* (Cory's Shearwater). In addition, six species were recorded in the period from September 2017 to December 2020 during field expeditions without a defined frequency, being them: *Tringa solitaria* (Solitary Sandpiper), *Tringa melanoleuca* (Greater Yellowlegs), *Tringa semipalmata* (Eastern Willet), *Tringa flavipes* (Lesser Yellowlegs), *Jacana jacana* (Wattled Jacana) and *Parabuteo unicinctus* (Harris's Hawk). Based on records made in field (excluded those made by pictures and after September 2017), accumulation curve for Ilha do Cardoso stabilizes from 23th sampling (Figure 2). Among recorded species, 28 are considered endemic from Brazil (Pacheco et al. 2021) and 33 threatened with extinction whether in state level (Silveira et al. 2009), national (MMA 2014) or worldwide (IUCN 2018) (Table 2).

Was captured 628 birds from 81 species belonging to eight orders and 29 families. From total captures, 88 correspond to recaptures. Concerning ecosystems, on mangrove were captured 263 birds belonging to 46 species, on restinga 209 birds belonging to 45 species and 156 on forest belonging to 30 species.

Ecosystems species richness showed significant differences between sampled years (df = 1, *F* = 4,82, *P* = 0,0026) and seasons (df = 3, *F* = 7,27, *P* = 0,0004). Between September to December (spring) was possible to register the largest number of species in both years, with a peak on November. In contrast, between May to August (winter) was recorded the lowest numbers of species in the area, with minimum values on June (Figure 3).

Between sampled ecosystems (including the six species recorded after September 2017), the restinga showed largest richness (N = 232), followed by forest (N = 145), mangrove (N= 118), beach (N = 42), sandbar (N = 30), marine (N = 15) and flying (N = 11). Except sandbar, all others present species recorded only in their own ecosystem: restinga (N = 77), forest (N = 36), beach (N = 20), mangrove (N = 14), flying (N = 11) and marine (N = 3) (more details on Table 1).

Regarding occurrence frequency (FO) (excluded 20 species identified by pictures records or after September 2017), 73 species were recorded in all sampled months (FO = 100%), while 46 showed records in just a month (FO = 4%) (APPENDIX 1). Concerning occurrence status, 55% of species are residents, 20% occasional visitors, 13% unusual residents, 6% migratory, 4% visitors from North hemisphere and 2% visitors from South hemisphere (Table 1).

According to 335 recorded species added to the six species recorded after September 2017, was possible recognize 25 guilds based in food items, body size and strata that commonly forage, being the most representative in the community, the guilds "Medium-Strata Insectivores" (N = 55), "Canopy Omnivores" (N = 33), "Piscivores" (N= 31) and "Aquatic Invertebrate Consumers" (N = 31) (Table 3). When analyzing the distribution inside of mangrove, restinga and forest ecosystems, the predominant guild is "Medium-Strata Insectivores" as well, while in the sandbar, beach and marine was "Piscivores". In flying it was possible to observe the predominance of "Daytime Aerial Insectivores".

Discussion

The expressive number of recorded species in Parque Estadual Ilha do Cardoso (PEIC; N= 335) represents well the biome's diversity and the Atlantic Forest remnants of South coastal region of São Paulo's State, corresponding to 35.4% avifauna of the biome in the Brazil Southern and 17% of all Brazilian's avifauna. Furthermore, the sum of the different ecosystems (mangrove, restinga, forest, beach, sandbar and marine) and in a good conservation state, contribute directly to the highly richness. Still stands out the fact of to shelter 28 endemic species, 33 species in some level endangered and species which indicates that the environment still in a good conservation's state, as *Aburria jacutinga* (Black-fronted

Table 1. List of species recorded in Parque Estadual Ilha do Cardoso (SP/Brazil). Caption: **En** – Endangered Species; **E** – Endemic Species; [1] record made by local residents; [2] recorded made by Projeto Monitoramento de Praias – Bacia de Santos. Occurrence ecosystem: **M**- Mangrove; **R** – Restinga; **F** – Forest; **B** – Beach; **S** – Sandbar; **Mr** – Marine; **Fl** – Flying. Status: **Re** - Resident; **UR** - Unusual Resident; **OV** - Occasional Visitor; **VS** - Visitors from South Hemisphere; **VN** - Visitors from North Hemisphere; **Mi** – Migratory. Guilds: **LSF** - Large Soil Frugivores; **LCF** - Large Canopy Frugivores; **SMF** - Small and Medium Canopy Frugivores; **MSF** - Medium Strata Frugivores; **AO** - Aquatic Omnivores; **SO** - Soil Omnivores; **UO** - Understory Omnivores; **MSO** - Medium-Strata Omnivores; **CO** - Canopy Omnivores; **P** – Piscivores; **DC** - Daytime Carnivores; **NC** - Nocturnal Carnivores; **AIC** - Aquatic Invertebrate Consumers; **TIC** - Terrestrial Invertebrates Consumers; **SI** - Soil Insectivores; **MSI** - Medium-Strata Insectivores; **UI** - Understory Insectivores; **CI** - Canopy Insectivores; **DAI** - Daytime Aerial Insectivores; **NAI** - Nocturnal Aerial Insectivores; **S** – Scavengers; **SG** - Soil Granivores; **UG** - Understory Granivores; **MSG** - Medium-Strata Granivores; **N** - Nectarivores. Numbers in parenthesis after family name corresponds to the number of species that composing its.

Taxonomic Ordering	**Occurrence Ecosystem**							**Status**	**FO**	**Guild**
	M	**R**	**F**	**P**	**B**	**Mr**	**V**			
Order Tinamiformes										
Family Tinamidae (2)										
Tinamus solitarius (Vieillot, 1819)[a] – macuco – Solitary Tinamou	-	-	x	-	-	-	-	Re	48%	LSF
Crypturellus obsoletus (Temminck, 1815) – inhambuguaçu – Brown Tinamou	-	x	x	-	-	-	-	Re	100%	LSF
Order Anseriformes										
Family Anatidae (3)										
Dendrocygna viduata (Linnaeus, 1766) – irerê – White-faced Whistling-Duck	-	-	-	x	-	-	-	OV	4%	AO
Cairina moschata (Linnaeus, 1758) - pato-do-mato – Muscovy Duck	x	-	-	-	x	-	-	Re	36%	AO
Amazonetta brasiliensis (Gmelin, 1789)[1] – marreca-ananaí – Brazilian Teal	-	x	-	-	-	-	-	OV	-	AO
Order Galliformes										
Family Cracidae (3)										
Penelope superciliaris Temminck, 1815 – jacupemba – Rusty-margined Guan	-	x	-	-	-	-	-	Re	20%	LCF
Penelope obscura Temminck, 1815 – jacuguaçu – Dusky-legged Guan	-	x	x	-	-	-	-	Re	32%	LCF
Aburria jacutinga (Spix, 1825)[a]- jacutinga – Black-fronted Piping-Guan	-	-	x	-	-	-	-	UR	12%	LCF
Family Odontophoridae (1)										
Odontophorus capueira (Spix, 1825) – uru – Spot-winged Wood-Quail	-	-	x	-	-	-	-	Re	52%	LSF
Order Podicipediformes										
Family Podicipedidae (2)										
Tachybaptus dominicus Linnaeus, 1766) - mergulhão-pequeno – Least Grebe	-	-	-	-	-	x	-	OV	4%	P
Podicephorus major (Boddaert, 1783) - mergulhão-grande – Great Grebe	-	-	-	-	-	x	-	OV	4%	P
Order Columbiformes										
Family Columbidae (8)										
Patagioenas picazuro (Temminck, 1813) – pomba-asa-branca – Picazuro Pigeon	-	x	-	-	-	-	-	UR	12%	LCF
Patagioenas cayennensis (Bonaterre, 1792) – pomba-galega – Pale-vented Pigeon	x	x	x	-	-	-	-	Mi	64%	LCF
Patagioenas plumbea (Vieillot, 1818) – pomba-amargosa – Plumbeous Pigeon	x	x	x	-	-	-	-	Re	60%	LCF
Geotrygon montana (Linnaeus, 1758) – pariri – Ruddy Quail-Dove	-	x	x	-	-	-	-	Re	48%	LSF

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Leptotila verreauxi Bonaparte, 1856 – juriti-pupu – White-tipped Dove	x	x	-	-	-	-	-	Re	40%	LSF
Leptotila rufaxilla (Richard & Bernard, 1792) – juriti-de-testa-branca – Gray-founted Dove	x	x	-	-	-	-	-	Re	28%	LSF
Zenaida auriculata (Des Murs, 1847) – avoante – Eared Dove	-	x	-	-	-	-	-	OV	4%	SG
Columbina talpacoti (Temminck, 1810) – rolinha – Ruddy Ground-Dove	x	x	-	-	-	-	-	Re	64%	SG
Order Cuculiformes										
Family Cuculidae (6)										
Guira guira (Gmelin, 1788) – anu-branco – Guira Cuckoo	-	x	-	-	-	-	-	Re	24%	MSI
Crotophaga ani Linnaeus, 1758 – anu-preto – Smooth-billed Ani	-	x	-	-	-	-	-	Re	56%	MSI
Tapera naevia (Linnaeus, 1766) – saci – Striped Cuckoo	-	x	-	-	-	-	-	UR	12%	MSI
Dromococcyx pavoninus Pelzeln, 1870 – peixe-frito-pavonino – Pavonine Cuckoo	-	-	x	-	-	-	-	OV	4%	MSI
Piaya cayana (Linnaeus, 1766) – alma-de-gato – Squirrel Cuckoo	x	x	x	-	-	-	-	Re	76%	MSI
Coccyzus melacoryphus Vieillot 1817 – papa-lagarta-acanelado – Dark-billed Cuckoo	-	-	x	-	-	-	-	OV	8%	MSI
Order Nyctibiiformes										
Family Nyctibiidae (1)										
Nyctibius griseus (Gmelin, 1789) – urutau – Common Potoo	-	x	-	-	-	-	-	UR	28%	NAI
Order Caprimulgiformes										
Família Caprimulgidae (5)										
Lurocalis semitorquatus (Gmelin, 1789) – tuju – Short-tailde Nighthawk	x	x	-	-	-	-	-	Mi	44%	NAI
Nyctidromus albicollis (Gmelin, 1789) – bacurau – Common Pauraque	-	x	-	-	-	-	-	Re	68%	NAI
Hydropsalis parvula (Gould, 1837) – bacurau-chintã – Little Nightjar	-	x	-	-	-	-	-	OV	4%	NAI
Hydropsalis torquata (Gmelin, 1789) - bacurau-tesoura - Scissor-tailed Nightjar	-	x	-	-	-	-	-	Re	40%	NAI
Podager nacunda (Vieillot, 1817) – corucão – Nacunda Nighthawk	-	x	-	-	-	-	-	Re	36%	NAI
Order Apodiformes										
Family Apodidae (4)										
Streptoprocne zonaris (Shaw, 1796) – taperuçu-de-coleira-branca – Biscutate Swift	-	-	-	-	-	-	x	Re	28%	DAI
Chaetura cineireventris Sclater, 1862 – andorinhão-de-sobre-cinzento – Gray-rumped Swift	-	-	-	-	-	-	x	Re	24%	DAI
Chaetura meridionalis Hellmayr, 1907 – andorinhão-de-temporal – Sick's Swift	-	-	-	-	-	-	x	OV	8%	DAI
Panyptila cayennensis (Gmelin, 1789) – andorinhão-estofador – Lesser Swallow-tailed Swift	-	-	-	-	-	-	x	OV	4%	DAI
Family Trochilidae (14)										
Florisuga fusca (Vieillot, 1817) – beija-flor-preto – Black Jacobin	-	x	x	-	-	-	-	Re	24%	N
Ramphodon naevius (Gmelin, 1789)[e] – beija-flor-rajado – Saw-billed Hermit	x	x	x	-	-	-	-	Re	100%	N
Colibri serrirostris (Vieillot, 1816) – beija-flor-de-orelha-violeta – White-vented Violetear	-	x	-	-	-	-	-	UR	16%	N
Heliothryx auritus (Gmelin, 1788) – beija-flor-de-bochecha-azul – Black-eared Fairy	-	x	-	-	-	-	-	OV	8%	N
Anthracothorax nigricollis (Vieillot, 1817) – beija-flor-de-veste-preta – Black-throated Mango	-	x	-	-	-	-	-	UR	12%	N
Lophornis chalybeus (Temminck, 1821) – topetinho-verde – Festive Coquette	-	x	-	-	-	-	-	OV	4%	N
Chlorostilbon lucidus (Shaw, 1812) – besourinho-de-bico-vermelho – Glittering-bellied Emerald	x	x	-	-	-	-	-	Re	56%	N
Stephanoxis lalandi (Vieillot, 1818)[e] – beija-flor-de-topete-verde – Green-crowned Plovercrest	-	x	-	-	-	-	-	OV	4%	N
Thalurania glaucopis (Gmelin, 1788) – beija-flor-de-fronte violeta – Violet-capped Woodnymph	x	x	x	-	-	-	-	Re	100%	N

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Eupetomena macroura (Gmelin, 1788) – beija-flor-tesoura – Swallow-tailed Hummingbird	-	x	-	-	-	-	-	Re	28%	N
Aphantochroa cirrochloris (Vieillot, 1818) – beija-flor-cinza – Sombre Hummingbird	-	x	x	-	-	-	-	Re	40%	N
Chrysuronia versicolor (Vieillot, 1818) – beija-flor-de-banda-branca – Versicolored Emerald	-	x	x	-	-	-	-	Re	40%	N
Leucochloris albicollis (Vieillot, 1818) - beija-flor-de-papo-branco - White-throated Hummingbird	-	x	-	-	-	-	-	OV	4%	N
Chionomesa fimbriata (Gmelin, 1788) - beija-flor-de-garganta-verde - Glittering-throated Emerald	-	x	-	-	-	-	-	Re	40%	N
Order Gruiformes										
Famíly Aramidae (1)										
Aramus guarauna (Linnaeus, 1766) – carão – Limpkin	-	-	x	-	-	-	-	OV	4%	AIC
Family Rallidae (13)										
Rallus longirostris Boddaert, 1783[a] – saracura-matraca- Mangrove Rail	x	-	-	-	-	-	-	UR	16%	AIC
Porphyrio martinica (Linnaeus, 1766) – frango-d'água-azul – Purple Gallinule	x	-	-	-	-	-	-	OV	4%	AO
Laterallus melanophaius (Vieillot, 1819) – sanã-parda – Rufous-sided Crake	x	-	-	-	-	-	-	Re	32%	AIC
Laterallus exilis (Temminck, 1831) – sanã-do-capim – Gray-breasted Crake	x	x	-	-	-	-	-	OV	8%	AIC
Laterallus leucopyrrhus (Vieillot, 1819) – sanã-vermelha – Red-and-white Crake	x	x	-	-	-	-	-	OV	8%	AIC
Mustelirallus albicollis (Vieillot, 1819) – sanã-carijó – Ash-throated Crake	x	x	-	-	-	-	-	UR	16%	AIC
Pardirallus nigricans (Vieillot, 1819) – saracura-sanã – Blackish Rail	x	-	-	-	-	-	-	OV	8%	AIC
Amaurolimnas concolor (Gosse, 1847) – saracura-lisa – Uniform Crake	x	x	x	-	-	-	-	Re	44%	TIC
Aramides mangle (Spix, 1825)[a] – saracura-do-mangue – Little Wood-Rail	x	-	-	-	-	-	-	UR	16%	AIC
Aramides cajaneus (Statius Muller, 1776) – saracura-três-potes – Gray-nacked Wood-Rail	x	x	-	-	x	-	-	Re	100%	AIC
Aramides saracura (Spix, 1825) – saracura-do-mato – Slaty-breasted Wood-Rail	-	x	-	-	-	-	-	OV	8%	AIC
Gallinula galeata (Lichtenstein, 1818) – galinha-d´água – Common Gallinule	x	-	-	-	-	-	-	UR	12%	AO
Fulica rufifrons Philippi & Landbeck, 1861 – carqueja-de-escudo-vermelho – Red-fronted Coot	-	x	-	-	-	-	-	OV	4%	AO
Order Charadriiformes										
Family Charadriidae (5)										
Pluvialis dominica (Statius Muller, 1776) – batuiruçu – American Golden-Plover	-	x	-	x	-	-	-	VN	4%	AIC
Pluvialis squatarola (Linnaeus, 1758) – batuiruçu-de-axila-preta – Black-bellied Plover	-	-	-	x	-	-	-	VN	4%	AIC
Vanellus chilensis (Molina, 1782) – quero-quero – Southern Lapwing	-	x	-	x	x	-	-	Re	100%	TIC
Charadrius semipalmatus Bonaparte, 1825 – batuíra-de-bando – Semipalmated Plover	-	-	-	x	x	-	-	Re	20%	AIC
Charadrius collaris Vieillot, 1818 – batuíra-de-coleira – Collared Plover	-	-	-	x	x	-	-	OV	4%	AIC
Family Haematopodidae (1)										
Haematopus palliatus Temminck, 1820 – piru-piru – American Oystercatcher	-	-	-	x	-	-	-	Re	40%	AIC
Family Recurvirostridae (1)										
Himantopus melanurus (Statius Muller, 1776)[1] pernilongo -de-costas-brancas - White-backed Stilt	-	-	-	x	-	-	-	OV	-	AIC
Family Scolopacidae (11)										
Limosa haemastica (Linnaeus, 1758) – maçarico-de-bico-virado – Hudsonian Godwit	-	-	-	x	-	-	-	VN	-	AIC
Calidris canutus (Linnaeus, 1758) – maçarico-de-papo-vermelho – Red Knot	-	-	-	x	-	-	-	VN	4%	AIC
Calidris alba (Pallas, 1764) – maçarico-branco – Sanderling	-	-	-	x	-	-	-	VN	4%	AIC
Calidris fuscicollis (Vieillot, 1819) – maçarico-de-sobre-branco – White-rumped Sandpiper	-	-	-	x	-	-	-	VN	4%	AIC

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Calidris pusilla (Linnaeus, 1766) – maçarico-rasteirinho – Semipalmated Sandpiper	-	-	-	x	-	-	-	VN	8%	AIC
Gallinago paraguaiae (Vieillot, 1816) – narceja –South American Snipe	-	x	-	-	-	-	-	OV	8%	TIC
Actitis macularius (Linnaeus, 1766) – maçarico-pintado – Spotted Sandpiper	x	-	-	-	-	-	-	Mi	72%	AIC
Tringa solitaria Wilson, 1813 – maçarico-solitário – Solitary Sandpiper	-	-	-	x	x	-	-	VN	-	AIC
Tringa melanoleuca (Gmelin, 1789) – maçarico-grande-de-perna-amarela – Greater Yellowlegs	-	-	-	x	x	-	-	VN	-	AIC
Tringa semipalmata (Gmelin, 1789) – maçarico-de-asa-branca - Eastern Willet	-	-	-	x	-	-	-	VN	-	AIC
Tringa flavipes (Gmelin, 1789) – maçarico-de-perna-amarela – Lesser Yellowlegs	-	-	-	x	x	-	-	VN	-	AIC
Family Jacanidae (1)										
Jacana jacana (Linnaeus, 1766) – jaçanã - Wattled Jacana	-	x	-	-	-	-	-	OV	-	AO
Family Stercorariidae (1)										
Stercorarius chilensis Bonaparte, 1857 – mandrião-chileno – Chilean Skua	-	-	-	-	-	x	-	VS	4%	DC
Family Laridae (7)										
Larus dominicanus Lichtenstein, 1823 – gaivotão - Kelp Gull	-	-	-	x	x	x	-	Re	100%	S
Rynchops niger Linnaeus, 1758 – talha-mar – Black Skimmer	-	-	-	x	x	x	-	Re	40%	P
Sterna hirundo Linnaeus, 1758 – trinta-réis-boreal – Common Tern	-	-	-	x	-	-	-	VN	8%	P
Sterna hirundinacea Lesson, 1831[a] – trinta-réis-de-bico-vermelho – South American Tern	-	-	-	x	-	-	-	Re	32%	P
Sterna trudeaui Audubon, 1838 – trinta-réis-de-coroa-branca – Snowy-crowned Tern	-	-	-	-	-	-	x	OV	8%	P
Thalasseus acuflavidus (Cabot, 1847)[a] – trinta-réis-de-bando – Cabot's Tern	-	-	-	x	x	x	-	Re	40%	P
Thalasseus maximus (Boddaert, 1783)[a] – trinta-réis-real – Royal Tern	-	-	-	x	x	x	-	Re	40%	P
Order Sphenisciformes										
Family Spheniscidae (1)										
Spheniscus magellanicus (Forster, 1781)[2] - pinguim-de-magalhães – Magellanic Penguin	-	-	-	x	-	-	-	VS	-	P
Order Procellariiformes										
Family Diomedeidae (2)										
Thalassarche chlororhynchos (Gmelin, 1789)[2,a] - albatroz-de-nariz-amarelo –Yellow-nosed Albatross	-	-	-	x	-	-	-	VS	-	P
Thalassarche melanophris (Temminck, 1828)[2,a] - albatroz-de-sobrancelha – Black-browned Albatross	-	-	-	x	-	-	-	VS	-	P
Family Procellariidae (5)										
Macronectes giganteus (Gmelin, 1789)[2] - petrel-grande – Southern Giant-Petrel	-	-	-	x	-	-	-	VS	-	DC
Macronectes halli Mathews, 1912[2] - petrel-grande-do-norte – Northern Giant-Petrel9	-	-	-	x	-	-	-	VS	-	DC
Procellaria aequinoctialis Linnaeus, 1758[2,a] - pardela-preta – White-chinned Petrel	-	-	-	x	-	-	-	VS	-	P
Calonectris borealis (Cory, 1881)[2] - cagarra-grande – Cory's Shearwater	-	-	-	x	-	-	-	VN	-	P
Puffinus puffinus (Brünnich, 1764) - pardela-sombria – Manx Shearwater	-	-	-	x	-	x	-	VN	4%	P
Order Suliformes										
Family Fregatidae (1)										
Fregata magnificens Mathews, 1914 – fragata – Magnificent Frigatebird	-	-	-	-	-	-	x	Re	100%	P
Family Sulidae (1)										
Sula leucogaster (Boddaert, 1783) – atobá-pardo – Brown Booby	-	-	-	x	-	x	-	Re	100%	P

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Family Phalacrocoracidae (1)										
Nannopterum brasilianum (Gmelin, 1789) – biguá – Neotropic Cormorant	x	-	-	x	x	x	-	Re	100%	P
Family Anhingidae (1)										
Anhinga anhinga (Linnaeus, 1766) – biguatinga – Anhinga	x	-	-	x	-	x	-	OV	8%	P
Order Pelecaniformes										
Family Ardeidae (11)										
Tigrisoma lineatum (Boddaert, 1783) - socó-boi – Rufescent Tiger-Heron	x	x	x	-	-	-	-	Re	28%	P
Cochlearius cochlearius (Linnaeus, 1766)[a] – arapapá – Boat-billed Heron	x	-	-	-	-	-	-	UR	12%	AIC
Nycticorax nycticorax (Linnaeus, 1758) – socó-dorminhoco – Black-crowned Night-Heron	x	-	-	-	x	-	-	Re	100%	P
Nyctanassa violacea (Linnaeus, 1758)[a] – savacu-de-coroa – Yellow-crowned Night-Heron	x	-	-	-	x	-	-	Re	100%	P
Butorides striata (Linnaeus, 1758) – socozinho – Striated Heron	x	-	-	-	-	-	-	Re	24%	P
Bubulcus ibis (Linnaeus, 1758) – garça-vaqueira – Cattle Egret	x	x	-	-	-	-	-	OV	8%	TIC
Ardea cocoi Linnaeus, 1766 – garça-moura – Cocoi Heron	x	-	-	-	x	-	-	Re	100%	P
Ardea alba Linnaeus, 1758 – garça-branca-grande – Great Egret	x	x	-	-	x	-	-	Re	100%	P
Syrigma sibilatrix (Temminck, 1824) – maria-faceira – Whistling Heron	-	x	-	-	-	-	-	OV	4%	TIC
Egretta thula (Molina, 1782) – garça-branca-pequena – Snowy Egret	x	-	-	x	x	-		Re	100%	P
Egretta caerulea (Linnaeus, 1758) – garça-azul – Little Blue Heron	x	-	-	x	x	-	-	Re	100%	P
Family Threskiornithidae (4)										
Eudocimus ruber Linnaeus, 1758[a] – guará – Scarlet Ibis	x	-	-	-	x	-	-	Re	100%	AIC
Pimosus infuscatus (Lichtenstein, 1823) – tapicuru – Bare-faced Ibis	x	-	-	-	-	-	-	OV	8%	AIC
Theristicus caudatus (Boddaert, 1783) – curicaca – Buff-necked Ibis	-	x	-	-	-	-	-	OV	12%	TIC
Platalea ajaja Linnaeus, 1758 – colhereiro – Roseate Spoonbill	x	-	-	-	x	-	-	Re	32%	AIC
Order Cathartiformes										
Family Cathartidae (3)										
Coragyps atratus (Bechstein, 1793) – urubu-preto – Black Vulture	x	x	x	x	-	-	-	Re	100%	S
Cathartes aura (Linnaeus, 1758) – urubu-de-cabeça-vermelha – Turkey Vulture	x	x	x	x	-	-	-	Re	100%	S
Cathartes burrovianus Cassin, 1845 – urubu-de-cabeça-amarela – Lesser Yellow-headed Vulture	-	-	-	x	-	-	-	OV	4%	S
Order Accipitriformes										
Family Pandionidae (1)										
Pandion haliaetus (Linnaeus, 1758) – águia-pescadora – Osprey	-	-	-	-	-	-	x	VN	4%	P
Family Accipitridae (17)										
Elanus leucurus (Vieillot, 1818) – gavião-peneira – White-tailed Kite	-	-	-	-	-	-	x	OV	4%	DC
Chondrohierax uncinatus (Temminck, 1822) – gavião-caracoleiro – Hook-billed Kite	-	-	x	-	-	-	-	OV	4%	TIC
Elanoides forficatus (Linnaeus, 1758) – gavião-tesoura – Swallow-tailed Kite	-	x	x	-	-	-	-	Mi	20%	DC
Spizaetus tyrannus (Wied, 1820)[a] – gavião-pega-macaco – Black Hawk-Eagle	-	-	-	-	-	-	x	Re	28%	DC
Harpagus diodon (Temminck, 1823) – gavião-bombachinha – Rufous-thighed Kite	-	-	x	-	-	-	-	OV	4%	DC
Ictinia plumbea (Gmelin,1788) – sovi – Plumbeous Kite	-	-	-	-	-	-	x	OV	4%	DAI

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Accipiter poliogaster (Temminck, 1824) – tauató-pintado – Gray-bellied Hawk	-	-	x	-	-	-	-	OV	4%	DC
Accipiter striatus Vieillot, 1808 – tauató-miúdo – Sharp-shinned Hawk	-	x	-	-	-	-	-	OV	4%	DC
Accipiter bicolor (Vieillot, 1817) – gavião-bombachinha-grande – Bicolored Hawk	x	x	x	-	-	-	-	UR	12%	DC
Buteogallus aequinoctialis (Gmelin, 1788)[a] – gavião-caranguejeiro – Rufous Crab Hawk	x	-	-	-	-	-	-	OV	4%	TIC
Amadonastur lacernulatus (Temminck, 1827)[a,c] – gavião-pombo-pequeno – White-necked Hawk	-	x	x	-	-	-	-	UR	12%	DC
Urubitinga urubitinga (Gmelin, 1788) – gavião-preto – Great BlackHawk	x	x	x	-	-	-	-	Re	20%	DC
Rupornis magnirostris (Gmelin, 1788) – gavião-carijó – Roadside Hawk	x	x	x	-	-	-	-	Re	100%	DC
Parabuteo unicinctus (Temmincki, 1824) – gavião-asa-de-telha - Harris's Hawk	-	x	-	-	-	-	-	OV	-	DC
Geranoaetus albicaudatus (Vieillot, 1816) – gavião-de-rabo-branco – White-tailed Hawk	-	-	-	-	-	-	-	OV	8%	DC
Pseudastur polionotus (Kaup, 1847)[a] – gavião-pombo – Mantled Hawk	-	-	x	-	-	-	-	OV	8%	DC
Buteo brachyurus Vieillot, 1916 – gavião-de-cauda-curta - Short-tailed Hawk	-	-	-	-	-	-	x	UR	12%	DC
Order Strigiformes										
Family Tytonidae (1)										
Tyto furcata (Temminck, 1827) – suindara – American Barn Owl	-	x	-	-	-	-	-	OV	4%	NC
Família Strigidae (5)										
Megascops choliba (Vieillot, 1817) – corujinha-do-mato – Tropical Screech-Owl	-	x	-	-	-	-	-	UR	16%	NC
Megascops atricapilla (Temminck, 1822) – corujinha-sapo – Black-capped Screech-Owl	-	x	x	-	-	-	-	Re	20%	NC
Athene cunicularia (Molina, 1782) – coruja-buraqueira – Burrowing Owl	-	x	-	-	-	-	-	Re	24%	NC
Asio clamator (Vieillot, 1808) – coruja-orelhuda – Striped Owl	x	-	-	-	-	-	-	UR	12%	NC
Asio stygius (Wagler, 1832) – mocho-diabo – Stygian Owl	-	x	-	-	-	-	-	OV	4%	NC
Order Trogoniformes										
Family Trogonidae (1)										
Trogon viridis Linnaeus, 1766 – surucuá-de-barriga-amarela – Green-backed Trogon	x	x	x	-	-	-	-	Re	100%	MSO
Order Coraciiformes										
Family Momotidae (1)										
Baryphthengus ruficapillus (Vieillot, 1818) – juruva – Rufous-capped Motmot	-	-	x	-	-	-	-	UR	12%	TIC
Family Alcedinidae (4)										
Megaceryle torquata (Linnaeus, 1766) – martim-pescador-grande – Ringed Kingfisher	x	-	-	-	x	x	-	Re	100%	P
Chloroceryle amazona (Latham, 1790) – martim-pescador-verde – Amazon Kingfisher	x	-	-	-	x	x	-	Re	100%	P
Chloroceryle americana (Gmelin, 1788) – martim-pescador-pequeno – Green Kingfisher	x	x	x	-	x	x	-	Re	100%	P
Chloroceryle inda (Linnaeus,1766) – martim-pescador-da-mata – Green-and-rufous Kingfisher	-	x	-	-	-	-	-	UR	12%	P
Order Galbuliformes										
Family Bucconidae (3)										
Nonnula rubecula (Spix, 1824) – macuru – Rusty-breasted Nunlet	-	-	x	-	-	-	-	UR	12%	MSI
Malacoptila striata (Spix, 1824)[c] – barbudo-rajado – Crescent-chested Puffbird	-	-	x	-	-	-	-	OV	4%	MSI
Notharchus swainsoni (Gray 1846) – macuru-de-barriga-castanha – Buff-bellied Puffbird	-	x	-	-	-	-	-	OV	-	CI
Order Piciformes										
Family Ramphastidae (4)										
Ramphastos toco Statius Muller, 1776 – tucanuçu – Toco Toucan	x	x	-	-	-	-	-	OV	4%	CO

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Ramphastos vitellinus Lichtenstein, 1823[a,e] – tucano-de-bico-preto – Channel-billed Toucan	-	-	x	-	-	-	-	Re	100%	CO
Ramphastos dicolorus Linnaeus, 1766[1] – tucano-de-bico-verde- Red-brested Toucan	-	-	x	-	-	-	-	OV	-	CO
Selenidera maculirostris (Lichtenstein, 1823)[a,e] – araçari-poca – Spot-billed Toucanet	-	x	x	-	-	-	-	Re	60%	MSO
Family Picidae (10)										
Picumnus temminckii Lafresnaye, 1845 – picapauzinho-de-coleira – Ochre-collared Piculet	x	x	x	-	-	-	-	Re	100%	MSI
Melanerpes candidus (Otto, 1796) – pica-pau-branco – White Woodpecker	x	x	-	-	-	-	-	OV	8%	MSI
Melanerpes flavifrons (Vieillot, 1818) – benedito-de-testa-amarela – Yellow-tufted Woodpecker	-	-	x	-	-	-	-	Re	36%	MSI
Veniliornis spilogaster (Wagler, 1827) – picapauzinho-verde-carijó – White-spotted Woodpecker	x	x	x	-	-	-	-	Re	80%	MSI
Campephilus robustus (Lichtenstein, 1818) – pica-pau-rei – Robust Woodpecker	x	x	x	-	-	-	-	Re	64%	MSI
Dryocopus lineatus (Linnaeus, 1766) – pica-pau-de-banda-branca – Lineated Woodpecker	-	x	x	-	-	-	-	Re	36%	MSI
Celeus flavescens (Gmelin, 1788) – pica-pau-de-cabeça-amarela – Blond-crested Woodpecker	x	x	x	-	-	-	-	Re	100%	MSI
Piculus flavigula (Boddaert, 1783) – pica-pau-bufador – Yellow-throated Woodpecker	x	x	x	-	-	-	-	Re	84%	MSI
Colaptes melanochloros (Gmelin, 1788) – pica-pau-verde-barrado – Green-barred Woodpecker	-	x	-	-	-	-	-	Re	28%	MSI
Colaptes campestris (Vieillot, 1818) – pica-pau-do-campo – Campo Flicker	-	x	-	-	-	-	-	Re	28%	SI
Order Falconiformes										
Family Falconidae (7)										
Herpetotheres cachinnans (Linnaeus, 1758) – acauã – Laughing Falcon	x	x	-	-	-	-	-	UR	16%	DC
Micrastur ruficollis (Vieillot, 1817) – falcão-caburé – Barred Forest-Falcon	-	-	x	-	-	-	-	UR	16%	DC
Micrastur semitorquatus (Vieillot, 1817) – falcão-relógio – Collared Forest-Falcon	-	x	x	-	-	-	-	Re	60%	DC
Caracara plancus (Miller, 1777) – carcará – Southern Caracara	x	x	-	x	-	-	-	Re	92%	DC
Milvago chimachima (Vieillot, 1816) – carrapateiro – Yellow-headed Caracara	x	x	x	x	x	x	-	Re	100%	DC
Milvago chimango (Vieillot, 1816) – chimango – Chimango Caracara	x	-	-	-	x	-	-	OV	8%	DC
Falco femoralis Temminck, 1822 – falcão-de-coleira – Aplomado Falcon	-	x	-	-	-	-	-	OV	4%	DC
Order Psittaciformes										
Family Psittacidae (7)										
Touit melanonotus (Wied, 1829)[a,e] – apuim-de-costas-pretas –Brown-backed Parrotlet	-	x	x	-	-	-	-	UR	12%	SMF
Brotogeris tirica (Gmelin, 1788)[e] – periquito-rico – Plain Parakeet	x	x	x	-	-	-	-	Re	80%	SMF
Pionopsitta pileata (Scopoli, 1769) – cuiú-cuiú – Pileated Parrot	-	x	x	-	-	-	-	Re	48%	SMF
Pionus maximiliani (Kuhl, 1820) – maitaca-verde – Scaly-headed Parrot	-	x	x	-	-	-	-	Re	88%	LCF
Amazona brasiliensis (Linnaeus, 1758)[a,e] – papagaio-da-cara-roxa – Red-tailed Parrot	x	x	x	-	-	-	-	Re	100%	LCF
Forpus xanthopterygius (Spix, 1824) – tuim – Blue-winged Parrotlet	x	x	x	-	-	-	-	Re	100%	SMF
Pyrrhura frontalis (Vieillot, 1817) – tiriba – Maroon-bellied Parakeet	-	x	x	-	-	-	-	Re	40%	SMF
Ordem Passeriformes										
Família Thamnophilidae (11)										
Terenura maculata (Wied, 1831) – zidedê – Streak-capped Antwren	-	-	x	-	-	-	-	OV	8%	MSI
Myrmotherula unicolor (Ménétriès, 1835)[a,e] – choquinha-cinzenta – Unicolored Antwren	-	x	x	-	-	-	-	Re	28%	MSI
Dysithamnus mentalis (Temminck, 1823) – choquinha-lisa – Plain Antvireo	-	x	x	-	-	-	-	Re	88%	MSI
Herpsilochmus rufimarginatus (Temminck, 1822) – chororozinho-de-asa-vermelha – Rufous-winged Antwren	-	x	x	-	-	-	-	Re	92%	MSI
Thamnophilus caerulescens Vieillot, 1816 – choca-da-mata – Variable Antshrike	x	x	-	-	-	-	-	Re	100%	MSI

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Hypoedaleus guttatus (Vieillot, 1816) – chocão-carijó – Spot-backed Antshrike	-	x	x	-	-	-	-	Re	44%	MSI
Mackenziaena leachii (Such, 1825) – borralhara-assobiadora – Large-tailed Antshrike	-	-	x	-	-	-	-	UR	12%	UI
Mackenziaena severa (Lichtenstein, 1823) – borralhara – Tufted Anthrike	-	x	x	-	-	-	-	UR	12%	UI
Myrmoderus squamosus (Pelzeln, 1868)[e] – papa-formiga-de-grota – Squamate Antbird	-	x	x	-	-	-	-	Re	84%	UI
Pyriglena leucoptera (Vieillot, 1818) – papa-taoca-do-sul – White-shouldered Fire-eye	-	x	x	-	-	-	-	Re	100%	UI
Drymophila squamata (Lichtenstein, 1823)[e] – pintadinho – Scaled Antbird	-	x	x	-	-	-	-	Re	100%	UI
Family Conopophagidae (2)										
Conopophaga melanops (Vieillot, 1818)[e] – cuspidor-de-máscara-preta – Black-cheeked Gnateater	-	-	x	-	-	-	-	OV	4%	UI
Conopophaga lineata (Wied, 1831) – chupa-dente – Rufous Gnateater	-	x	x	-	-	-	-	Re	24%	UI
Family Grallariidae (1)										
Cryptopezus nattereri (Pinto, 1937) – pinto-do-mato – Speckle-breasted Antpitta	-	-	x	-	-	-	-	OV	4%	UI
Family Rhinocryptidae (2)										
Psilorhamphus guttatus (Ménétriès, 1835) – tapaculo-pintado – Spotted Bamboowren	-	-	x	-	-	-	-	UR	12%	UI
Eleoscytalopus indigoticus (Wied, 1831)[e] – macuquinho – White-breasted Tapaculo	-	x	-	-	-	-	-	UR	16%	UI
Family Formicariidae (2)										
Formicarius colma Boddaert, 1783 – galinha-do-mato – Rufous-capped Antthrush	-	x	x	-	-	-	-	Re	100%	SI
Chamaeza meruloides Vigors, 1825[e] – tovaca-cantadora – Such's Antthrush	-	-	x	-	-	-	-	OV	4%	SI
Family Scleruridae (1)										
Sclerurus scansor (Ménétriès, 1835) – vira-folhas – Rufous-breasted Leaftosser	-	-	x	-	-	-	-	Re	28%	SI
Family Dendrocolaptidae (5)										
Sittasomus griseicapillus (Vieillot, 1818) – arapaçu-verde – Olivaceous Woodcreeper	-	x	x	-	-	-	-	Re	84%	MSI
Dendrocincla turdina (Lichtenstein, 1820) – arapaçu-liso – Plain-winged Woodcreeper	-	x	x	-	-	-	-	Re	100%	MSI
Dendrocolaptes platyrostris Spix, 1824 – arapaçu-grande – Planalto Woodcreeper	-	x	x	-	-	-	-	Re	52%	MSI
Xiphocolaptes albicollis (Vieillot, 1818) arapaçu-de-garganta-branca -White-throated Woodcreper	-	x	x	-	-	-	-	Re	84%	MSI
Xiphorhynchus fuscus (Vieillot, 1818) – arapaçu-rajado – Lesser Woodcreeper	-	x	x	-	-	-	-	Re	84%	MSI
Family Xenopidae (2)										
Xenops minutus (Sparrman, 1788) – bico-virado-miúdo – Plain Xenops	-	x	x	-	-	-	-	Re	88%	MSI
Xenops rutilans Temminck, 1821 – bico-virado-carijó – Streaked Xenops	x	-	-	-	-	-	-	Re	24%	MSI
Family Furnariidae (9)										
Furnarius rufus (Gmelin, 1788) – joao-de-barro – Rufous Hornero	x	x	-	-	-	-	-	Re	100%	SI
Lochmias nematura (Lichtenstein, 1823) – joão-porca – Sharp-tailed Streamcreeper	-	-	x	-	-	-	-	Re	20%	SI
Cichlocolaptes leucophrus (Jardine & Selby, 1830)[e] – trepador-sobrancelha – Pale-beowned Treehunter	-	x	x	-	-	-	-	Re	72%	MSI
Philydor atricapillus (Wied, 1821) – limpa-folhas-coroado - Black-capped Foliage-gleaner	-	x	x	-	-	-	-	Re	88%	MSI
Anabacerthia amaurotis (Temminck, 1823) – limpa-folha-miúdo – White-browed Foliage-gleaner	-	x	-	-	-	-	-	OV	4%	UI
Anabacerthia lichtensteini (Cabannis & Heine, 1859) – limpa-folhas-ocráceo – Ochre-breasted Foliage-gleaner	-	x	x	-	-	-	-	Re	28%	MSI
Automolus leucophthalmus (Wied, 1821) – barranqueiro-de-olho-branco - White-eyed Foliage-gleaner	-	x	x	-	-	-	-	Re	100%	UI
Synallaxis ruficapilla Vieillot, 1819 – pichororé – Rufous-capped Spinetail	-	x	-	-	-	-	-	UR	12%	UI
Synallaxis spixi Sclater, 1856 – joão-teneném - Spix's Spinetail	-	x	-	-	-	-	-	Re	25%	UI
Family Pipridae (2)										
Chiroxiphia caudata (Shaw & Nodder, 1793) – tangará – Swallow-tailed Manakin	-	x	x	-	-	-	-	Re	100%	MSF

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Manacus manacus (Linnaeus, 1766) – rendeira – White-bearde Manakin	-	x	-	-	-	-	-	Re	100%	MSF
Family Cotingidae (3)										
Carpornis melanocephala (Wied, 1820)[a,e] – sabiá-pimenta – Black-headed Berryeater	-	-	x	-	-	-	-	Re	68%	LCF
Pyroderus scutatus (Shaw, 1792)[a] – pavó – Red-ruffed Fruitcrow	-	x	x	-	-	-	-	Re	24%	LCF
Procnias nudicollis (Vieillot, 1817)[a] – araponga – Bare-throated Bellbird	x	x	x	-	-	-	-	Re	72%	LCF
Family Tityridae (6)										
Schiffornis virescens (Lafresnaye, 1838) – flautim – Greenish Schiffornis	-	x	x	-	-	-	-	Re	100%	MSO
Tityra inquisitor (Lichtenstein, 1823) – anambé-branco-de-bochecha-parda – Black-crowned Tityra	-	-	x	-	-	-	-	OV	4%	CO
Tityra cayana (Linnaeus, 1766) – anambé-branco-de-rabo-preto – Black-tailed Tityra	-	-	x	-	-	-	-	OV	4%	CO
Pachyramphus polychopterus (Vieillot, 1818) – caneleiro-preto – White-winged Becard	-	x	-	-	-	-	-	Re	24%	MSI
Pachyramphus marginatus (Lichtenstein, 1823) – caneleiro-bordado – Black-capped Becard	-	x	-	-	-	-	-	UR	12%	MSI
Pachyramphus validus (Lichtenstein, 1823) – caneleiro-de-chapéu-preto – Crested Becard	x	x	-	-	-	-	-	Re	36%	MSI
Family Oxyruncidae (1)										
Oxyruncus cristatus Swainson, 1821 – araponga-do-horto - Sharpbill	-	x	-	-	-	-	-	UR	12%	CO
Family Onychorhynchidae (1)										
Myiobius barbatus (Gmelin, 1789) – assanhadinho – Whiskered Flycatcher	-	x	x	-	-	-	-	Re	88%	UI
Family Platyrinchidae (2)										
Platyrinchus mystaceus Vieillot, 1818 – patinho – White-throated Spadebill	-	x	x	-	-	-	-	Re	80%	MSI
Platyrinchus leucoryphus Wied, 1831[a] – patinho-de-asa-castanha – Russet-winged Spadebill	-	x	x	-	-	-	-	Re	16%	MSI
Family Rhynchocyclidae (10)										
Mionectes rufiventris Cabanis, 1846 – abre-asa-de-cabeça-cinza – Gray-hooded Flycatcher	x	x	x	-	-	-	-	Re	48%	MSI
Leptopogon amaurocephalus Tschudi, 1846 – cabeçudo – Sepia-capped Flycatcher	x	x	x	-	-	-	-	Re	100%	MSI
Phylloscartes ventralis (Temminck,1824) – borboletinha-do-mato – Mottle-cheeked Tyrannulet	-	x	-	-	-	-	-	Re	20%	CI
Phylloscartes kronei Willis & Oniki, 1992[a,e] – maria-da-restinga – Restinga Tyrannulet	-	x	-	-	-	-	-	Re	24%	CI
Phylloscartes paulista Ihering & Ihering, 1907[a] – não-pode-parar – Sao Paulo Tyrannulet	-	x	x	-	-	-	-	UR	12%	CI
Phylloscartes oustaleti (Sclater, 1887)[e] – papa-moscas-de-olheiras – Oustalet's Tyrannulet	-	-	x	-	-	-	-	UR	12%	CI
Tolmomyias sulphurescens (Spix, 1825) – bico-chato-de-orelha-preta – Yellow-olive Flycatcher	-	x	x	-	-	-	-	Re	100%	MSI
Todirostrum poliocephalum (Wied, 1831)[e] – teque-teque – Gray-headed Tody-Flycatcher	-	x	x	-	-	-	-	Re	20%	MSI
Myiornis auriculares (Vieillot, 1818) – miudinho – Eared Pygmy-Tyrant	-	x	-	-	-	-	-	Re	28%	UI
Hemitriccus orbitatus (Wied, 1831)[e] – tiririzinho-do-mato – Eye-ringed Tody-Tyrant	-	x	-	-	-	-	-	Re	44%	MSI
Family Tyrannidae (35)										
Hirundinea ferruginea (Gmelin, 1788) – gibão-de-couro – Cliff Flycatcher	-	x	-	-	-	-	-	OV	4%	CI
Camptostoma obsoletum (Temminck, 1824) – risadinha – Southern Beardless-Tyrannulet	x	x	-	-	-	-	-	Re	100%	MSI
Elaenia flavogaster (Thunberg, 1822) – guaracava-de-barriga-amarela – Yellow-bellied Elaenia	x	x	-	-	-	-	-	Mi	64%	CO
Elaenia parvirostris Pelzeln, 1868 – tuque-pium – Small-billed Elaenia	x	x	-	-	-	-	-	OV	8%	CO
Elaenia mesoleuca (Deppe, 1830) – tuque – Olivaceous Elaenia	x	x	-	-	-	-	-	Mi	36%	CO
Elaenia obscura (d´Orbigny & Lafresnaye, 1837) – tucão – Highland Elaenia	x	x	-	-	-	-	-	Mi	44%	CO
Myiopagis caniceps (Swainson, 1835) – guaracava-cinzenta – Gray Elaenia	-	-	x	-	-	-	-	UR	12%	CI
Capsiempis flaveola (Lichtenstein, 1823) – marianinha-amarela – Yellow Tyrannulet	-	x	x	-	-	-	-	Re	20%	MSI
Phyllomyias fasciatus (Thunberg, 1822) – piolhinho – Planalto Tyrannulet	-	x	x	-	-	-	-	Re	20%	MSI
Phyllomyias griseocapilla Sclater, 1862[e] – piolhinho-serrano – Gray-capped Tyrannulet	-	x	x	-	-	-	-	Re	20%	MSI

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Serpophaga subcristata (Vieillot, 1817) – alegrinho – White-crested Tyrannulet	-	x	-	-	-	-	-	Re	24%	CI
Attila rufus (Vieillot, 1819)^{c} – capitão-de-saíra – Gray-hooded Attila	x	x	x	-	-	-	-	Re	100%	MSI
Legatus leucophaius (Vieillot, 1818) – bem-te-vi-pirata – Piratic Flicatcher	-	x	-	-	-	-	-	Re	24%	CO
Myiarchus swainsoni Cabanis & Heine, 1859 – irré – Swainson's Flycatcher	x	x	-	-	-	-	-	Re	92%	MSI
Myiarchus ferox (Gmelin, 1789) – maria-cavaleira – Short-crested Flycatcher	x	x	-	-	-	-	-	Re	28%	MSI
Sirystes sibilator (Vieillot, 1818) – gritador – Sibilant Sirystes	-	x	x	-	-	-	-	Re	100%	CI
Rhytipterna simplex (Lichtenstein, 1823) – vissiá – Grayish Mourner	-	-	x	-	-	-	-	UR	12%	CI
Pitangus sulphuratus (Linnaeus, 1766) – bem-te-vi – Great Kiskadee	x	x	-	-	x	-	-	Re	100%	CO
Machetornis rixosa (Vieillot, 1819) – suiriri-cavaleiro – Cattle Tyrant	-	x	-	-	-	-	-	Mi	20%	SI
Myiodinastes maculatus (Statius Muller, 1776) – bem-te-vi-rajado – Streaked Flycatcher	x	x	-	-	-	-	-	Mi	44%	CO
Megarynchus pitangua (Linnaeus, 1766) – neinei – Boat-billed Flycatcher	x	x	x	-	-	-	-	Re	76%	CO
Myiozetetes similis (Spix, 1825) – bentevizinho-de-penacho-vermelho – Social Flycatcher	-	x	x	-	-	-	-	Re	44%	CO
Tyrannus melancholicus Vieillot, 1819 – suiriri – Tropical Kingbird	x	x	-	-	-	-	-	Mi	64%	CI
Tyrannus savana Daudin, 1802 – tesourinha – Fork-tailed Flycatcher	-	x	-	-	-	-	-	Mi	28%	CI
Empidonomus varius (Vieillot, 1818) – peitica – Variegated Flycatcher	x	x	-	-	-	-	-	Mi	36%	CO
Conopias trivirgatus (Wied, 1831) – bem-te-vi-pequeno – Three-striped Flycatcher	-	x	x	-	-	-	-	Re	44%	CI
Colonia colonus (Vieillot, 1818) – viuvinha – Long-tailed Tyrant	-	x	-	-	-	-	-	OV	4%	CI
Fluvicola nengeta (Linnaeus, 1766) – lavadeira-mascarada – Masked Water-Tyrant	x	x	-	x	x	-	-	Re	100%	SI
Pyrocephalus rubinus (Boddaert, 1783) – príncipe – Vermilion Flycatcher	-	x	-	-	-	-	-	Mi	24%	MSI
Muscipipra vetula (Lichtenstein, 1823) – tesoura-cinzenta - Shear-tailed Gray Tyrant	-	x	-	-	-	-	-	UR	12%	CI
Myiophobus fasciatus (Statius Muller, 1776) – filipe – Bran-colored Flycatcher	-	x	-	-	-	-	-	Mi	32%	UI
Cnemotriccus fuscatus (Wied, 1831) – guaracavuçu – Fuscous Flycatcher	-	x	x	-	-	-	-	Mi	60%	UI
Lathrotriccus euleri (Cabanis, 1868) – enferrujado – Euler's Flycatcher	-	x	x	-	-	-	-	Mi	60%	UI
Contopus cinereus (Spix, 1825) – papa-moscas-cinzento - TropicalPewee	-	x	-	-	-	-	-	Re	20%	CI
Satrapa icterophrys (Vieillot, 1818) – suiriri-pequeno – Yellow-browed Tyrant	-	x	-	-	-	-	-	UR	16%	MSI
Family Vireonidae (2)										
Hylophilus poicilotis Temminck, 1822 – verdinho-coroado – Rufous-crowned Greenlet	-	x	-	-	-	-	-	UR	16%	MSO
Vireo chivi (Vieillot, 1817) – juruviara – Chivi Vireo – Azure Jay	x	x	-	-	-	-	-	Mi	56%	CO
Family Corvidae (1)										
Cyanocorax caeruleus (Vieillot, 1818) – gralha-azul	x	x	x	-	-	-	-	Re	100%	MSO
Family Hirundinidae (6)										
Pygochelidon cyanoleuca (Vieillot, 1817) – andorinha-pequena-de-casa - Blue-and-white Swallow	-	x	-	-	-	-	-	Re	20%	DAI
Stelgidopteryx ruficollis (Vieillot, 1817) – andorinha-serradora – Southern Rough-winged Swallow	x	x	-	-	-	-	-	Mi	64%	DAI
Progne tapera (Vieillot, 1817) – andorinha-do-campo – Brown-chested Martin	x	x	-	-	-	-	-	Re	28%	DAI
Progne chalybea (Gmelin, 1789) – andorinha-grande – Gray-breasted Martin	x	x	-	-	-	-	-	Re	68%	DAI
Tachycineta leucorrhoa (Vieillot, 1817) – andorinha-de-sobre-branco – White-rumped Swallow	x	x	-	-	-	-	-	Re	20%	DAI
Hirundo rustica Linnaeus, 1758 – andorinha-de-bando – Barn Swallow	-	x	-	-	-	-	-	OV	4%	DAI
Family Troglodytidae (2)										
Troglodytes musculus Naumann, 1823 – curruira – Southern House Wren	x	x	-	-	-	-	-	Re	100%	UI
Cantorchilus longirostris (Vieillot, 1819)^{c} – garrinchão-de-bico-grande – Long-billed Wren	x	x	x	-	-	-	-	Re	100%	UI
Family Polioptilidae (1)										
Ramphocaenus melanurus Vieillot, 1819 – chirito – Long-billed Gnatwren	-	x	-	-	-	-	-	UR	12%	UI

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Family Turdidae (5)										
Turdus flavipes Vieillot, 1818 – sabiá-una – Yellow-legged Thrush	x	x	x	-	-	-	-	Re	92%	MSO
Turdus leucomelas Vieillot, 1818 – sabiá-barranco – Pale-breasted Thrush	x	x	x	-	-	-	-	UR	12%	MSO
Turdus rufiventris Vieillot, 1818 – sabiá-laranjeira – Rufous-bellied Thrush	x	x	x	-	x	-	-	Re	100%	MSO
Turdus amaurochalinus Cabanis, 1850 – sabiá-poca – Creamy-bellied Thrush	x	x	-	-	x	-	-	Re	100%	MSO
Turdus albicollis Vieillot, 1818 – sabiá-coleira – White-necked Thrush	x	x	x	-	-	-	-	Re	100%	MSO
Família Mimidae (1)										
Mimus saturninus (Lichtenstein, 1823) – sabiá-do-campo – Chalk-browed Mockingbird	-	x	-	-	-	-	-	UR	16%	MSO
Family Motacillidae (1)										
Anthus chii Vieillot, 1818 – caminheiro-zumbidor - Yellowish Pipit	-	x	-	-	-	-	-	OV	8%	SI
Family Fringillidae (4)										
Spinus magellanicus (Vieillot, 1805) – pintassilgo – Hooded Siskin	-	x	-	-	-	-	-	OV	4%	UG
Euphonia chalybea (Mykan, 1825)[a] – cais-cais – Green-throated Euphonia	-	x	x	-	-	-	-	Re	20%	MSO
Euphonia violacea (Linnaeus, 1758) – gaturamo-verdadeiro – Violaceous Euphonia	x	x	x	-	-	-	-	Re	100%	MSO
Euphonia pectoralis (Latham, 1801) – ferro-velho – Chestnut-bellied Euphonia	-	x	x	-	-	-	-	Re	100%	MSO
Family Passerellidae (1)										
Zonotrichia capensis (Statius Muller, 1776) – tico-tico – Rufous-collared Sparrow	x	x	-	-	-	-	-	Re	100%	SG
Family Icteridae (4)										
Sturnella superciliaris (Bonaparte, 1850) – polícia-inglesa-do-sul – White-browed Meadowlark	-	x	-	-	-	-	-	OV	-	MSG
Cacicus haemorrhous (Linnaeus, 1766) – guaxe – Red-rumped Cacique	x	x	x	-	-	-	-	Re	100%	CO
Icterus pyrrhopterus (Vieillot, 1819) – encontro – Variable Oriole	-	x	-	-	-	-	-	UR	12%	CO
Molothrus bonariensis (Gmelin, 1789) – chupim – Shiny Cowbird	x	x	-	-	-	-	-	Mi	60%	SO
Family Parulidae (4)										
Geothlypis aequinoctialis (Gmelin, 1789) – pia-cobra – Masked Yellowthroat	x	x	-	-	-	-	-	Re	100%	UI
Setophaga pitiayumi (Vieillot, 1817) – mariquita – Tropical Parula	x	x	-	-	-	-	-	Re	100%	MSI
Myiothlypis rivularis (Wied, 1821) – pula-pula-ribeirinho – Neotropical River Warbler	x	x	x	-	-	-	-	Re	100%	SI
Basileuterus culicivorus (Deppe, 1830) – pula-pula – Golden-crowned Warbler	x	x	x	-	-	-	-	Re	100%	UI
Family Mitrospingidae (1)										
Orthogonys chloricterus (Vieillot, 1819)[e] – catirumbava – Olive-green Tanager	-	-	x	-	-	-	-	Re	28%	CO
Family Cardinalidae (1)										
Habia rubica (Vieillot, 1817) – tiê-de-bando – Red-crowned Ant-Tanager	-	x	x	-	-	-	-	Re	100%	UO
Family Thraupidae (31)										
Chlorophanes spiza (Linnaeus, 1758) – saí-verde – Green Honeycreeper	-	x	x	-	-	-	-	Re	40%	CO
Hemithraupis ruficapilla (Vieillot, 1818)[e] – saíra-ferrugem – Rufous-headed Tanager	-	x	x	-	-	-	-	Re	24%	CO
Tersina viridis (Illiger, 1811) – saí-andorinha – Swallow Tanager	x	x	x	-	-	-	-	Re	40%	MSO
Dacnis cayana (Linnaeus, 1766) - saí-azul – Blue Dacnis	-	x	x	-	-	-	-	Re	44%	CO
Saltator similis d´Orbigny & Lafresnaye, 1837 – trinca-ferro – Green-winged Saltator	-	x	-	-	-	-	-	Re	28%	MSO
Saltator fuliginosus (Daudin, 1800) – bico-de-pimenta – Black-throated Grosbeak	-	x	-	-	-	-	-	OV	4%	MSO
Coereba flaveola (Linnaeus, 1758) – cambacica – Bananaquit	x	x	x	-	-	-	-	Re	100%	N
Asemospiza fuliginosa (Wied, 1830) – cigarra-preta – Sooty Grassquit	-	-	x	-	-	-	-	Re	20%	UG
Volatinia jacarina (Linnaeus, 1766) – tiziu – Blue-black Grassquit	-	x	-	-	-	-	-	OV	8%	UG
Trichothraupis melanops (Vieillot, 1818) – tiê-de-topete – Black-goggled Tanager	x	x	x	-	-	-	-	Re	100%	UO
Loriotus cristatus (Linnaeus, 1766) – tiê-galo – Flame-crested Tanager	x	x	x	-	-	-	-	Re	100%	MSO

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Tachyphonus coronatus (Vieillot, 1822) – tiê-preto – Ruby-crowned Tanager	x	x	x	-	-	-	-	Re	100%	MSO
Ramphocelus bresilius (Linnaeus, 1766)[e] – tiê-sangue – Brazilian Tanager	x	x	-	-	-	-	-	Re	100%	MSO
Sporophila lineola (Linnaeus, 1758) – bigodinho – Lined Seedeater	-	x	-	-	-	-	-	UR	16%	UG
Sporophila frontalis (Verreaux, 1869)[a] – pixoxó – Buffy-fronted Seedeater	-	x	-	-	-	-	-	Re	28%	MSG
Sporophila caerulescens (Vieillot, 1823) – coleirinho – Double-collared Seedeater	x	x	-	-	-	-	-	Re	100%	UG
Sporophila angolensis (Linnaeus, 1766)[a] – curió – Chestnut-bellied Seed-Finch	-	x	-	-	-	-	-	Re	24%	UG
Thlypopsis sordida (d´Orbigny & Lafresnaye, 1837) – saí-canário – Orange-headed Tanager	-	-	x	-	-	-	-	UR	12%	CO
Conirostrum speciosum (Temminck, 1824) – figuinha-de-rabo-castanho – Chestnut-vented Conebill	-	-	x	-	-	-	-	UR	16%	CO
Conirostrum bicolor (Vieillot, 1809)[a] – figuinha-do-mangue - BicoloredConebill	x	-	-	-	-	-	-	Re	100%	MSI
Sicalis flaveola (Linnaeus, 1766) – canário-da-terra – Saffron Finch	x	x	-	-	-	-	-	Re	100%	SG
Haplospiza unicolor Cabanis, 1851 – cigarra-bambu – Uniform Finch	-	x	-	-	-	-	-	Re	36%	UG
Pipraeidea melanonota (Vieillot, 1819) – saíra-viúva – Fawn-breasted Tanager	-	x	x	-	-	-	-	Re	24%	CO
Schistoclamys ruficapillus (Vieillot, 1817)[1] – bico-de-veludo – Cinnamon Tanager	-	x	-	-	-	-	-	OV	-	MSG
Thraupis sayaca (Linnaeus, 1766) – sanhaço-cinzento – Sayaca Tanager	x	x	x	-	-	-	-	Re	100%	CO
Thraupis cyanoptera (Vieillot, 1817) – sanhaço-de-encontro-azul – Azure-chouldered Tanager	-	x	x	-	-	-	-	Re	56%	CO
Thraupis palmarum (Wied, 1821) – sanhaço-do-coqueiro – Palm Tanager	x	x	x	-	-	-	-	Re	100%	CO
Thraupis ornata (Sparrman, 1789)[e] – sanhaço-de-encontro-amarelo – Golden-chevroned Tanager	-	-	x	-	-	-	-	UR	12%	CO
Stilpnia peruviana (Desmarest, 1806)[a,e] – saíra-sapucaia – Chestnut-backed Tanager	x	x	x	-	-	-	-	Re	100%	CO
Tangara seledon (Statius Muller, 1776) – saíra-sete-cores – Green-headed Tanager	x	x	x	-	-	-	-	Re	100%	CO
Tangara cyanocephala (Statius Muller, 1776) – saíra-militar- Red-necked Tanager	x	x	x	-	-	-	-	Mi	32%	CO

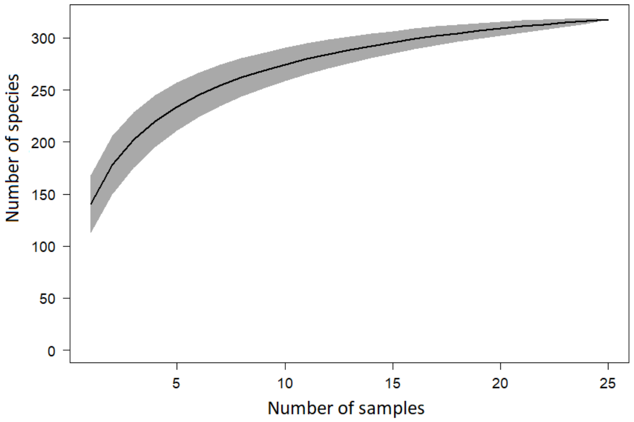

Figure 2. Species accumulation curve to Parque Estadual Ilha do Cardoso (PEIC) avifauna based on 321 species recorded in field (excluded those was recorded by pictures and after to September 2017).

Piping-Guan), *Touit melanonotus* (Brown-backed Parrotlet) and *Carpornis melanocephala* (Black-headed Berryeater) (IUCN 2017).

Through use mist-nets, were captured 81 bird's species, which corresponds to 24.3% of the total species recorded to Parque Estadual Ilha do Cardoso. Despite only one species has been recorded solely by mist-nets (*Baryphthengus ruficapillus* – Rufus-capped Motmot), is important to pay attention in fact that their utilization made it possible to capture birds which, in certain occasions, would go unnoticed, either by the influence of the year period that turns they less conspicuous or by their ecological habits, as some representatives of families Xenopidae, Onychorhynchidae, Platyrinchidae and Rhynchocyclidae. Accordingly, it is worth mentioning that obtaining a more complete inventory is only possible by using techniques such as visual and auditory contact and mist-nets, reducing possible biases that could be generated, for example, variations in the auditory and visual accuracy of researchers (Karr 1981, Pearman 2002, Dunn & Ralph 2004).

The restinga was the ecosystem which gathered the biggest number of species (N = 232). This expressive richness could be explained by the fact that in the Northeast portion of the island (sampled area), restinga is between mangrove and forest, receiving influences by both ecosystems for compose its own avifauna. Thus, it is possible that some bird's species occur only eventually in the ecosystem, which are attracted by resources or just by the fact that been moving through the different sections, as occur, for example, with *Automolus leucophthalmus* (White-eyed Foliage-gleaner) which is seen commonly in forest, but in two occasions was detected as part of a mixed flock feeding on insects in restinga's understory.

The high avian richness recorded in mangrove (N = 118), included some species which are typical from forest, such as *Xenops rutilans* (Streaked Xenops), *Mionectes rufiventris* (Grey-hooded Flycatcher), *Leptopogon amaurocephalus* (Sepia-capped Flycatcher), *Turdus flavipes* (Yellow-legged Thrush) e *Ramphodon naevius* (Saw-billed Hermit). Sick (2001) had already highlighted how great is richness of species which inhabits mangrove, included some Passeriformes and, mainly, species which feed from aquatic invertebrates and fish. The record of species typically considered forest reinforce the fact of the specie richness on mangrove could be underestimated, what increases the importance of new studies be developed in such ecosystem. In addition, the fact of species considered typical from a specific ecosystem were also recorded in mangrove, could reflect in a bigger plasticity on their part to explore environment, resulting in a better comprehension of their ecological questions.

Concerning forest's richness (N = 145), based on assumption that the sampling was made in the plains, with a maximum lift of 50 m above sea level, it is possible that in slopes and hilltop, could exist more species which are not recorded in this study, once higher altitudes was explored sporadically. Such hypothesis is based on the fact of the avifauna's composition vary in diversity and abundance along altitudinal gradience (Blake & Loiselle 2000, Mallet-Rodrigues 2010). Therefore, even though an expressive number of species was being recorded, such richness may still be underestimated.

The species richness showed a significative difference between years and seasons (spring, summer, fall and winter). Concerning richness differences detected between years, there is emphasis on the first sampled year (September 2015 to August 2016) which concentrated a higher amount of rain if compared to the next year (personal observation). Thus, even if the sampling has been made in corresponding weeks and months, adverse weather conditions may have influenced on behavior, and consequently, in species detectability.

Analyzing monthly within seasons, the highest richness was obtained from September to December (spring), justified by the fact that, in this period, the most Brazilian birds start their reproductive activity (Sick 2001), when they end up exposing themselves more (visually or by their vocalizations), what facilitates their record. In PEIC was possible to detect the increase of conspicuity of many species in this time, mainly by the increase of their vocalization, such as *Tinamus solitarius* (Solitary Tinamou), *Odontophorus capueira* (Spot-winged Wood-Quail), *Trogon viridis* (Green-backed Trogon), *Pyriglena leucoptera* (White-shouldered Fire-Eyed), *M. rufiventris*, *Chiroxiphia caudata* (Swallow-tailed Manakin), *Turdus* spp. and *Euphonia* spp.

Another important factor which directly contributed to the richness increase in the period from September to March (Spring and Summer) is the arrival of migratory species, coming search warmer areas and with food offer, good factors to breed (Alves 2007). In PEIC stands out the arrival of species from genus *Elaenia*, *Tyrannus savanna* (Fork-tailed Flycatcher), *Tyrannus melancholicus* (Tropical Kingbird), *Vireo chivi* (Chivi Vireo), *Stelgidopteryx ruficollis* (Southern Rough-winged Swallow), *Cnemotriccus fuscatus* (Fuscus Flycatcher), *Lathrotriccus euleri* (Euler's Flycatcher), *Myiodynastes maculatus* (Streaked Flycatcher) and *Empidonomus varius* (Variegated Flycatcher). Furthermore, the richness is increased by species in migration routes which uses beaches and sandbar to rest and feed themselves (identified here as "Visitors from South and North Hemisphere"), such as some Procellariiformes and Charadriiformes.

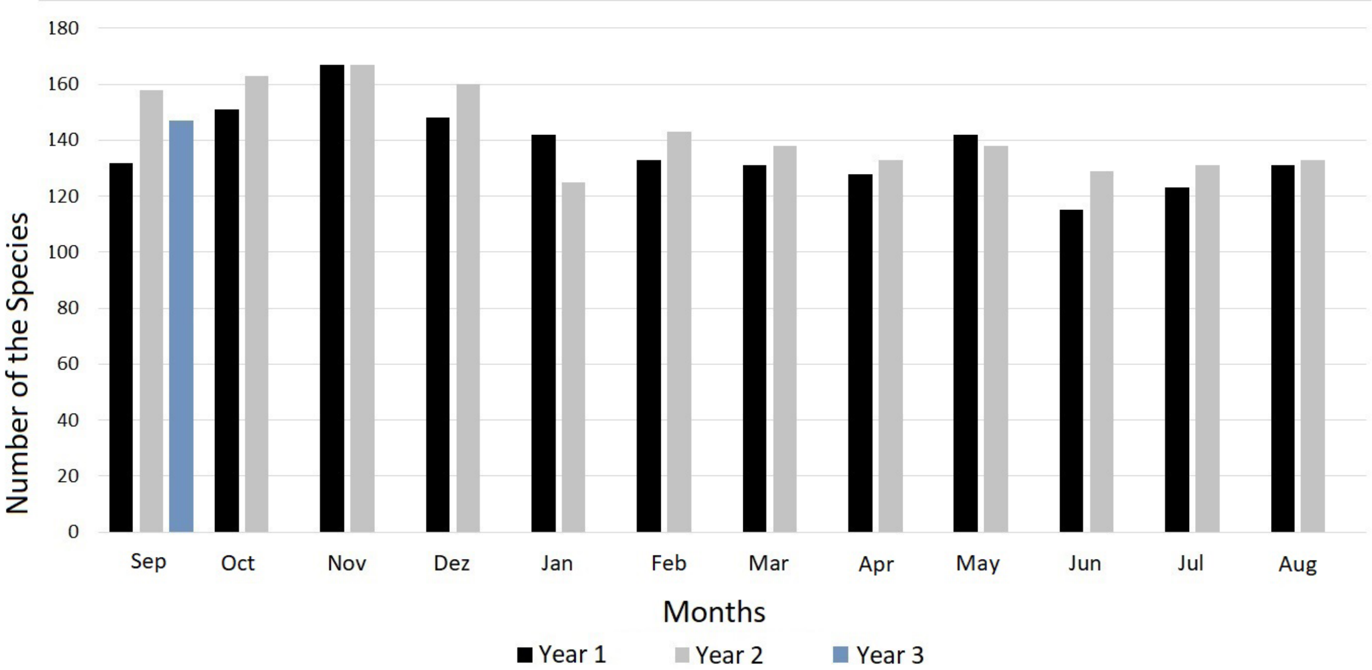

Figure 3. Number of species recorded among sampled months in the period from September 2015 to September 2017 in Parque Estadual Ilha do Cardoso, South coast of São Paulo's State.

The high number of identified guilds (N = 25) could be a reflex of ecosystem diversity that compose the studied area, what provides a vary of niches to be occupied. The fact of the main type of guild, both in mangrove, forest and restinga, be composed of insectivores, is justified, in a first moment, by the fact such resource is abundant in tropical ecosystems (Erwin 1982, Stork 1988, Basset 2012) thus ensuring coexistence of different species. A second factor would be the conservation status of the island, once insectivore birds are susceptible to environmental degradation (Wills 1979, Bierregaard Jr & Stouffer 1997, Goerck 1997), since in altered environments has a decrease of micro habitats, which leads to a decrease in insect's abundance (Sodhi et al. 2004). In the case of the second more common guild, composed by omnivores, its higher number was expected, since those birds explores a wider range of food items and tend to widely occupy ecosystems, once they don't depend of a specific resource availability (Willis 1979, Anjos & Bóçon 1999).

In mangrove, the number of omnivores (N = 30) was very similar to the insectivores (N = 31). Such result is interesting because some of these species shows a strongly tendency to frugivory (Sick 2001), and, of these, ten show FO = 100%. Therefore, added to the fact of eight frugivore species also occur in mangrove, it is emphasizing that the richness in such ecosystem could be underestimated if we only evaluate the environmental features when checking species that occur there. These species can regularly occupy this ecosystem (probably with a mostly compound diet by insects) and moving to restinga areas around searching for fruits that complement their diet. Similarly, species which occur in restinga and forest, eventually can moving to the mangrove searching for insects and other invertebrates that are abundant in these ecosystems.

Frugivores, nectivores and granivores species had their occurrence restricted to ecosystems as mangrove, restinga and forest. Between nectivores, four species occurrences in mangrove (*Chlorostilbon lucidus* – Glittering-bellied Emerald, *Ramphodon naevius* – Saw-billed Hermit, *Thalurania glaucopis* – Violet-capped Woodnymph and *Coereba flaveola* – bananaquit) indicates a higher plasticity by them to explore this environment, once other 11 species of Trochilidae are concentered in restinga and forest. In the *C. flaveola* (Thraupidae) case, the specie was commonly seen caching insects among vegetation; while the three species of Trochilidae were seen exploring flowers from *Vrisea*, very common in PEIC's mangroves (Barros et al. 1991). There is more frugivores in restinga and forest, ecosystems which reunite many fruitful species that provides food to birds, whereas its occurrence in mangrove can occur only eventually, as previously mentioned. Granivores species recorded are typical from open environment, occur in PEIC mainly in shrub restinga areas and in the transition range with mangrove. In forest ecosystem, the only granivore recorded was *Asemospiza fuliginosa* (Sooty Grassquit), specie from understory which diet is based mainly on bamboo seeds (Sick 2001).

Carnivores species belonging to Falconiformes and Accipitriformes orders were recorded in all sampled ecosystems, while Strigiformes were seen only on mangrove, restinga and forest. From an ecological point of view, the presence of carnivore birds contributes directly to the ecosystem's balance, once they influence in populational dynamics of their preys (Terborgh 1992, Jaksik 2002). Similar ecological importance applies to scavengers, in PEIC represented by four species (*Coragyps atratus* – Black Vulture, *Cathartes aura* – Turkey Vulture, *Cathartes burrovianus* – Lesser Yellow-headed Vulture and *Larus dominicanus* – Kelp Gull). The scavengers decrease is taken as a

Table 2. Species with some level of threat recorded in Parque Estadual Ilha do Cardoso (PEIC) in State Level (S), National Level (N) and Worldwide Level (W). Threat category: CR (Critically Endangered), EN (Endangered), VU (Vulnerable), NT (Near Threatened), LC (Least Concern) and DD (Data Deficient).

Species	**S**	**N**	**W**
Tinamus solitarius	VU	-	NT
Aburria jacutinga	CR	EN	EN
Thalassarche chlororhynchus	VU	EN	EN
Thalassarche melanophris	VU	-	NT
Sterna hirundinacea	NT	VU	LC
Procellaria aequinoctialis	VU	VU	VU
Cochlearius cochlearius	EN	-	LC
Nyctanassa violacea	VU	-	LC
Eudocimus ruber	EN	-	LC
Buteogallus aequinoctialis	CR	-	NT
Amadonastur lacernulatus	VU	VU	VU
Pseudastur polionotus	VU	-	NT
Spizaetus tyrannus	VU	-	LC
Rallus longirostris	LC	-	LC
Aramides mangle	DD	-	LC
Thalasseus acuflavidus	VU	-	LC
Thalasseus maximus	VU	EN	LC
Touit melanonotus	VU	VU	VU
Amazona brasiliensis	EM	-	VU
Ramphastos vitellinus	CR	-	VU
Selenidera maculirostris	NT	-	LC
Myrmotherula unicolor	VU	-	NT
Carpornis melanocephala	CR	VU	VU
Pyroderus scutatus	VU	-	LC
Procnias nudicollis	VU	-	VU
Platyrinchus leucoryphus	VU	-	VU
Phylloscartes kronei	VU	-	VU
Phylloscartes paulista	VU	-	NT
Stilpnia peruviana	EM	VU	VU
Conirostrum bicolor	LC	-	NT
Sporophila frontalis	CR	VU	VU
Sporophila angolensis	VU	-	LC
Euphonia chalybea	VU	-	NT

serious ecological problem in some parts of the globe, where their decay triggers a trophic cascade process in all community (Buechley & Şekercioğlu 2016). Thus, the abundance of scavengers in PEIC (except *C. burrovianus*, with occasional occurrence), is an indicator that nutrient cycling in the environment occur intensively, thus contributing to its balance.

The record of piscivores and invertebrate consumer's species is clustered mainly in beach, sandbar and mangrove ecosystems. Such result reflects richness that characterizes waters around the island and ensure a wide food source to bird species, mainly from Pelecaniformes, Gruiformes, Suliformes and Charadriiformes orders. Here we include the Procellariiformes within the "Piscivore" guild, however, it is worth mentioning that the composition of the diet of this taxon varies seasonally, with the predominance of fish or cephalopods (Vooren & Fernandes 1989).

The richness of species mentioned in this study, highlights the importance of preserving forest remnants in coastal regions of São Paulo's State, aiming to maintain Atlantic Forest diversity. Finally, this study, made through a great field effort (25 months) bring a better knowledge of PEIC's avifauna, such effort that should serve as basis for establishing actions aimed ate the management of the park as well as the conservation units that are distributed in its surroundings.

Table 3. Total number of species and specie/ecosystem recorded in Parque Estadual Ilha do Cardoso (SP) among each trophic guild. Bold values correspond to ecosystem which take the biggest number of specie within each guild. In the squares values correspond to the main guild in each ecosystem. Caption: **C** – Whole Community; **M** – Mangrove; **R** – Restinga; **F** – Forest; **Sb** – Sand-bar; **B** – Beach; **Mr** – Marine; **Fl** – Flying.

Guilds	C	M	R	F	Sb	B	Mr	Fl
Medium-Strata Insectivores	55	18	**47**	38	0	0	0	0
Canopy Omnivores	33	15	**25**	21	1	0	0	0
Piscivores	31	13	4	2	13	**16**	12	3
Aquatic Invertebrates Consummers	31	**14**	5	1	7	10	0	0
Daytime Carnivores	24	7	**12**	11	1	5	2	3
Understory Insectivores	23	4	**19**	14	0	0	0	0
Medium-strata Omnivores	20	12	**20**	13	1	0	0	0
Canopy Insectivores	16	1	**13**	6	0	0	0	0
Nectarivores	15	4	**15**	6	0	0	0	0
Large Canopy Frugivores	11	4	**9**	**9**	0	0	0	0
Daytime Aerial Insectivores	11	4	**6**	0	0	0	0	5
Soil Insectivores	10	3	**7**	5	1	1	0	0
Terrestrial Invertebrates Consummers	8	2	**6**	3	1	1	0	0
Understory Granivores	7	1	**6**	1	0	0	0	0
Large Soil Frugivores	6	2	**4**	**4**	0	0	0	0
Nocturnal Aerial Insectivores	6	1	**6**	0	0	0	0	0
Nocturnal Carnivores	6	1	**5**	1	0	0	0	0
Aquatic Omnivores	6	2	**3**	0	1	1	0	0
Small-Medium Canopy Frugivores	5	2	**5**	**5**	0	0	0	0
Scavengers	4	3	2	2	2	**4**	1	0
Soil Granivores	4	3	**4**	0	0	0	0	0
Medium-strata Granivores	3	0	**3**	0	0	0	0	0
Medium-strata Frutivores	2	0	**2**	1	0	0	0	0
Understory Omnivores	2	1	**2**	**2**	0	0	0	0
Soil Omnivores	1	**1**	**1**	0	0	0	0	0

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Author Contributions

Henrique Chupil: 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.

Emygdio Leite de Araujo Monteiro-Filho: Substantial contribution in the concept and design of the study; Contribution to data analysis and interpretation; Contribution to critical revision, adding intelectual content.

Conflicts of Interest

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

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Short Communication

Molecular confirmation of twinning in the West Indian Manatee (*Trichechus manatus*)

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Abstract: Few twinning events have been recorded in the West Indian manatee (*Trichechus manatus*, Sirenia: Trichechidae) and no previous published study has provided confirmation of this phenomenon based in molecular tools. Here we investigate a possible case of twinning in an endangered Brazilian population of *T. manatus* using molecular tools. We analyzed two male neonates found stranded in Ceará State, on the northeastern coast of Brazil. The DNA of both individuals was isolated, and 10 microsatellite loci were amplified and genotyped. Following the identification of the alleles, the probabilities of identity by descent (Δ_7 and Δ_8) and relatedness (r_{xy}) were calculated using estimators that evaluate inbreeding. The two individuals shared most of the alleles, with differences in the genotypes being identified in only two loci. All the estimators identified a level of relatedness compatible with that found between siblings (selfed or outbred), indicating they were dizygotic twins. This is the first confirmed case of fraternal twins in free-ranging West Indian manatees in South America. The recognition of this type of twinning provides elements to improve actions for the rehabilitation of stranded animals and their subsequent release to the environment.

Keywords: *Sirenians; relatedness; microsatellite; stranding; dizygotic twins.*

Confirmação molecular de gemelaridade em peixes-boi marinho (*Trichechus manatus*)

Resumo: Poucos eventos de gemelaridade foram registrados para o peixe-boi marinho (*Trichechus manatus*, Sirenia: Trichechidae) e nenhum estudo previamente publicado confirmou esse fenômeno com base em ferramentas moleculares. Aqui investigamos um possível caso de gemelaridade em uma ameaçada população brasileira de *T. manatus* utilizando ferramentas moleculares. Foram analisados dois neonatos machos encontrados encalhados no Ceará, costa nordeste do Brasil. O DNA dos indivíduos foi isolado e 10 loci microsatélites foram amplificados e genotipados. Após a identificação dos alelos, as probabilidades de identidade por descendência (Δ_7 e Δ_8) e relação (r_{xy}) foram calculadas usando estimadores que avaliam endogamia. Os dois indivíduos partilharam a maioria dos alelos, com diferenças nos genótipos sendo identificadas em apenas dois loci. Todos os estimadores identificaram um nível de parentesco compatível com o encontrado entre irmãos (com e sem endogamia), o que aponta para o fato de serem gêmeos dizigóticos. Este é o primeiro caso confirmado de gêmeos fraternais em peixes-boi marinho de vida livre na América do Sul. O reconhecimento deste tipo de gemelaridade fornece elementos para aprimorar ações que visem a reabilitação de animais encalhados e sua posterior soltura ao ambiente.

Palavras-chave: *Sirênios; parentesco; microssatélites; encalhe; gêmeos dizigóticos.*

Introduction

The West Indian manatee (*Trichechus manatus* Linnaeus, 1758) is an aquatic mammal which is considered vulnerable to extinction due to the natural processes and anthropogenic impacts that have affected its populations, primarily over the past two centuries (Deutsch et al. 2008). The traditional classification of the species includes two subspecies, one of which is restricted to the Florida peninsula (*Trichechus manatus latirostris*), and the other to the Caribbean and South America (*Trichechus manatus manatus*). A recent study found this classification inadequate, pointing to the existence of two Evolutionary Significant Units - ESUs (Ryder 1986), one in the Atlantic (on the Brazilian coast), and the other in the Caribbean, between Venezuela and Florida (Lima et al. 2021). The Brazilian and Guiana populations should be of the highest priority for conservation due to its isolation and high level of differentiation (Garcia-Rodriguez et al. 1998, Vianna et al. 2006, Barros et al. 2017, Lima et al. 2021). Reproductive rates in *T. manatus* are low, with females reaching sexual maturity from around three years of age, and only begin breeding between five and eight years of age (Marmontel 1995, O`shea & Hartley 1995). Births are almost invariably of singletons, following a gestation of 12-14 months (Marmontel 1995, Rathbun et al. 1995). The females typically select a sheltered environment, such as estuaries, to give birth (Silva et al. 2016). However, the unregulated human occupation of these environments (Borges et al. 2007, Silva et al. 2016) has been the principal factor leading to the high rate of neonate strandings on the northeastern coast of Brazil (Meirelles 2008, Balensiefer et al. 2017).

Reports of twins in free-ranging West Indian manatees are scant, and monitoring data indicate that multiple births occur extremely infrequently (Marmontel 1995, Rathbun et al. 1995). Records of twinning events are often restricted to unpublished reports and graduate thesis, which limits the potential for a more systematic analysis of the phenomenon, since the data is of limited access. In the present study, we investigate a rare report of possible case of twinning in two neonate West Indian manatees found stranded in Ceará State, on the northeastern coast of Brazil, contributing to a broader understanding of this phenomenon and proposing the use of an applicable methodology for future similar reports.

Material and Methods

On October 15th 2014, two male West Indian manatees (Aquasis registers #02S0111/65 and #02S0111/66, here denominated Tma65 and Tma66, respectively) were found stranded on Agulhas beach (04º23'13.4" S, 37º49'50.0" W) in the municipality of Fortim, on the eastern coast of Ceará State. The two individuals were found 10 meters apart, and both had umbilical stumps and fetal folds, and were similar in size and weight (Tma65 = 1.16 m and 27.3 kg; Tma66 = 1.21 m and 30.1 kg), all indicatives that they were newborn. The animals were rescued by the Association for Research and the Preservation of Aquatic Ecosystems (Aquasis) and sent for rehabilitation in the Marine Mammal Rehabilitation Center (CRMM) in Caucaia, Ceará State.

During the rescue and handling of these animals, skin samples were obtained from each individual tail under local anesthetic (lidocaine 2%) using the protocol described by Bonde et al (2012). The samples were stored in ethanol 90% at -20 ºC at the Genetic and Conservation Laboratory, Federal University of Pará (UFPA). The total DNA was isolated using the Wizard Genomic DNA protocol (PROMEGA), with a final concentration of 712 ng/µl for Tma65 and 251 ng/µl for Tma66. Ten microsatellite loci extensively using with *Trichechus* (*TmaE08*, *TmaE11*, *TmaA09* - Garcia-Rodriguez et al. 2000, and *TmaE1, TmaSC5*, *TmaJ02*, *TmaKb60*, *TmaSC13, TmaE14*, *TmaE07* - Pause et al. 2007) were amplified by PCR. The amplified products were genotyped in an ABI 3500XL (Applied Biosystems) fragment analyzer and the alleles were identified using GeneMapper 4.1, with the default parameters and autobins, and inspected visually to confirm the reads.

To estimate the relatedness of the individuals, the genotypes of two other rescued manatees from the coast of Ceará State (#02S0112/69 and #02S0111/72) were incorporated into the database. These four samples were analyzed as part of a bank of 55 genotyped West Indian manatees (data not shown) to check for possible genotyping errors using the Micro-Checker software (Van Oosterhout et al. 2004), and none of the estimated errors were evident. Prior the analysis, a simulation was run in the Coancestry program (Wang 2011) to determine the best estimator of relatedness for the data. This simulation included two estimators of probability (TrioML and DyadML), which consider inbreeding, and two moment estimators (Wang and LynchLi), which are appropriate for samples of small size (Wang 2011). A total of 10,000 genotypes were simulated based on the observed allele frequencies, with 1,000 dyads (pairs of individuals) being analyzed for each of the five categories of relatedness (unrelated, half siblings, full siblings, parent-offspring, and monozygotic twins), as defined by Bonin et al. (2012). The probabilities of identity by descent (Δ_7 and Δ_8) and relatedness (r_{xy}) were calculated to estimate the degree of relatedness between the two manatee calves, with the values being compared with those of each category of relatedness defined by Wang (2011).

Results and Discussion

Alleles of all 10 microsatellites were identified in both individuals, which shared the same genotypes for almost all the loci, except for *TmaE14* and *TmaE08*, for which Tma65 was homozygous in both cases and Tma66 was heterozygous, also in both cases (Figure 1). The signal intensity for the *TmaE14* locus was 24.739 RFU for individual Tma65 (allele A), and 8.916 RFU and 6.996 RFU for individual Tma66 (alleles A and B, respectively). For the *TmaE08* locus, the intensities were 31.815 RFU for individual Tma65 (allele C), and 22.611 RFU and 15.705 RFU for individual Tma66 (alleles C and D respectively). The genotypes of these two markers were confirmed by repeating the genotyping, with a new amplified product (additional amplification by PCR), and the nucleotide sequencing of the alleles of the two samples.

The DyadML and TrioML probability estimator were the most adequate for the dataset, given that they returned the lowest variances (Table 1), although all four estimators provided highly similar values for the three parameters analyzed, with values of Δ_7 and r_{xy} of around 0.5, and those of Δ_8 equal to zero. The comparison of these values with the reference values defined by Wang (2011) allows us to reject the hypotheses that the two animals are monozygotic twins (r_{xy}=1.0) or unrelated (r_{xy}=0). The most adequate category for the observed r_{xy} value was sibs, with and without inbreeding (selfed outbred sibs and full-sibs, respectively), or between parent-offspring. The latter relationship was discarded, given that both individuals were newborn. The Δ_7 and Δ_8 values indicate the occurrence of inbreeding in the population. It is important to note here that the relatedness probability values for dizygotic twins are the same as those for full siblings, given that the latter also originate from distinct gametes produced by the same parents.

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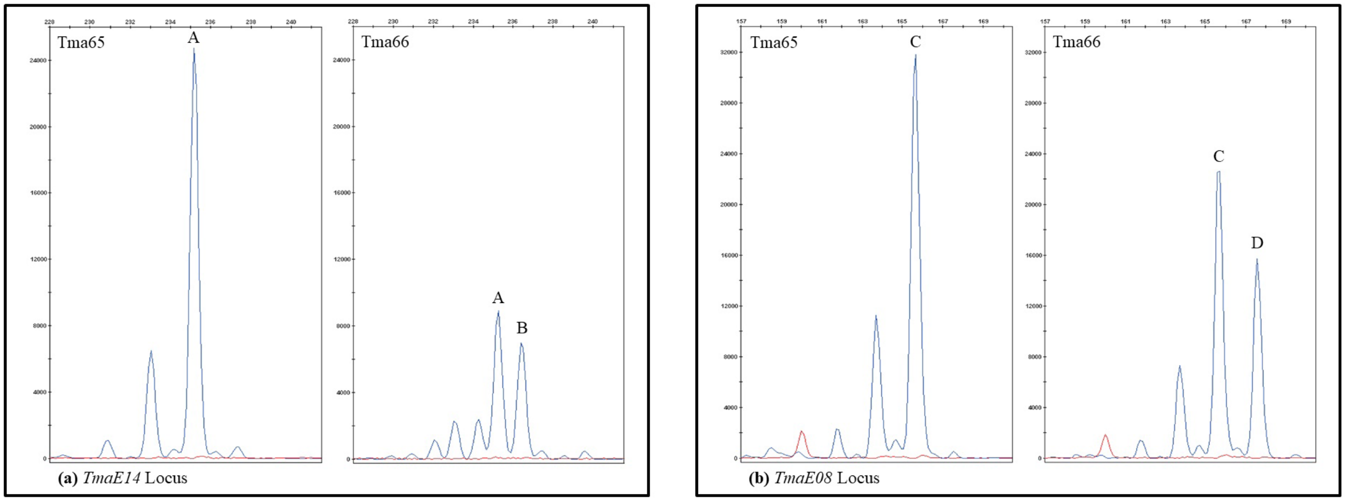

(a) *TmaE14* Locus

(b) *TmaE08* Locus

Figure 1. Electropherograms showing the alleles (A, B, C and D) of the loci *TmaE14* (box a) and *TmaE08* (box b). The x-axis indicates the position while the y-axis shows the signal intensity of the identified alleles.

Table 1. Variance recorded in the simulation of 1.000 dyads for each of the five categories of relatedness, and the probabilities of identity by descent (Δ_7 and Δ_8) and relatedness (r_{xy}) between Tma65 and Tma66 recorded by each estimator. *The LynchLi estimator does not provide probabilities of identity by descent (Δ_7 and Δ_8).

Estimator	**Variance**	$\boldsymbol{\Delta_7}$	$\boldsymbol{\Delta_8}$	$\boldsymbol{r_{xy}}$
DyadML	0.11441	0.5260	0.0000	0.4332
TrioML	0.11474	0.5393	0.0000	0.5393
LynchLi	0.21732	*	*	0.6854
Wang	0.21858	0.4895	0.0000	0.7160

Overall, then, these findings, together with the morphometric data and the estimated ages of the individuals at the timing of their stranding, indicate that the two manatee calves are dizygotic (fraternal) twins.

This is the first published report of the use of molecular tools to confirm twinning in the West Indian manatee as well as to determine the type of twins observed. In addition to the fact that multiple births are extremely rare in this species (Rathbun et al. 1995), the simultaneous stranding of two infants is an even rarer event, which reinforces the exceptional nature of the record presented here. Data on possible twinning events in *T. manatus* are very scant in general, and many cases may only be available in unpublished reports, which limits attempts to comprehend the broader scenario. Example of this are the theses of Bonde (2009) and Luna (2013), which presented evidence of twinning events in free-living manatees in Florida and in a rehabilitation center in northeastern Brazil, respectively. While the relatedness of the twins was confirmed using molecular tools (microsatellites, in both cases), these data have yet to be published. Other evidence confirming twin gestations and multiple births based on carcass analyses were published in Florida more than two decades ago (Marmontel 1995, Rathbun et al. 1995).

The confirmation of twinning in free-ranging aquatic mammals based on monitoring and necropsies, while relatively frequent, is nevertheless occasional and often imprecise (IJsseldijk et al. 2014, Davison et al. 2016). Estimates of twinning rates in sirenians are invariably low, with 1.4-4.0% of the pregnant female *T. m. latirostris* carcasses necropsied in Florida bearing twins (Marmontel 1995, Rathbun et al. 1995). In the dugong (*Dugong dugong*), the only record is of a free-ranging female being accompanied by two calves (Lanyon et al. 2009), although it was not known whether they were in fact siblings. Any estimates based on observations are obviously vulnerable to behavioral phenomena, such as adoptions (involving abandoned or orphaned infants), as well as stochastic events, such as the death of one of the fetuses or neonates (Meirelles et al. 2016). In the Antarctic fur seal (*Arctocephalus gazella*), for example, the molecular analysis of supposed twins confirmed cases of fraternal twinning, but also revealed adoptions and even heteropaternity (Bonin et al. 2012, Hoffman & Forcada 2009). Adoption cases have already been recorded in captive West Indian manatees in Brazil (Luna & Passavante 2010). This reinforces the need for molecular analyses, like that presented here, associated with long-term monitoring, to confirm the occurrence of twinning events and provide estimates of their frequency, which will be important for the better understanding of the social structure and behavior of the species.

The present study has also validated the viability of the use of genetic tools of biparental inheritance and manatee rescue data to confirm the occurrence of twinning in these mammals, as well as identifying the type of twins. The application of this molecular approach at the population level makes it possible to clarify the relatedness between individuals and also allows the quantification of inbreeding rates. These findings will provide valuable insights into the reproductive and demographic parameters of the endangered Brazilian population of West Indian manatees, which is genetically (Lima et al. 2021), cytogenetically, and morphologically (Barros et al. 2017) distinct from the other populations, which will be important for the development of adequate management strategies. The reliable determination of the relatedness of manatees in rehabilitation will be essential for the adequate planning of ongoing release strategies on the northeastern coast of Brazil (Luna et al. 2012). As the stranding of neonates is one of the principal threats to the manatee population of the Brazilian coast (Meirelles 2008, Balensiefer et al. 2017), the recognition of twins in rescued individuals may also contribute to a better understanding of the factors driving calf strandings process, such as the inexperience or incapacity of the mother during the puerperium, especially following multiple births.

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Sávia Moreira: 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.

Ana Carolina O. de Meirelles: Substantial contribution in the concept and design of the study; Contribution to data collection; Contribution to critical revision, adding intellectual content

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Juliana Araripe: 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 declares that they have no conflict of interest related to the publication of this manuscript.

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Effects of hydrological cycles and water body connectivity on abundance and co-occurrence of two Neotropical Curculionidae species

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Abstract: The effect of the hydrological cycle on the abundance of adults and larvae of the weevils *Cyrtobagous salviniae* and *Cyrtobagous singularis* in the Pantanal was tested and related to the host-plant abundance, limnological variables, and hydrological connectivity of 10 "bays" (lakes and ponds) along the Cuiabá River. Adults and larvae of *C. salviniae* were more abundant than *C. singularis*, and larvae and adult abundance differed significantly both within and between the two species. Adults and larvae of both species were more abundant in connected bays, but only *C. salviniae* responded to both connectivity and hydrological cycle, with the highest abundances during the high-water and rising-water periods for adults and larvae, respectively. Abundance of *C. singularis* was negatively related to the predominance of *C. salviniae*, and populations of adults and larvae of both species were slightly and negatively related to the limnological variables and host-plant abundance. The results showed that the temporal variation in larval and adult abundance and dominance of *C. salviniae* is influenced by hydrological cycle and connectivity, but not by limnological variables and host-plant abundance.

Keywords: *Aquatic macrophytes; Coleoptera; interspecific competition; sympatric species.*

Efeito do ciclo hidrológico e da conectividade das baías do Pantanal na abundância e co-ocorrência de duas espécies de Curculionidae Neotropical

Resumo: O efeito do ciclo hidrológico sobre a abundância de adultos e larvas dos curculionídeos *Cyrtobagous salviniae* e *Cyrtobagous singularis* do Pantanal foi testado e relacionado com a abundância das plantas hospedeiras, variáveis limnológicas e conectividade hidrológica de 10 baías do rio Cuiabá. Os adultos e larvas de *C. salviniae* foram mais abundantes do que *C. singularis*, e a abundância de larvas e adultos diferiu significativamente intra e interespecificamente. Adultos e larvas de ambas espécies foram mais abundantes em baías conectadas, mas apenas *C. salviniae* respondeu ao ciclo hidrológico, com a maior abundância de adultos durante o período de cheia, e maior abundância de larvas na enchente. A abundância de *C. singularis* foi relacionada negativamente com a predominância de *C. salviniae*, e a abundância de adultos e larvas de ambas espécies foi fraca e negativamente relacionada com as variáveis limnológicas e abundância das plantas hospedeiras. Os resultados demonstraram que a variação temporal na abundância de larvas e adultos e a dominância de *C. salviniae* foram influenciadas pela conectividade e ciclo hidrológico, mas não pelas variáveis limnológicas e abundância das macrófitas aquáticas.

Palavras-chave: *Espécies simpátricas; Coleoptera; competição interespecífica; macrófitas aquáticas.*

Introduction

The Pantanal is a Neotropical floodplain influenced by the regular monomodal hydrological cycle, as is common in wetlands of the South American tropics and subtropics. Due to the fluctuation between terrestrial and aquatic phases, the level of the rivers shows a predictable monomodal flood curve, and this hydrological cycle is of fundamental ecological importance (Oliveira & Calheiros 2000, Junk et al. 2006, Alho et al. 2011, Junk et al. 2011, Penatti, et al. 2015). In the northern region of the Pantanal, periodic flooding occurs during the rainy season in areas along river courses, due to topography, low soil drainage capacity, and pronounced precipitation concentrated in a single period of the year (Junk et al. 1989, Hamilton 2002, Junk et al. 2011). The flood pulse is mainly responsible for changes across the hydrological periods (receding water, dry period, rising water, high water) in the chemical, physical, and biotic components of the aquatic environments of floodplains (Junk 1997, Junk et al. 2006) and the biota responds with morphological, anatomical, physiological, phenological, and ethological adaptations (Junk et al. 2011).

Among the aquatic environments in the Pantanal floodplain, the lakes and ponds, locally known as "bays", are influenced physically, chemically, and biologically by the monomodal hydrological cycle and degree of hydrological connectivity (Thomaz et al. 2003, Wantzen et al. 2005, Girard 2011). The connectivity of Pantanal bays to the main channel ranges from permanently connected bays to those that exchange surface water only during the highest flood peaks (Wantzen et al. 2005). Shifts in hydrological connectivity among different floodplain bays vary with flood intensity, influencing the heterogeneity of habitats and the community distribution and composition at the local and regional levels, as well as population size, segregation of many plant and animal species, and consequently trophic interactions (Pott & Pott 2000, Hamilton 2002, Alho 2008, Pott 2011).

The Pantanal floodplain bays harbor a complex vegetation mosaic with many species of aquatic macrophytes, of which *Eichhornia crassipes* (Mart.) Solms. (Pontederiaceae) and *Salvinia auriculata* Aubl. (Salviniaceae) are the most abundant (Pott & Pott 2003, Wantzen et al. 2005). These macrophytes significantly affect the ecological attributes of other biological assemblages (Thomaz & Da Cunha 2010). The vegetation has high productivity rates, with growth depending on the supply of nutrients during high water (Pozer & Nogueira 2004, Nogueira et al. 2011), and the competition among plants depends on the water level (Murillo et al. 2019).

The temporal variation in the abundance of these plant species limits the population dynamics of oligophagous insects (Junk & Piedade 1997), e.g., during the dry period, insect populations are sharply reduced due to lack of food, and population densities may become much lower in subsequent hydrological periods (Vieira & Adis 1992). Co-occurrence and competition between oligophagous insects can be influenced by the hydrological cycle, temporal variations in the growth of aquatic macrophytes, and allocation of resources to phytophagous insects. Therefore, changes across seasonal periods resulting from the water-level fluctuations, monomodal hydrological cycle, and variations in connectivity influence the dynamics of the macrophyte community and abundance of co-distributed herbivore species, due to changes in the supply of nutrients for plant growth and of habitats for food or reproduction of herbivores (Neiff & Poi de Neiff 2003, Thomaz & Da Cunha 2010, Hernandez et al. 2020).

Studies of the invertebrates associated with aquatic macrophytes in natural environments are restricted to communities or groups that inhabit the submerged parts of these plants (Junk & Robertson 1997, Takeda et al. 2003, Junk et al. 2006). These organisms increase or decrease in abundance throughout the year, directly influenced by particular characteristics of the seasons, such as variation in rainfall and temperature, stress caused by the hydrological cycle (water-level fluctuations), and degree of connection of the bays to the main river channel, which affect factors such as the dissolved-oxygen content of the water (Franco & Takeda 2000, 2002, Melo et al. 2002, 2004, Benetti & Cueto 2004, Clemente et al. 2018, Prellvitz & Albertoni 2004, Wantzen et al. 2011, Zilli 2012). Little attention has been given to the impacts of the monomodal hydrological cycle, seasonal periods, and hydrological connectivity of these bays on the population attributes of phytophagous insects that inhabit aquatic macrophytes (Agostinho et al. 2000, Gopal et al. 2001, Murphy et al. 2003, Fortney et al. 2004, Pott & Pott 2004, Loyola et al. 2006).

Cyrtobagous Hustache, 1929 is a genus of aquatic weevils, and its species can be considered good biological models for investigating regional and local ecological patterns and floodplain attributes (e.g., flood periodicity, connectivity, environmental and biological factors), since the genus has a wide geographic distribution in South America and its species are native to Brazil, Bolivia, Paraguay, Uruguay, and Argentina (Wibmer & O'Brien 1986). It was introduced as a potential control agent of *Salvinia* spp. in 16 localities outside its native range (Parys & Johnson 2013), because of its levels of specificity and behavioral and morphological characteristics that promote survival in aquatic environments, feeding underwater on the developing leaf buds and petioles, new leaves, and roots of species of *Salvinia* Ség. (DeLoach 1975, O'Brien 1976, Forno et al. 1983, Calder and Sands, 1985, May & Sands 1986, Julien et al. 2002, Tipping & Center 2005, Madeira et al. 2006).

Considering the impacts of the monomodal hydrological cycle on the population attributes of phytophagous insects that inhabit emergent aquatic macrophytes, this study investigated the effects of the hydrological cycle, hydrological connectivity, host-plant abundance, and limnological variations across four seasonal periods (high water, receding water, dry period, and rising water) on the abundance and co-occurrence of the adults and larvae of two *Cyrtobagous* species associated with floating mats of *Salvinia* species in Pantanal floodplain bays. We tested the hypothesis that abundance and co-occurrence in the adults and larvae of two *Cyrtobagous* species are affected by the monomodal flood pulse and seasonality, predicting that larval and adult abundances would be higher in the high-water than in the dry period, in connected than in non-connected bays, and according to the host-plant abundance.

Materials and Methods

1. *Study area*

We sampled ten bays along a 32.6-km stretch of the Cuiabá River downstream from Porto Cercado, in the area of the Private Natural Heritage Reserve (RPPN-SESC Pantanal), Poconé, Mato Grosso, Brazil (Figure 1). Five of the bays were connected to the main channel of the Cuiabá River throughout the four hydrological periods (high water, receding water, dry period, and rising water) in 2009, and are here termed connected bays. The other five bays were connected only during the 2009 high-water period, and are here termed periodically connected bays, regardless of the position of the bays downstream and upstream along the river. The regional climate is tropical savannah with dry winters and rainy summers. The temperature ranges from 22 °C to 32 °C (Hasenack et al. 2003) and rainfall occurs from October to April, with the driest months in June through August. Annual rainfall varies between 1,100 and 1,200 mm, with 85% falling during the rainy season (Wantzen et al. 2005, Dourojeanni 2006).

2. *Environmental and biological data sampling*

Collections were made quarterly during the 2009 hydrological cycle and corresponded to the four seasonal periods of the Pantanal of Mato Grosso: high water (February/March), receding water (July), dry period (September), and rising water (December). In each bay, three transects were randomly established, with three sampling points each, totaling nine sampling points per bay. The transects were established perpendicular to the shore of the bay and extended from the littoral zone,

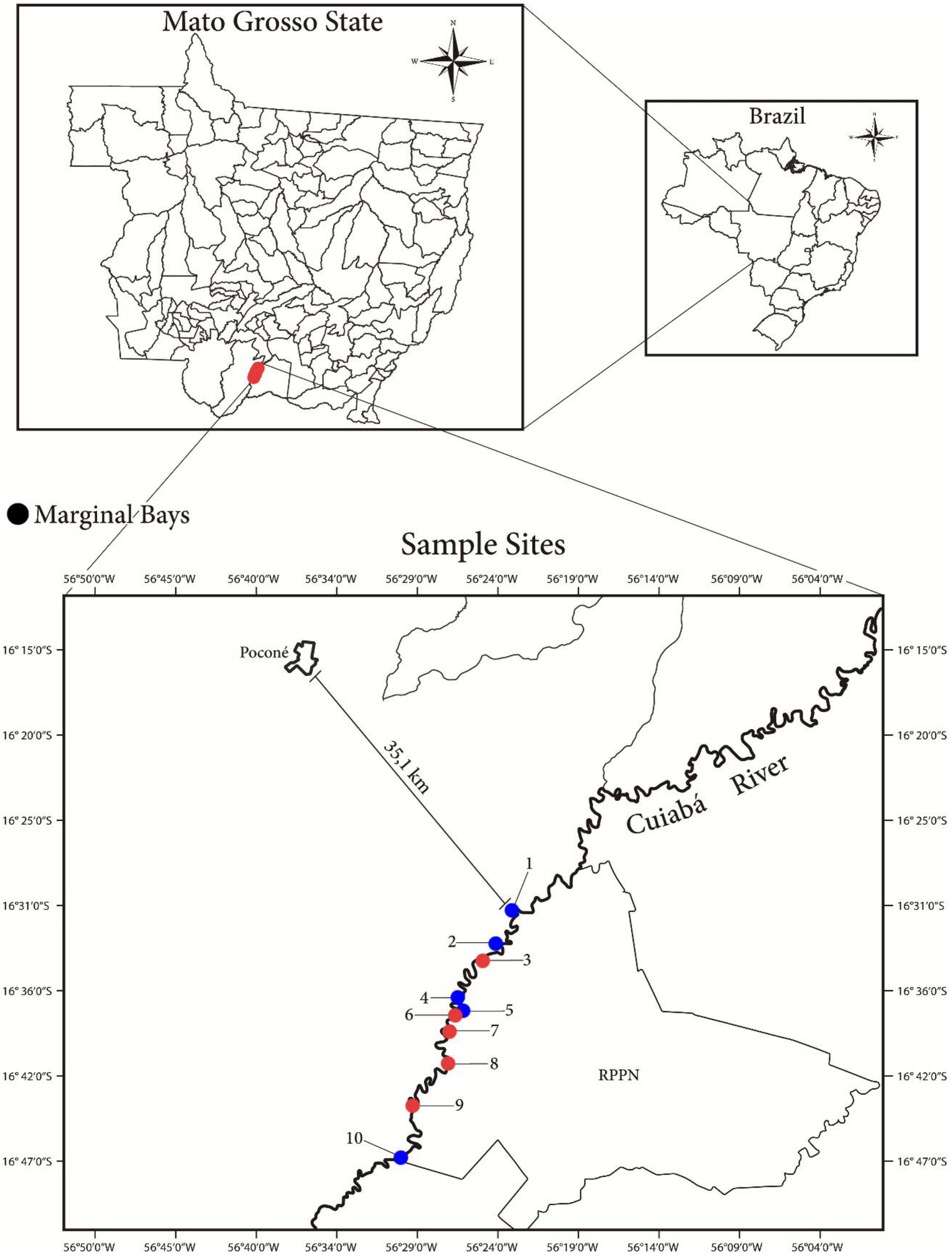

Figure 1. Study area and location of sampling sites along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso. Circles indicate the bays along the Cuiabá River selected for this study. Private Natural Heritage Reserve (RPPN). Blue circle - connected and red circle - periodically connected bays.

with a saturated substrate, to the area of maximum depth, 3 m to 5 m (Thomaz et al. 2003, Fortney et al. 2004). This method ensured that the size of the transects and number of sampling points were standardized in each bay (Brower & Zar 1984). Sample independence was assumed by establishing minimum distances of 2 m between each transect and 1 m between each point.

Limnological and biological variables were measured at each sampling point. Water depth was measured with a graduated ruler, water temperature and electrical conductivity with a Mettler-Toledo MC126 thermistor and conductivity meter, and pH with a Mettler-Toledo MP120 meter. Plants and weevils were collected using an aluminum cage 0.5 m square × 0.5 m high with an area of 0.25 m^2, screened on the sides, with the bottom open and the upper part with a lid (Vieira & Adis 1992). The cage was placed over a macrophyte bank, with the upper lid closed, trapping the community of arthropods and macrophytes inside. The lid was then opened, and *Salvinia* spp. were manually removed, stored in labeled 20 × 20 cm plastic bags, and transported to the Advance Base for Pantanal Research (Base Avançada de Pesquisas do Pantanal – BAPP) of the Federal University of Mato Grosso, where they were weighed and fixed in 98% ethanol for subsequent sorting of insects. The *Salvinia* fresh mass from each sampling point per 0.25 m^2 was used as a measure of coverage and abundance, to quantify the variation across seasonal periods. Weevil adults and larvae were sorted from the ethanol-fixed *Salvinia* samples under a stereomicroscope. Because the ethanol fixation stimulated the weevils to exit the plant tissues, the *Cyrtobagous* species could not be accurately associated with *Salvinia auriculata* and *S. minima* Baker. Therefore, the plants were evaluated based on the combined fresh mass and were treated in the analyses and discussion as *Salvinia* spp. This does not affect the interpretation of the results, as both species were present in the macrophyte banks and both are hosts of *Cyrtobagous salviniae* Hustache, 1929 and *Cyrtobagous singularis* Calder & Sands, 1985 (Madeira et al. 2006).

Adults of *C. singularis* and *C. salviniae* were identified based on Calder and Sands (1985), and the larvae were identified based on May (1994) and May and Sands (1986). The vegetation was identified based on the key by Pott and Pott (2000), later confirmed by the specialist Vali Joana Pott, M.Sc. (Embrapa Gado de Corte, Mato Grosso do Sul).

3. *Data analysis*

To evaluate the phenological (adults and larvae) differences in the mean abundances of *C. salviniae* and *C. singularis*, the chi-squared (χ^2) test was employed for both species together and for each individually. Repeated-measures (two-factor) ANOVA was used with and without interactions to explore the temporal effects (among seasonal periods: high water, receding water, dry period, and rising water), spatial variations (between bays), and permanently and periodically connected bay groups on mean abundances of adults and larvae of *C. salviniae* and *C. singularis*, fresh weight of *Salvinia* spp., and limnological variables (water depth, water temperature, pH, and electrical conductivity), considering the points of the three transects as subsamples (N = 9), the bays as sampling units (N = 10), and the hydrological periods (N = 4) and connectivity of bays (N = 2) as treatments. The effect of the hydrological connectivity of bays was analyzed by t test, using the mean abundances of adults and larvae of *C. salviniae* and *C. singularis* and the fresh weight of the *Salvinia* spp. macrophytes between the permanently and periodically connected bay groups, considering bays (N = 10) as sample units, the seasonal periods as repetitions (N = 4), and the permanently and periodically connected bay groups (N = 2) as treatments.

Spearman correlation was performed between all the predictor variables to detect autocorrelations, and linear regression analyses were used to explore the relationships between the abundances of adults and larvae, biological variables (*Salvinia* fresh mass), and limnological variables (water depth, water temperature, pH, and electrical conductivity). To determine if the presence and abundance of *C. salviniae* and *C. singularis* were influenced by the co-occurrence of the species in the sample, the abundance data were transformed to probability of occurrence in the sample, and the probability of occurrence of each species was then analyzed using nonlinear exponential regression. Thus, it was possible to determine if the abundance of one species was related to the proportion of the other. The level of significance adopted in all analyses was 0.05. The normality assumption was tested by observing distribution probability plots and by the Kolmogorov-Smirnov test, and when appropriate, the raw data were square-root +0.5 transformed. The analyses were performed and the graphs created in SYSTAT 11 (Wilkinson 2004) and SigmaPlot 12.

Results

A total of 1,790 *Cyrtobagous* individuals were collected. For the two species combined, larvae (59.94%) were significantly more abundant than adults (40.06%) ($\chi^2 = 47.41$, df = 18, p = 0.001). For *C. salviniae*, larval abundance (968 ind., 66.12%) was higher than adult abundance (496 ind., 33.88%) ($\chi^2 = 102.64$, df = 18, p = 0.001). For *C. singularis*, adults (221 ind., 67.80%) were more abundant than larvae (105 ind., 32.20%) ($\chi^2 = 27.91$, df = 18, p = 0.001) (Figure 2).

Only the abundances of *C. salviniae* adults and larvae varied significantly among the four seasonal periods (Figure 3), with the highest larval abundance in the rising-water period and the lowest in the receding-water period. Adults were most abundant during the high-water and least abundant during the dry period. The larval and adult abundances of *C. singularis* were highest in the rising-water period, and gradually decreased during the high-water, receding-water, and dry periods (Figure 4).

The highest abundances of *C. salviniae* and *C. singularis* larvae were recorded in bay 1 and the lowest in bay 7. Adult *C. salviniae* were most abundant in bay 8 and least abundant in bay 7. The highest abundance of adult *C. singularis* was recorded in bay 2 and the lowest in bay 3. These spatial variations were not significant for either species (Figure 5). The highest larval abundances for both species were recorded in the group of connected bays (Figure 6 and 7). For *C. salviniae*, the mean abundance in the periodically connected bays was 27.65 and higher in the connected bays, 45.55 (t = –2.95; df = 18, p = 0.049) for adults and larvae, both together and separately. For *C. singularis*, the mean abundance in the periodically connected bays was 5.65 and in the connected bays was 10.65 (t = –2.74; df = 18, p < 0.03) for adults and larvae together; only larvae showed a difference in relation to bay connectivity (Figure 7).

The environmental variables of conductivity, pH, water temperature, and water depth, as well as the fresh mass of *Salvinia* spp. varied significantly between the bays and among the four seasonal periods; only the limnological variables varied between the groups of connected and periodically connected bays (Table 1). These variables also showed

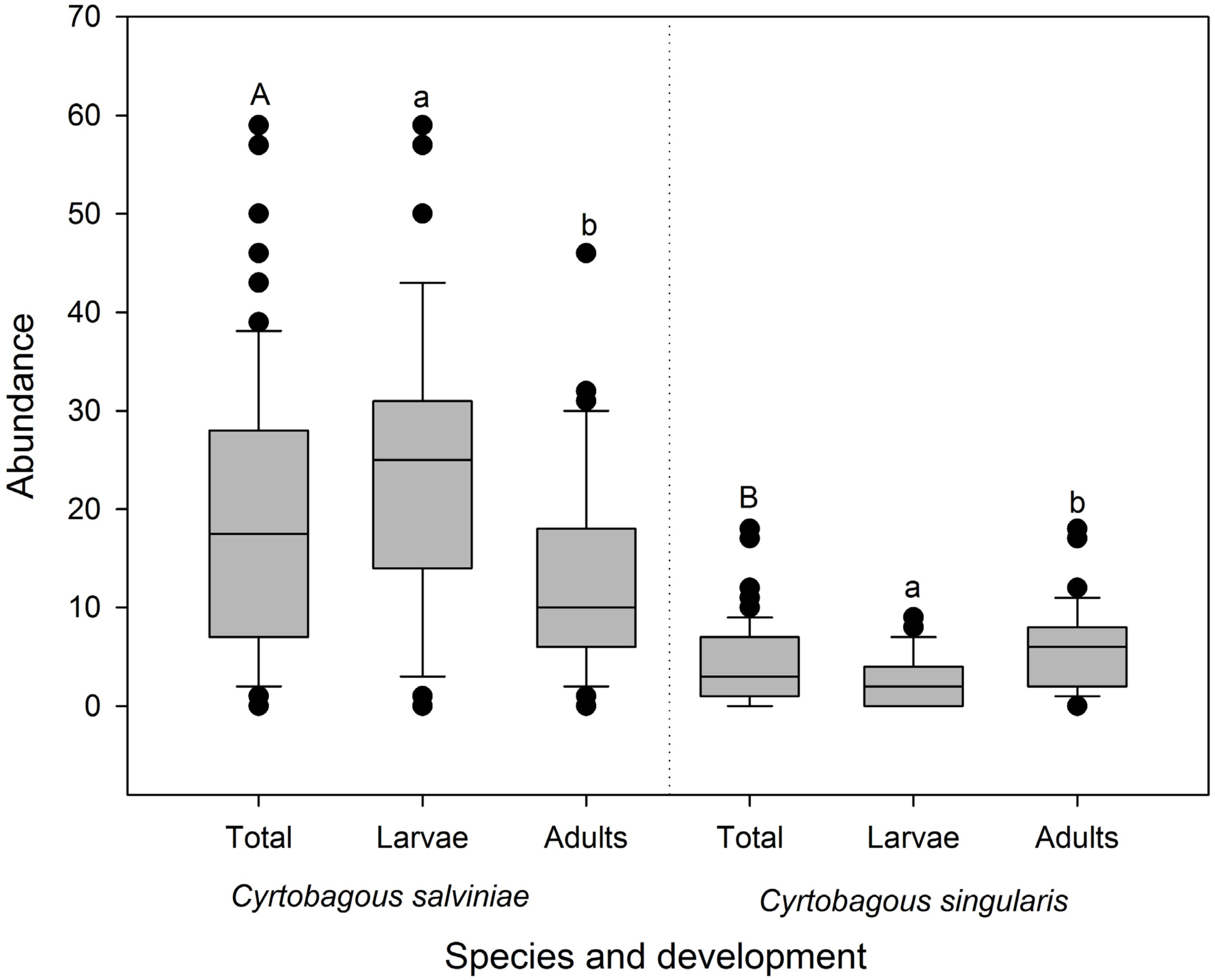

Figure 2. The phenological (adults and larvae) differences in the abundances of *Cyrtobagous* for both species together and for each individually, collected from bays along the Cuiabá River during a hydrological cycle (2009) in the Pantanal of Barão de Melgaço, Mato Grosso. Different letters indicate significant diferences.

significant interactions between the bays and seasonal periods and between bay connectivity and seasonal periods (Table 1 and 2). The abundances of *C. salviniae* ($R^2 = 0.16$, $p < 0.01$) and *C. singularis* ($R^2 = 0.35$, $p < 0.01$) adults and larvae were positively related only to bay water depth and water temperature (Figure 8 and 9). The abundances of adults and larvae of both species were negatively related to conductivity, pH, and fresh mass of Salvinia spp (Figure 8 and 9). No interaction between the variables contributed significantly to explaining the abundances of *C. salviniae* and *C. singularis* adults and larvae.

Cyrtobagous singularis showed a low probability of occurrence in the samples (10%), whereas *C. salviniae* was dominant. As the probability of occurrence of *C. salviniae* increased to more than 30%, the probability of occurrence of *C. singularis* decreased significantly ($R^2 = 0.12$, $p < 0.05$) (Figure 10); i.e., *C. singularis* was more frequent when *C. salviniae* was uncommon in the samples.

Discussion

Our results are consistent with the assumption that the flood pulse is the main factor responsible for regional changes across the seasonal periods (high water, receding water, dry period, and rising water), bay connectivity, and at the local level of the chemical, physical, and biotic components of the aquatic environments of floodplains (Junk et al. 2011) in the abundance of larvae and adults and co-occurrence of the two *Cyrtobagous* species. Conductivity, pH, water temperature, water depth, and fresh mass of *Salvinia* spp. varied significantly among seasonal periods and bays, and with connectivity. The seasonal variations in the abundances of *C. salviniae* and *C. singularis* larvae and adults were positively related to the seasonal variations in water depth, water temperature, and connectivity of the bays, confirming our hypothesis that adults and larvae of the two species would be most abundant during high water and in connected bays.

Hydrological seasonality affects the environmental determinants and biomass of aquatic macrophytes, with connectivity and water depth being the main variables (Junk et al. 1989, Junk & Piedade 1993, Pott & Pott 2003, Padial et al. 2009, Bornette & Puijalon 2011, Schneider et al. 2018). In general, aquatic macrophytes are most productive during the rising-water and high-water periods (Pozer & Nogueira 2004, Nogueira et al. 2011) and show reduced productivity during the receding-water period (Furch & Junk 1997). Our results for *Salvinia* fresh mass diverged from this pattern, with the lowest levels of fresh mass obtained in the rising-water and high-water periods and the highest levels in the

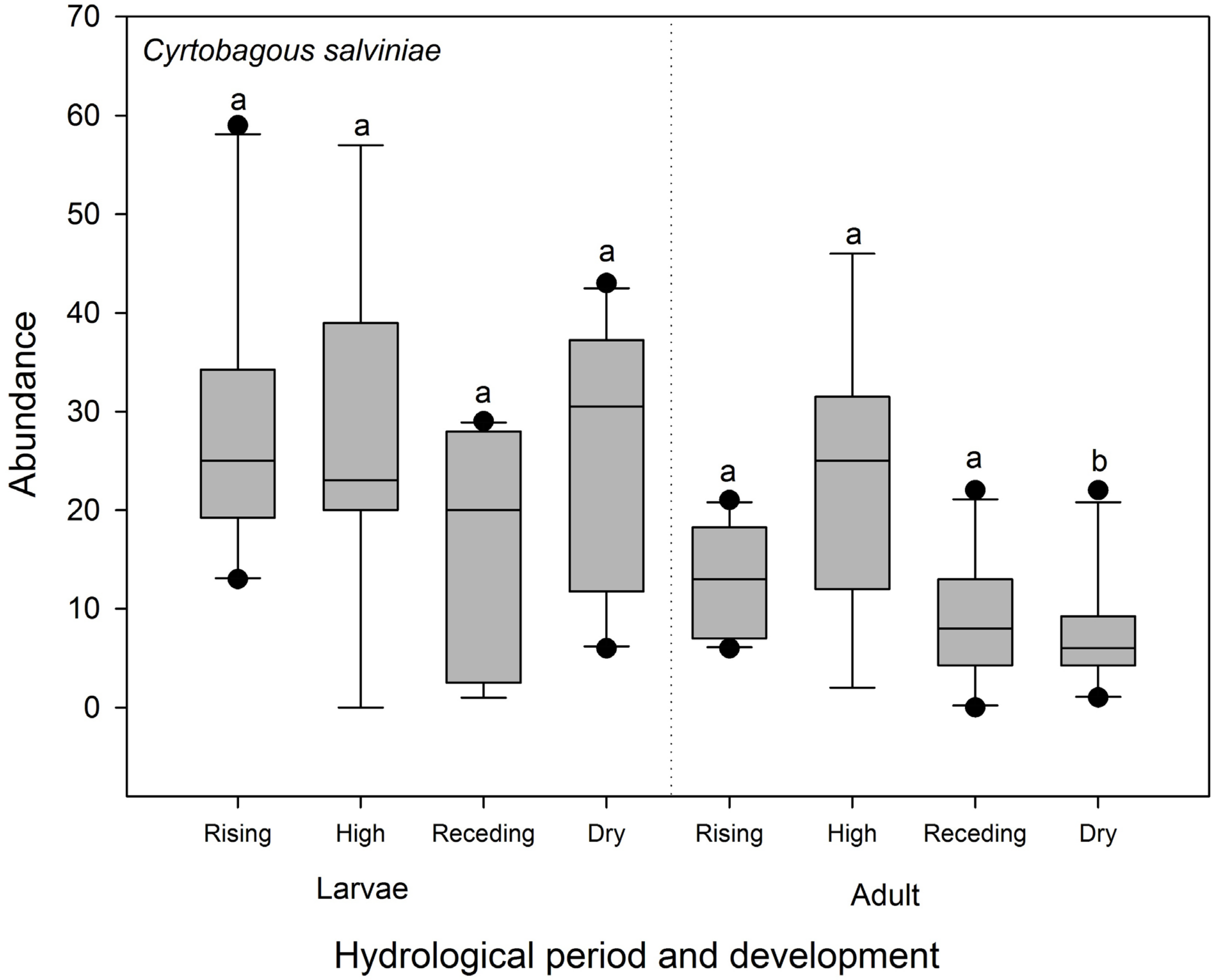

Figure 3. Temporal distribution of abundances of *Cyrtobagous salviniae* larvae and adults in bays along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso, during the 2009 hydrological cycle. Different letters indicate significant differences.

receding-water and dry periods, contrasting with the greater abundance of larvae and adults of *C. salviniae* and *C. singularis* in the rising-water and high-water periods, which were negatively related to the fresh mass of the host plants. Conditions such as high water-body connectivity and intense flooding tend to increase macrophyte abundance (e.g., Santos & Thomaz 2008, De Sousa et al. 2011) in certain locations or during certain periods of the hydrological cycle. These conditions may have occurred in the bays studied here, which contributed the largest fresh mass of *Salvinia* in the receding-water and dry periods. High-water or flooding events severely disrupt the spatial patterns of macrophyte communities, and floating plants tend to drift and can be easily carried out of a water body by currents in high-water periods, resulting in the removal of most of the plant biomass. Such events homogenize floodplain habitats at different spatial scales (Thomaz et al. 2007, De Sousa et al. 2011), leading to decreased fresh mass of *Salvinia* during high-water periods. Thus, the negative relationship between host-plant biomass and weevil abundance of larvae and adults is not a function of plant biomass, illustrating the complex ecology of phytophagous insects and aquatic plants on the Neotropical floodplain. A lack of relationship or a negative relationship between the abundance of phytophagous insects and the biomass of aquatic macrophytes has been observed in previous studies (e.g., De Sousa et al. 2011, Tipping et al. 2012). This can occur as a function of the delay in the response of the weevil to changes in macrophyte biomass, as supported by ecological theories such optimal foraging (Pyke at al. 1997; Pyke 1984). Since the fresh biomass of the roots of *Salvinia* spp. is almost 2.5 times larger than the aerial part (Room and Julien 1994), and the receding phase favors colonization and biomass increase of structures such as the roots that are necessary for later investment of aerial biomass due to resource allocation (Medeiros et al., 2016). When larvae and adult weevils are common, however, they are dispersed in the amount of fresh mass of the macrophytes, which then does not reflect the true biomass size of the plant. However, environmental factors that affect populations (e.g., stress factors such as flood pulses) have been identified as potential modulators of interactions between herbivores and plants (Cubit 1984, Menge & Sutherland 1987, O'Connor 2009, Wood et al. 2017).

Bays with a direct river connection are strongly affected by variations in water level (Neiff & Poi de Neiff 2003, Santos & Thomaz 2007). Directly connected bays show pronounced physico-chemical changes over the different hydrological periods, which alters the habitat

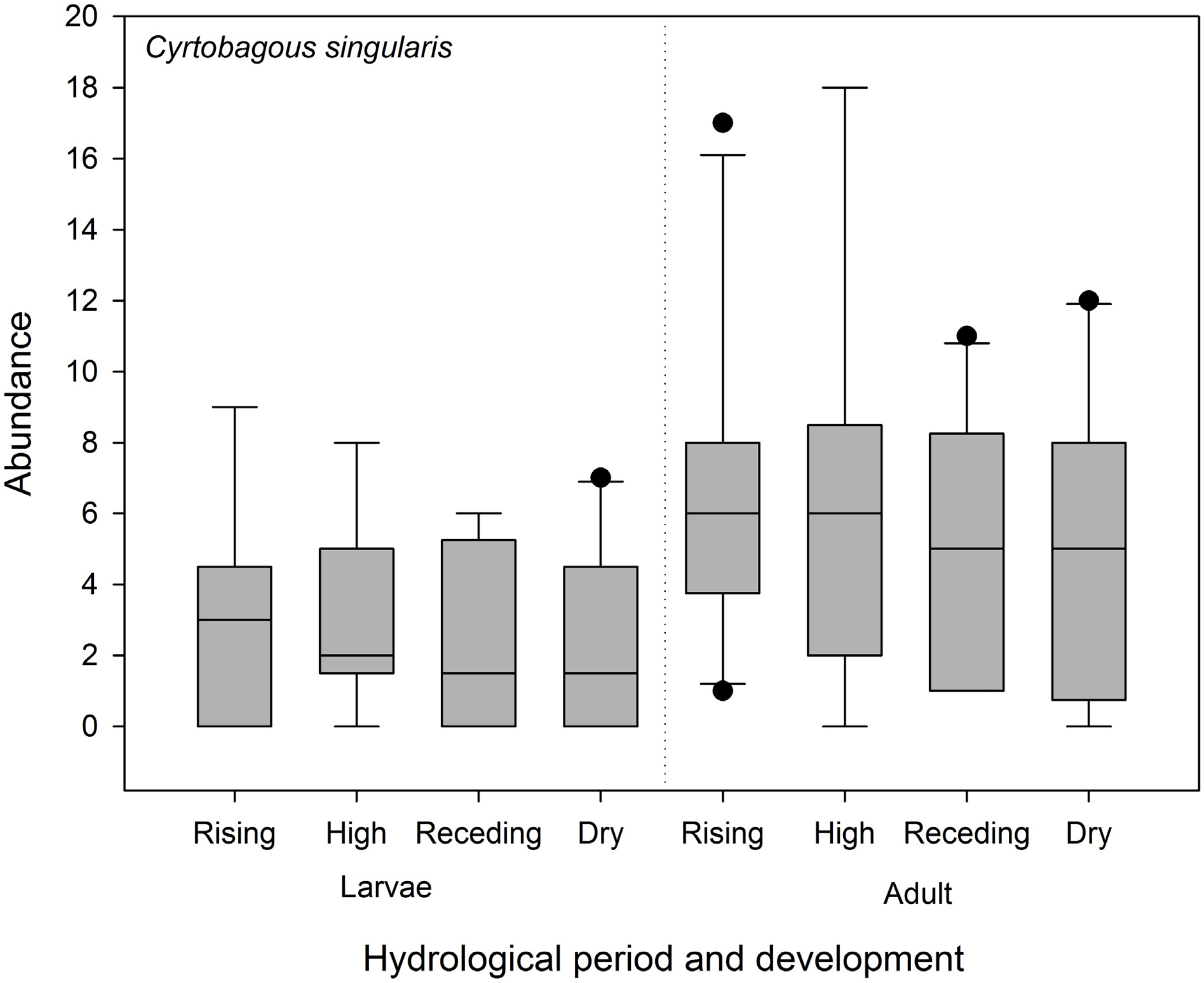

Figure 4. Temporal distribution of abundances of *Cyrtobagous singularis* larvae and adults in bays along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso, during the 2009 hydrological cycle.

available for groups of animals that use aquatic macrophytes for feeding or reproduction (e.g., Neiff & Poi de Neiff 2003). These changes promote morphological, anatomical, physiological, phenological, and ethological adaptations in organisms (Junk et al. 2011). The results of this study are consistent with the assumption of a connectivity effect, since the abundances of larval and adult *C. salviniae* and *C. singularis* were higher in the permanently connected bays and were related to water depth, reinforcing the effects of the flood pulse and connectivity on the life cycles of these phytophagous insects.

These results also illustrate the effect of the flood pulse on the local factors of conductivity, pH, water temperature, water depth, and fresh mass of *Salvinia* spp., which varied significantly among the bays (Junk 1997, Neiff & Poi de Neiff 2003). This finding supports the hypothesis that local factors and bay characteristics such as area and shape (Camargo et al. 2003, Neiff & Poi de Neiff 2003) influence the environmental characteristics of bays and the spatial distribution and colonization of aquatic macrophytes (Bianchini Jr. 2003, Thomaz & da Cunha 2010) and the reproductive dynamics of phytophagous insects associated with aquatic vegetation on floodplains. This effect was partially apparent in our study, since the spatial variations in abundance were significant for *C. salviniae* during its entire life cycle, but only for adults of *C. singularis*. The variations were largest in the group of connected bays, supporting our hypothesis of greater abundances of both species of *Cyrtobagous* in these water bodies.

The flood pulse causes changes across the seasonal periods, leading to phenological adaptations in organisms (Junk et al. 2011). This was apparent in the two *Cyrtobagous* species: larvae of *C. salviniae* were more abundant than adults, and larvae of *C. singularis* were less abundant than adults and occurred in all four periods, indicating that the flood pulse contributes to the maintenance of populations of *Cyrtobagous* in bays in the Pantanal of Mato Grosso, favoring more than one reproductive cycle in each of these two populations across seasonal periods (e.g., DeLoach & Cordo 1976a, Melo et al. 2002, Nunes et al. 2005, Franceschini et al. 2007). The higher abundances and temporal variations in the occurrence of larvae and adult *C. salviniae* indicate that the flood pulse affects the recruitment of individuals on spatial and temporal scales, directly affecting intra- and interspecific competition and resulting in density dependence (e.g., Speight et al. 1999). This is related to longevity and generational overlap in adults (e.g., DeLoach & Cordo 1976a) and to intraspecific competition in

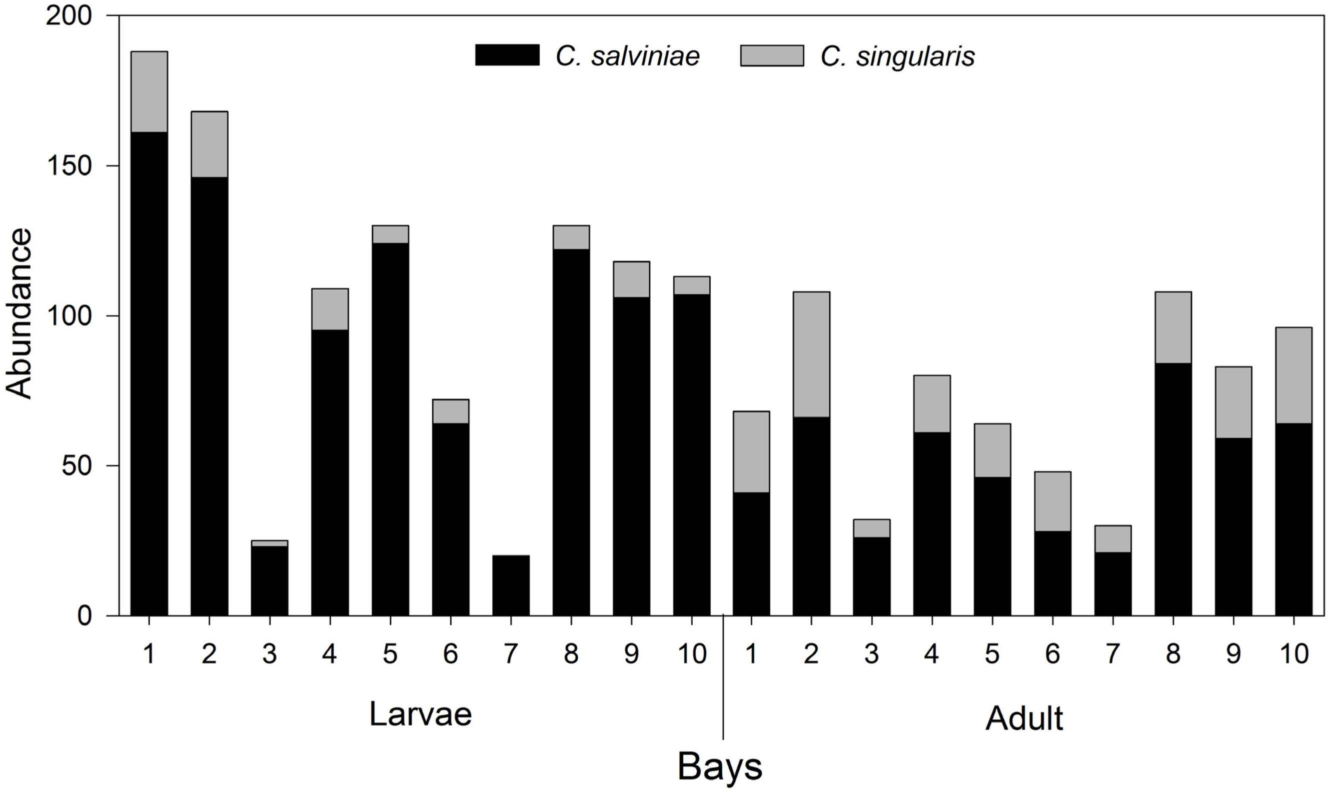

Figure 5. A- Spatial distribution of abundances of *Cyrtobagous salviniae* and *Cyrtobagous singularis* larvae and adults in bays (1-10) along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso, during the 2009 hydrological cycle.

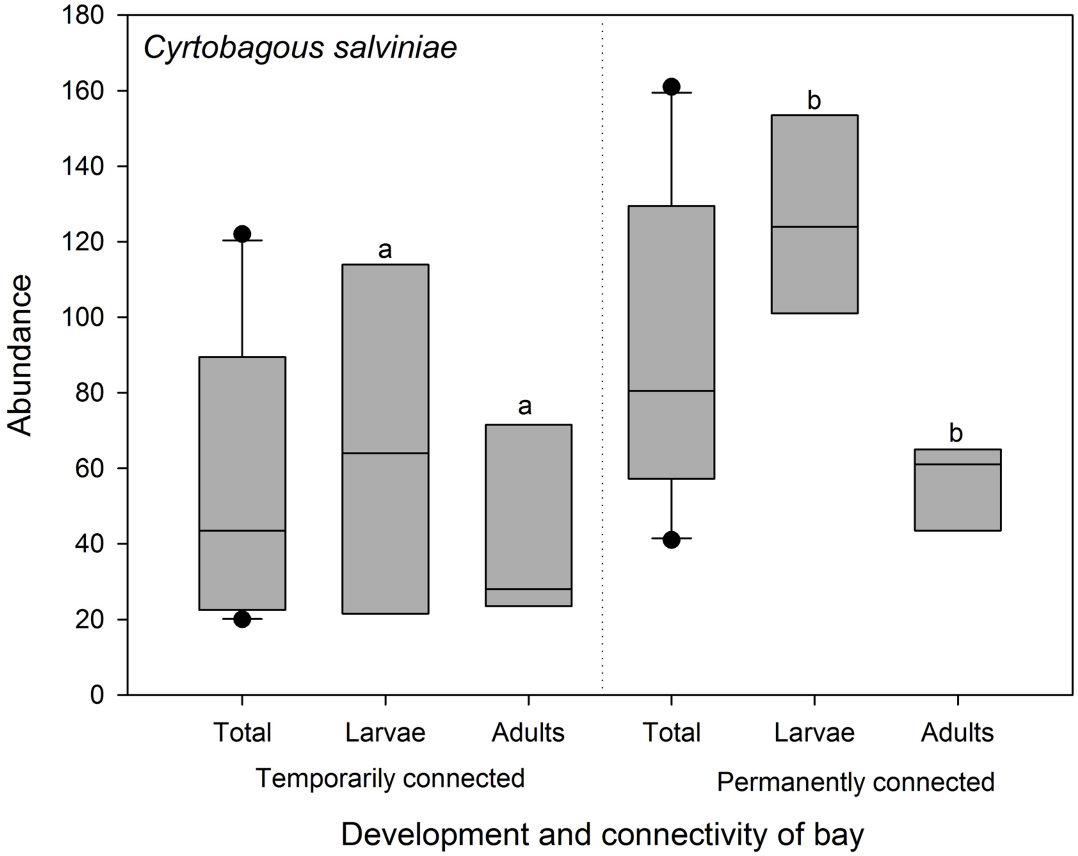

Figure 6. The phenological (adults and larvae) differences in the abundances of *Cyrtobagous salviniae* in connected and periodically connected bays along the Cuiabá River during a hydrological cycle (2009) in the Pantanal of Barão de Melgaço, Mato Grosso. Different letters indicate significant differences.

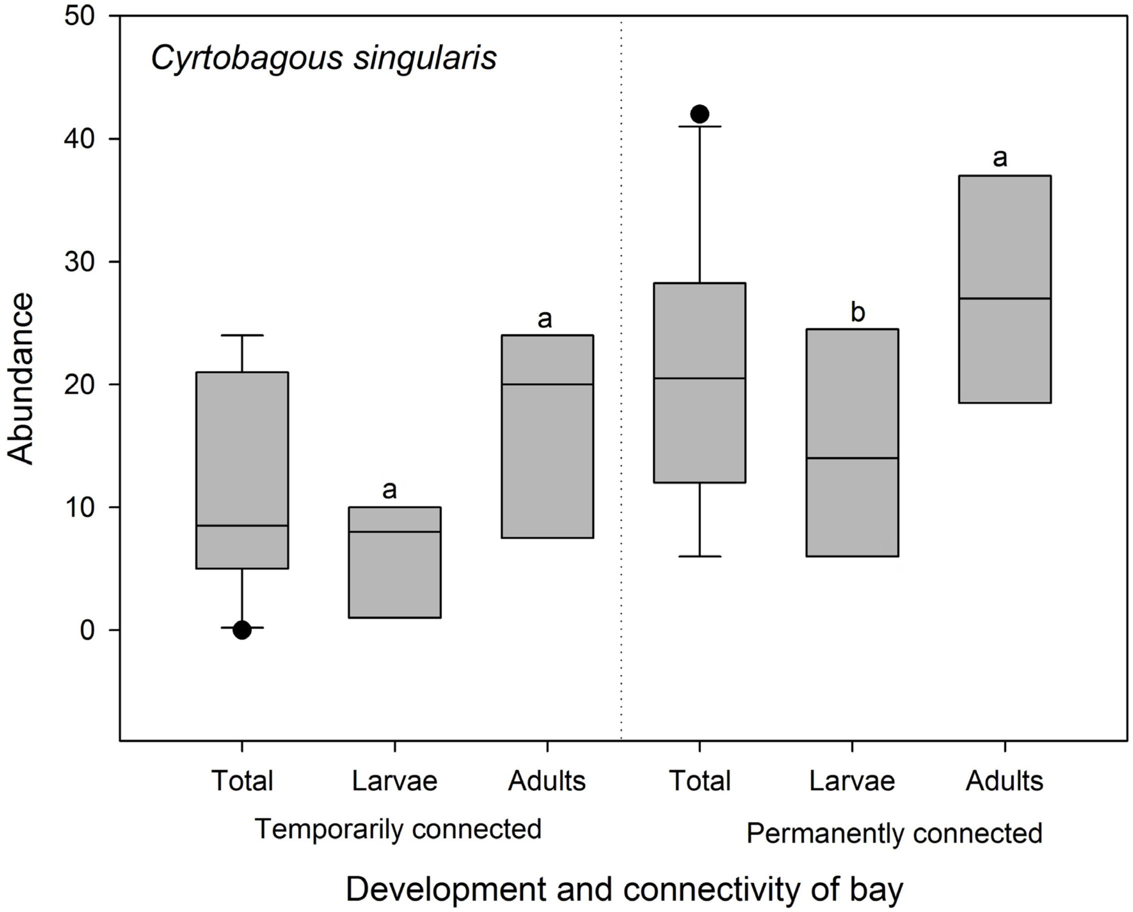

Figure 7. The phenological (adults and larvae) differences in the abundances of *Cyrtobagous singularis* in connected and periodically connected bays along the Cuiabá River during a hydrological cycle (2009) in the Pantanal of Barão de Melgaço, Mato Grosso. Different letters indicate significant differences.

Table 1. Spatial (bays) and temporal (rising water, high water, receding water, and dry period) effects on the means and standard deviations of environmental and biological variables per bay and seasonal period in the Pantanal of Barão de Melgaço, Mato Grosso. Connectivity: 1 – connected and 0 – periodically connected bays.

Connectivity	Bays	Conductivity (µS cm–1)	pH	Temperature	Depth (m)	Salvinia spp. (Kg)
1	1	(66.44 ±8.28)	(6.00 ±2.04)	(29.73 ±2.66)	(2.20 ±1.01)	(2.28 ±9.77)
1	2	(71.33 ±10.34)	(6.21 ±1.35)	(28.40 ±2.11)	(1.46 ±0.69)	(3.44 ±10.82)
0	3	(81.63 ±19.31)	(6.27 ±1.41)	(27.20 ±1.89)	(1.09 ±0.65)	(10.04 ±20.31)
1	4	(72.61 ±10.86)	(6.43 ±1.04)	(28.28 ±2.28)	(1.89 ±1.48)	(8.4 ±21.51)
1	5	(66.05 ±8.88)	(6.74 ±0.74)	(28.59 ±1.98)	(1.61 ±1.44)	(17.32 ±28.39)
0	6	(58.91 ±12.49)	(6.85 ±0.97)	(28.84 ±2.16)	(1.37 ±0.96)	(12.05 ±22.45)
0	7	(92.66 ±26.04)	(6.70 ±1.26)	(27. 32 ±3.88)	(0.99 ±0.61)	(3.65 ±14.03)
0	8	(61.33 ±11.72)	(6.11 ±1.44)	(28.84 ±1.81)	(1.53 ±0.83)	(8.22 ±20.67)
0	9	(73.25 ±11. 64)	(6.30 ±1.42)	(29.00 ±2.11)	(1.09 ±0.77)	(4.18 ±17.42)
1	10	(66.50 ±10.58)	(6.29 ±1.37)	(29.31 ±2.76)	(1.47 ±0.76)	(7.08 ±20.90)
	Bays (p)	<0.001	<0.001	<0.001	<0.001	<0.05
	Connectivity (p)	<0.05	<0.05	<0.005	<0.001	>0.05
	Rising water	(61.39 ±13.72)	(4.95 ±0.47)	(29.93 ±1.30)	(0.99 ±0.57)	(6.5 ±17.34)
	High water	(60.06 ±1.75)	(7.07 ±0.30)	(30.08 ±1.40)	(2.48 ±0.87)	(0.15 ±0.12)
	Receding water	(79.87 ±11.84)	(5.56 ±0.91)	(25.72 ±0.67)	(1.64 ±0.61)	(14.54 ±26.05)
	Dry	(82.99 ±19.09)	(8.00 ±0.66)	(28.71 ±2.07)	(0.71 ±0.57)	(9.48 ±21.66)
	Period (p)	<0.001	<0.001	<0.001	<0.001	<0.001
	Bays*Period (p)	<0.001	<0.001	<0.001	<0.001	<0.005
	Connectivity*Period (p)	<0.05	<0.005	<0.005	>0.05	>0.05

Table 2. Spearman correlation between environmental and biological variables and abundances of adults and larvae of *Cyrtobagous salviniae* and *Cyrtobagous singularis* in bays along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso.

	Conductivity	**pH**	**Temperature**	**Depth**	**Adult *C. salviniae***	**Immature *C. salviniae***	**Adult *C. singularis***	**Immature *C. singularis***	**Mass of *Salvinia* spp.**	**Water Level**
Conductivity	1.000									
pH	0.221	1.000								
Temperature	–0.517	0.114	1.000							
Depth	–0.337	–0.070	0.030	1.000						
Adult *C. salviniae*	–0.165	–0.029	0.181	0.228	1.000					
Immature *C. salviniae*	–0.152	0.004	0.248	0.132	0.426	1.000				
Adult *C. singularis*	–0.083	–0.087	0.086	0.137	0.429	0.329	1.000			
Immature *C. singularis*	–0.079	–0.083	0.107	0.158	0.257	0.356	0.292	1.000		
Salvinia spp. mass	0.069	–0.032	–0.216	–0.089	–0.113	–0.099	–0.057	–0.094	1.000	
Water Level	–0.532	–0.536	0.203	0.538	0.195	–0.043	0.068	0.052	–0.132	1.00

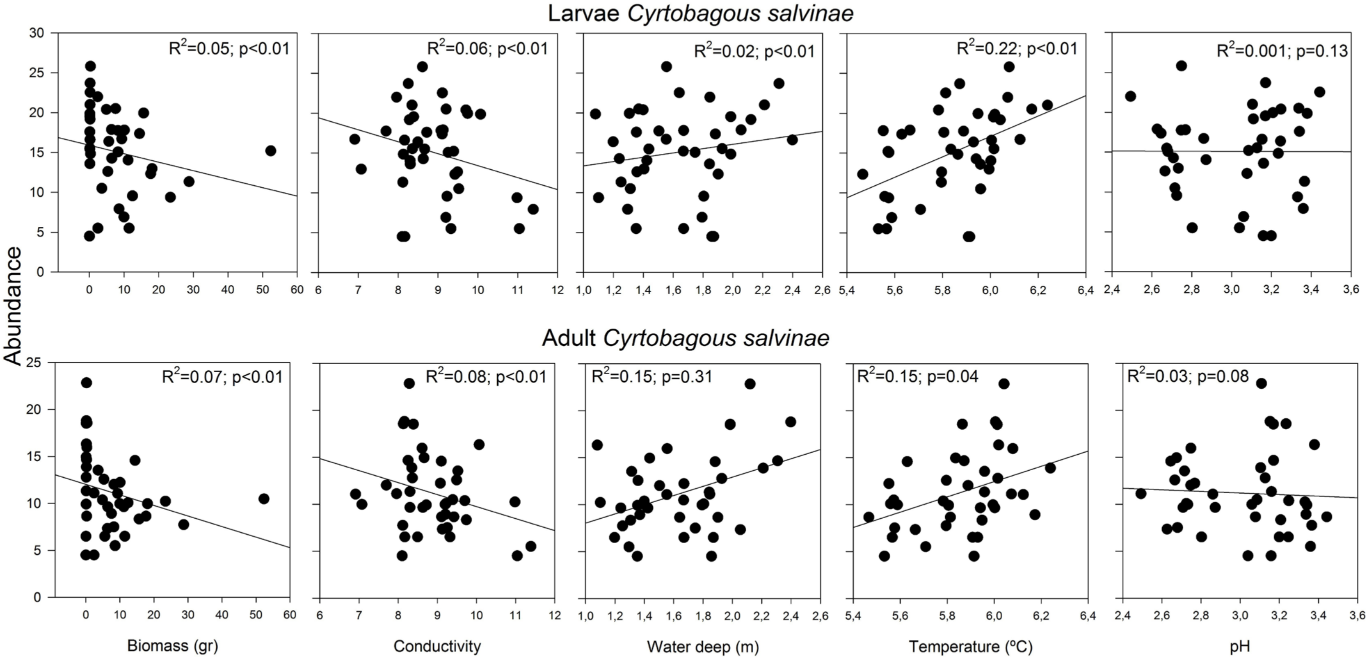

Figure 8. Linear regression between the biological variables (*Salvinia* fresh mass), limnological variables and abundances of *Cyrtobagous salvinae* larvae and adults in bays along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso, during the 2009 hydrological cycle.

larvae, which directly influences the birth and death rates in populations (e.g., McNeil et al. 2003), making *C. salviniae* more competitive than *C. singularis*. The co-occurrence analysis further indicated that C. salviniae is more competitive in banks of *Salvinia* spp. Other studies of aquatic curculionids have also reported the dominance of one species over another, e.g., *Neochetina eichhorniae* Warner, 1970 and *Neochetina bruchi* Hustache, 1926 (DeLoach & Cordo 1976a, 1976b, De Sousa et al. 2011). These imbalances may be associated with responses to environmental stress conditions as well as to the size and nutritional quality of the host plants (e.g., DeLoach & Cordo 1976b, Center & Dray 1992, Heard & Winterton 2000, Tipping & Center 2005), or to preferences for specific oviposition sites (e.g., DeLoach & Cordo 1976a, 1976b).

Greater competitiveness of *C. salviniae* in banks of *Salvinia* spp. can also be explained by the biology of the species. Adults of *C. salviniae* are smaller than those of *C. singularis*, and their larvae feed internally in rhizomes (Calder & Sands 1985); theoretically, they may be less vulnerable to generalist predators, which would likely feed on them despite their smaller size (Tipping et al. 2010). The adults and larvae of *C. singularis* are significantly larger than the adults and larvae of

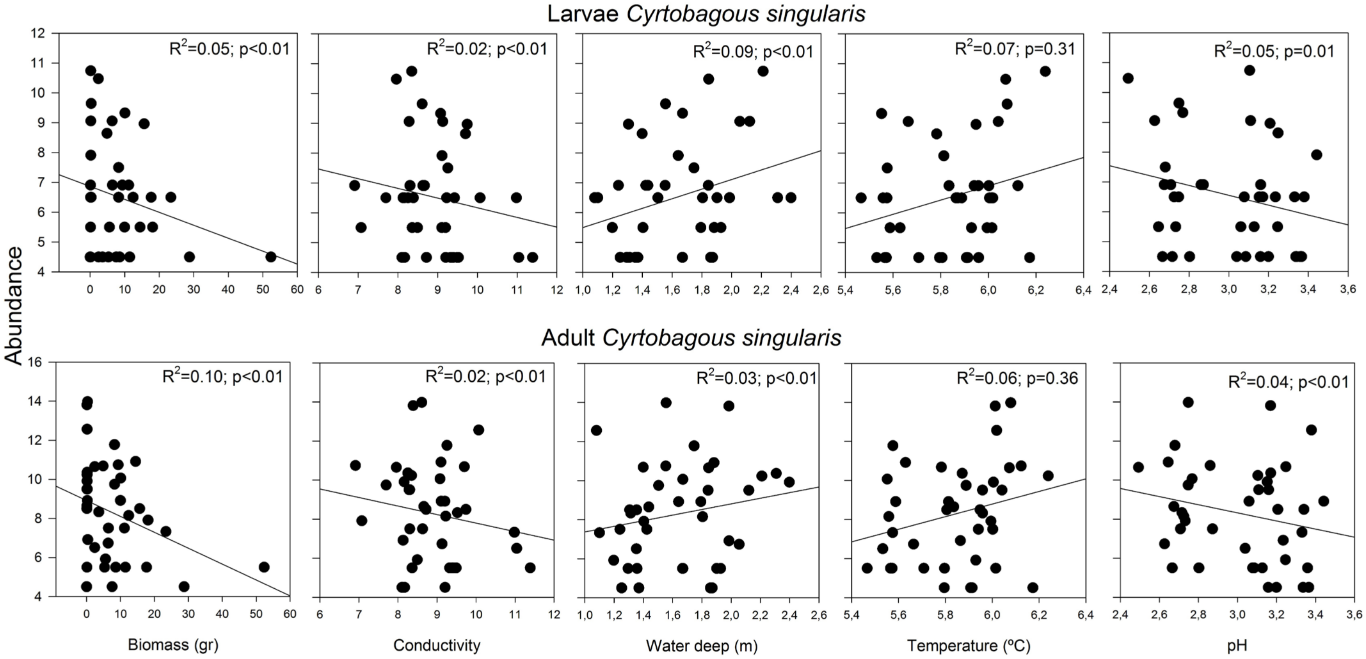

Figure 9. Linear regression between the biological variables (Salvinia fresh mass), limnological variables and abundances of Cyrtobagous singularis larvae and adults in bays along the Cuiabá River, Pantanal of Barão de Melgaço, Mato Grosso, during the 2009 hydrological cycle.

C. salviniae, and the larvae of the former feed externally rather than tunneling in the rhizome (Calder & Sands 1985, Sands et al. 1986); both attributes may make this species more vulnerable than *C. salviniae* to generalist predators. Furthermore, larger weevil larvae may be more sensitive than smaller larvae to plant size, as larger larvae may not be able to use small, narrow rhizomes, whereas smaller larvae may be able to burrow in a wider range of plant sizes (e.g., Tipping & Center 2005, Tipping et al. 2010).

Under laboratory conditions, *C. salviniae* laid seven times more eggs than *C. singularis*. These differences, along with differences in the survival of immature individuals, accounted for most of the differences in abundance between these species (Sands et al. 1986). Furthermore, *C. salviniae* requires favorable environmental conditions for pupation, which occurs in the submerged root hairs (e.g., Forno et al. 1983, Julien et al. 2002). During rising-water and high-water periods, the roots of floating *Salvinia* accumulate smaller amounts of organic matter and particles than during the other periods, and the physico-chemical characteristics of the water during rising- and high-water periods are more favorable for insect pupation in cocoons among the submerged root hairs. Accordingly, larvae and adults of both species reached higher abundances during the rising-water and high-water periods. The periods of low water levels are unfavorable for pupation among the underwater root hairs for both species, because the shallower water together with the isolation and high abundance of plants reduce water circulation, increase the accumulation of organic debris, and reduce water transparency. The abrupt decreases in oxygen and pH, coupled with the increased phosphorus and nitrogen concentrations in the inner bays likely reflected the storage of organic matter (e.g., De Sousa et al. 2011).

The significant variations in the abundances of larvae and adults of *C. salviniae* among the four seasonal periods demonstrated that the flood pulse influenced the population dynamics of this curculionid. Because the two species are congeners and probably phylogenetically proximate, the overlap in ecological niche requirements is accentuated, intensifying resource competition, which is further exacerbated by the high population densities in banks of *Salvinia* spp. In this respect, the data reinforce the supposition that the co-occurrence of these two curculionids can respond to spatial and temporal variations as a function of the hydrological periodicity, through resource partitioning (Kaplan & Denno 2007). The results suggest that the hydrological periodicity influences the evolutionary divergence of these phytophagous insects, making *C. salviniae* a superior competitor in a dynamic environment in terms of regional and local adaptations and dispersal ability, while allowing *C. singularis* to persist at low densities over time (e.g., Batzer & Wissinger 1996, Rozdilsky & Stone 2001, Roxburg et al. 2004, Urban et al. 2008).

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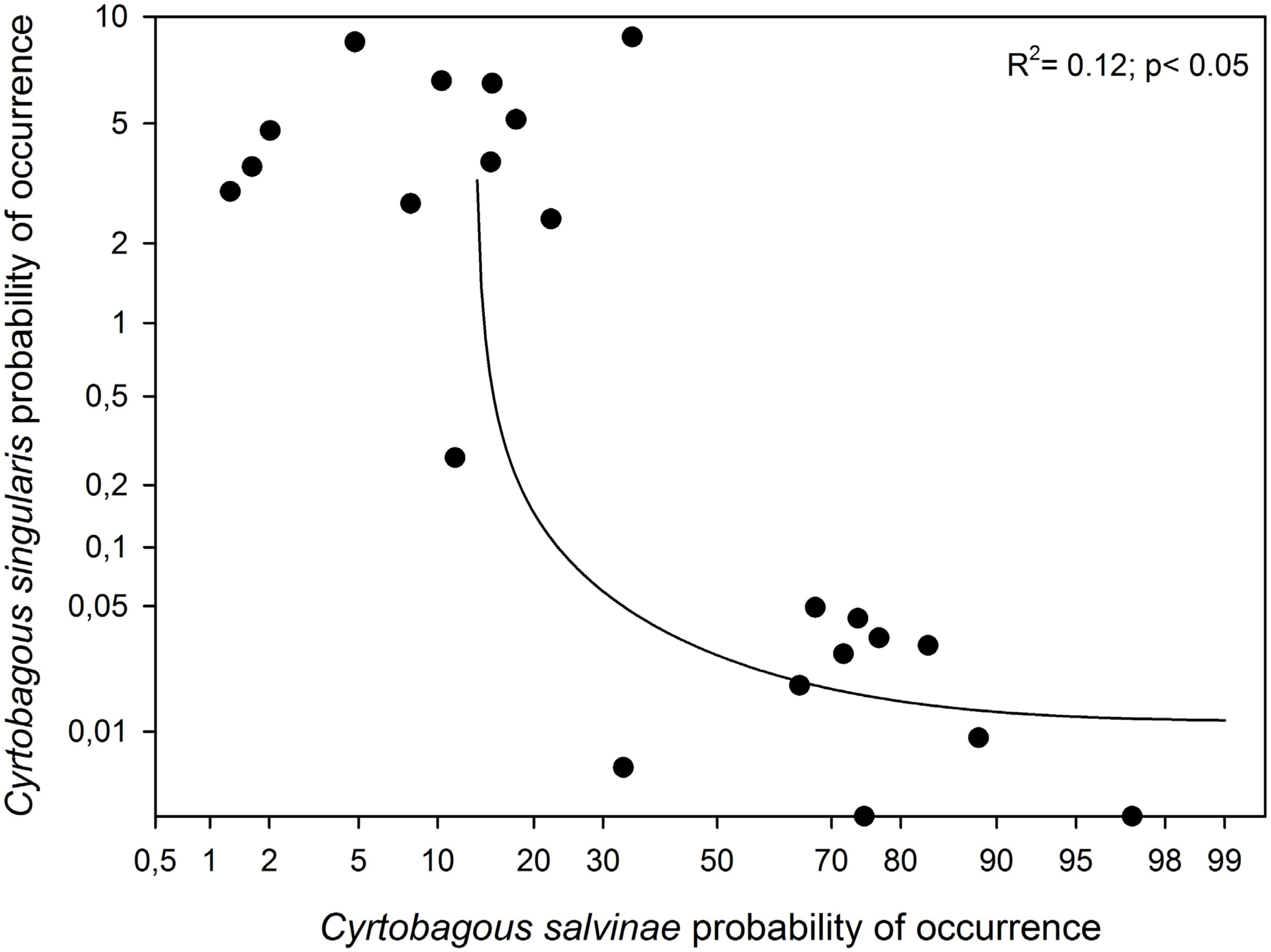

Figure 10. Nonlinear exponential regression of the occurrence probability of *Cyrtobagous singularis* in the presence of *Cyrtobagous salvinae.*

Associate Editor

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Authors Contributions

Wesley Oliveira de Sousa: substantial contribution in the concept and design of the study; data collection; analysis and interpretation; manuscript preparation and critical revision, adding intellectual content.

Nelson Vinicius Sabino Serra: substantial contribution in the data collection; analysis and interpretation; manuscript preparation and critical revision, adding intellectual content.

Geane Brizzola dos Santos: substantial contribution in the concept and design of the study; data collection; analysis and interpretation; manuscript preparation and critical revision, adding intellectual content.

Rodrigo Aranda: substantial contribution in the analysis and interpretation; manuscript preparation and critical revision, adding intelectual content.

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Conflicts of Interest

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

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Thematic Review

Insect galls on Asteraceae in Brazil: richness, geographic distribution, associated fauna, endemism and economic importance

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Abstract: An overview of insect galls on Asteraceae in Brazil is presented. We used the Web of Science database to find publications about insect galls from 1988 to 2020. We analyzed 88 publications and collected data from 51 of those. A total of 487 gall morphotypes were counted on 157 plant species of 42 genera. This value singled out Asteraceae as the richest plant family in number of gall morphotypes in Brazil. Most morphotypes were recorded in the Atlantic Forest (41%) and Cerrado (30.5%), the most surveyed biomes in Brazil. *Baccharis* L. supported the greatest gall richness (43.9%), which could be explained by the hypotheses of geographic area and taxon size. *Baccharis concinna* G.M. Barroso, B. *dracunculifolia* DC. and *B. platypoda* DC. were indicated as superhost species. Most galls were induced on stems (52.2%), a pattern known in Asteraceae for gall-inducing Tephritidae and Chloropidae, and extended in the present study to Cecidomyiidae. Most galls were fusiform (42.5%), which can be related to the highest number of gall on stems. Cecidomyiidae (Diptera) were the most frequent inducers, as observed worldwide. The presence of other dwellers - parasitoids, cecidophages, kleptoparasites, and successors - were reported in 8.8% of the gall morphotypes, being parasitoids the most frequent, as found in other Brazilian publications. Most host plants (58%) are endemic to Brazil, 14% are useful and few are vulnerable or endangered (six and four species, respectively). Due to the high host specificity, the gall-inducers associated with these plants can also be considered either endemic, important, vulnerable and/or endangered, respectively.

Keywords: *Gall-inducing insects; insect-plant interaction; Compositae.*

Galhas de insetos em Asteraceae no Brasil: riqueza, distribuição geográfica, fauna associada, endemismo e importância econômica

Resumo: Um panorama geral das galhas de insetos em Asteraceae no Brasil é apresentado. Usamos a base de dados "Web of Science" para encontrar publicações sobre galhas de insetos de 1988 a 2020. Analisamos 88 publicações no total, porém obtivemos dados de apenas 51. Um total de 487 morfotipos de galhas foi contabilizado em 157 espécies de plantas de 42 gêneros. Este valor indica as Asteraceae como a família botânica mais rica em número de morfotipos de galhas no Brasil. A maioria dos morfotipos foi registrada na Mata Atlântica (41%) e no Cerrado (30.5%), biomas mais investigados no Brasil. *Baccharis* L. suportou a maior riqueza de galhas (43.9%), o que poderia ser explicado pelas hipóteses de área geográfica e tamanho do táxon. *Baccharis concinna* G.M. Barroso, B. *dracunculifolia* DC. E B. *platypoda* DC. foram indicadas como as espécies super hospedeiras. A maioria das galhas foi induzida em caules (52.2%), um padrão conhecido em Asteraceae para Tephritidae e Chloropidae indutores de galhas, e estendido no presente estudo para Cecidomyiidae. A maioria das galhas foi fusiforme (42.5%), o que pode estar relacionado ao maior número de galhas em caule. Os Cecidomyiidae (Diptera) foram os indutores mais frequentes, como no mundo inteiro. A presença de outros ocupantes - parasitoides, cecidófagos, cleptoparasitas e sucessores - foi assinalada em 8.8% dos morfotipos de galhas, sendo os parasitoides os mais frequentes, como em outras publicações no país. A maioria das plantas hospedeiras são endêmicas do Brasil (58%), 14 são úteis e algumas são vulneráveis ou ameaçadas (seis e quatro, respectivamente). Devido à alta especificidade de plantas hospedeiras, os indutores de galhas associados a estas plantas podem ser considerados endêmicos, de importância econômica, vulneráveis e/ou ameaçados, respectivamente.

Palavras-chave: *Insetos indutores de galhas; interação inseto-planta; Compositae.*

Introduction

The Asteraceae family presents the largest number of species recorded in the world, totaling 32,581 species (Willis 2017). This group is found in all continents, except Antarctica, being more common in rural environments than in forests (Anderberg et al. 2007). In Brazil, Asteraceae are the third family in species diversity among Angiosperms and in number of endemisms (BFG 2018) with approximately 289 genera and 2,173 species, of which 71 genera and 1,367 species are endemic (Flora do Brasil 2020), occurring preferentially in open environments, such as savannahs and rupestrian fields (Hind & Miranda 2008).

Insect galls are pathological structures that originate new formations in plant tissues, as a result of mechanical and/or chemical stimulation by insects (Bronner 1992). According to Mani (1964), galls are induced in any part of a plant, both in vegetative and reproductive organs, providing food and shelter for the larva until its adult stage. Although the entomogenous galls occur in any part of the plants, they are most common on leaves and branches (Maia & Siqueira 2020).

Several plants of economic interest are vulnerable to damage by gall-inducing insects, including ornamental, edible, medicinal and pesticidal plants, as well as species used in carpentry, cosmetics, cabinet making, and agroforestry (Maia 2018). Most galling pests have been reported from the Old World and North America, whereas few examples are known from South America, especially in plants of the Asteraceae family.

In the last 30 years, several insect gall inventories in different Brazilian phytophysiognomies have been published (Araújo 2018). Most of them comprise galls on Asteraceae and indicate this family as one of the most important in gall richness. Other families are also important, such as Fabaceae (Santos-Silva & Araújo 2020) and Myrtaceae (Maia 2019). As data are scattered in the literature, the number of host plant species and gall-inducing species are unknown, as well as data on their associated fauna, distribution, endemism, and economic importance.

The main goal of this paper is to present a panoramic view of insect galls on Asteraceae in Brazil. The specific objectives are: (1) to inventory the gall-morphotypes on Asteraceae species in Brazil; (2) register the distribution of the galls phytogeographic domains; (3) determine the most frequent morphotype as well as the most attacked plant organ and the presence or absence of pubescence; (4) provide information about the identification of the inducers and the guilds of associated fauna; (5) obtain information about the origin, endemism, status of conservation, and the economic importance of the host plants and their gall inducing species.

Material and Methods

Insect gall scientific articles sampling Brazilian areas published from 1988 until 2020 were analyzed in order to collect information about insect galls on Asteraceae in Web of Science between August, 2020 and October, 2020 (www.periodicos.capes.gov.br). The following descriptors were used: Brazil/*Brasil*, Galls/*Galhas*, Insect galls/*Galhas de insetos*, Gall-inducing insects/*Insetos galhadores*, Cecidomyiidae, Asteraceae, Compositae. We only considered host plants identified to species level, to avoid overestimating gall morphotypes number.

Botanical names and synonyms were verified in Flora do Brasil (2020), as well as data on plant origin, geographic distribution and conservation categories (EN - endangered, LC - least concern, NE - not evaluated, NT - near threatened, VU - vulnerable) (IUCN 2020). Plant uses were verified using the websites Useful Tropical Plants Database (2014 - http://tropical.theferns.info) and EMBRAPA (https://www.embrapa.br/). The following categories were considered: agroforestry, edible, medicinal and other uses. Phytogeographic domains were retrieved from the original papers or verified in vegetation maps of Brazil (IBGE 2004). Host plants that occur exclusively in one single domain were considered as endemic of this domain. The plant species that presented the highest number of galls were considered galls' super-hosts.

Gall shapes were standardized based on Isaias et al. (2013), whenever possible. When published data were insufficient for standardization, we kept the terminology of the original papers. To establish the number of gall morphotypes per plant species, we compared gall morphology. Whenever we found galls with similar morphological characterization, but listed as different morphotypes in the same paper, we adopted the author(s)' concept.

In the present study, all records of Cecidomyiidae (Diptera) as gall-inducer were converted in Cecidomyiinae records, since this is the single subfamily of Cecidomyiidae which includes cecidogenous species (Gagné & Jaschhof 2021). Concerning the associated fauna, records of inquilines were converted in records of cecidophages and kleptoparasites, according to the criteria proposed by Luz & Mendonça-Júnior (2019).

Results

1. *Inventory of galls*

We found 88 articles reporting insect galls sampled from Brazilian areas: 64 with gall morphotypes on Asteraceae, 51 with identified host plant species and 24 of them without Asteraceae as hosts. Asteraceae species appear as hosts of insect galls in 34 (31%) of these studies, and this family is indicated as one of the super hosts in 21 inventories. They covered five (83%) Brazilian phytogeographic domains, with the Atlantic Forest and Cerrado the most studied, with 33 and 29 inventories, respectively. The surveys totaled 487 insect gall morphotypes in association with 157 species of 42 genera (Figure 1). *Baccharis* L. and *Mikania* Willd. showed the highest number of galled species, 42 (26.7%) and 29 (18.5%), and the greatest richness of galls, 218 (43.9%) and 76 (15.6%) morphotypes, respectively. The super-host plant species were: *Baccharis concinna* G.M.Barroso (n=18 morphotypes; 3.7%), *B. dracunculifoli*a DC. (n=17; 3.5%), *B. platypoda* DC. (n=17; 3.5%), *B. reticulari*a DC. (n=17; 3.5%), *B. retusa* DC. (n=16; 3.3%), *B. minutiflora* Mart. ex Baker. (n=14; 2.9%), *Eremanthus erythropappus* (DC.) MacLeish (n=12; 2.5%) and *Mikania glomerata* Spreng. (n=10; 2.1%) (Supplementary Material).

2. *Gall richness by phytogeographic domains*

The Atlantic Forest was the domain with the highest number of gall morphotypes, 200 (41%), followed by Cerrado with 150 (30.5%), Pampa with five, Pantanal with three and Amazon rainforest with two. Caatinga (dry forest) presented the lowest number of galls, with only one morphotype. Eighty-seven (55%) host plant species were recorded only in the Atlantic Forest, sixty-seven only in the Cerrado (42%), eighty in the Atlantic Forest + Cerrado, one in the Caatinga + Cerrado, and two in the Amazon Forest. Thirty-eight host plant species (24.2%) were more widespread than their galls, 28 were as widespread as their galls and 19 host plant species were reported in phytogeographic domains outside their distribution area according to Flora do Brasil (2020) (Table 1), showing that there is still no data on galls for Brazil.

3. *Gall morphotypes*

Thirteen distinct gall shapes were found, the most frequent were fusiform (42.5%) and globoid (35.1%) (Figures 1 and 2). The shape of two gall morphotypes was not informed. The most attacked organs were stems (n=254; 52.2%), leaves (n=165; 33.9%), bud galls (n=92; 14.2%) and flower (n=14; 2.8%) (Table 2). Galls on flowers (1.3%) were found only in Atlantic Forest and Cerrado. The majority of the galls were glabrous (58.4%), except in Pantanal where among the three galls found, two presented trichomes. Data on pubescence in 28.2% of the morphotypes (n=102) were not informed by the authors as well as the phytogeographic domains of 132 gall morphotypes. In addition, some of the galls occurred simultaneously in more than one plant organ so that the results shown in table 3 have totals higher than the overall number.

4. *Gall-inducing insects and associated fauna*

The inducers belong to four orders: Diptera (64.7%), Lepidoptera (6%), Hemiptera (3.3%) and Coleoptera (2%). Furthermore, inducers of 117 gall morphotypes (24%) were not determined (Table 3). Two families represented Diptera: Cecidomyiidae (61.8%) and Tephritidae (2.9%). Fifteen genera of gall midges have been recorded, most of

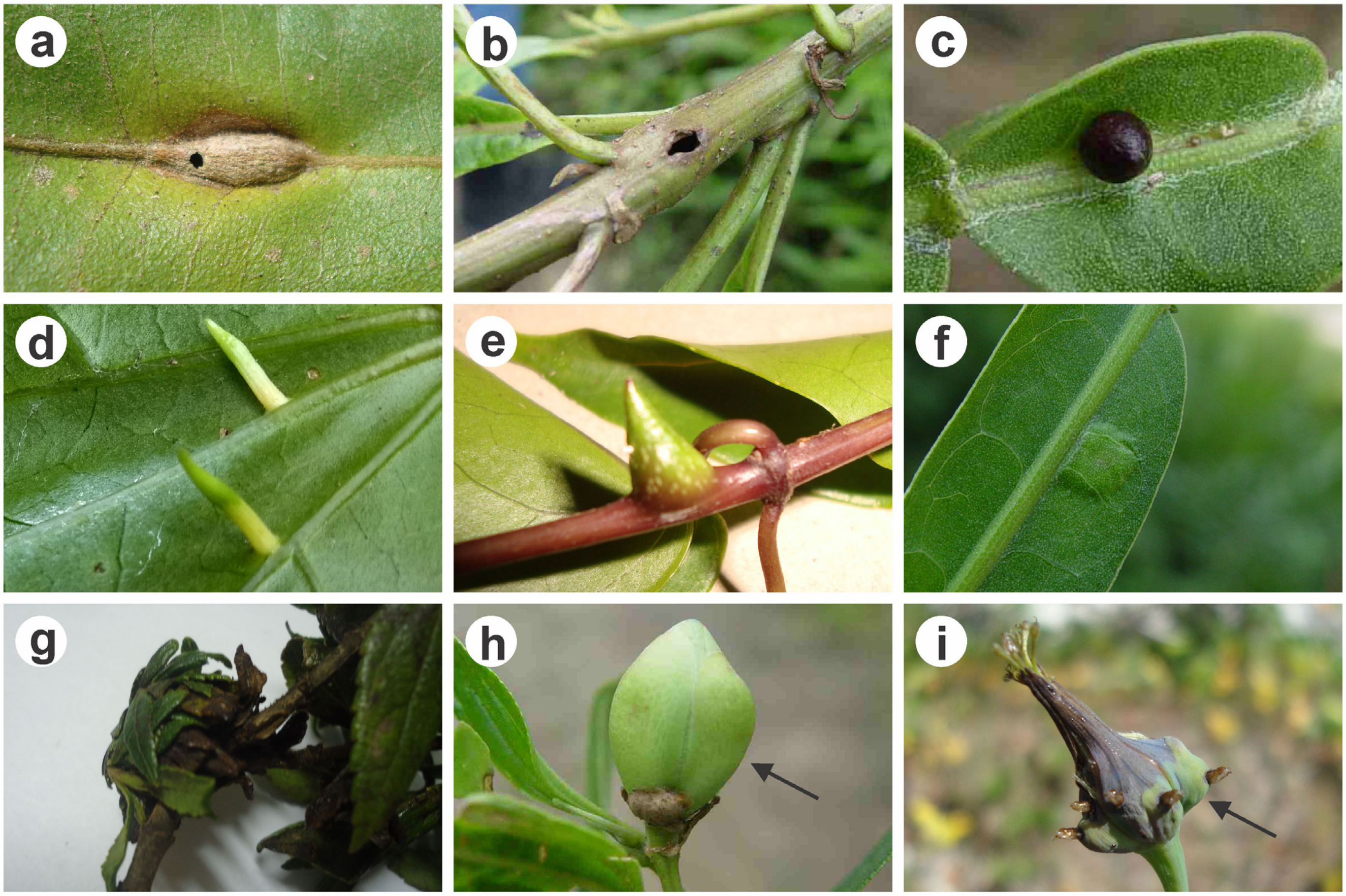

Figure 1. Galls on Asteraceae in Brazil. a) fusiform gall on leaf of *Mikania argyreiae* DC.; b) fusiform gall on stem of *Vernonia beyrichii* Less.; c) globoid gall on leaf of *Baccharis conyzoides* (Less.) DC.; d) cylindrical gall on leaf of *Mikania glomerata* Spreng.; e) conical gall on stem of *Mikania micrantha* Kunth; f) lenticular gall on leaf of *Baccharis conyzoides* (Less.) DC.; g) rosette gall on lateral bud of *Grazielia gaudichaudiana* (DC.) R.M.King & H.Rob.; h) globoid gall on apical bud of *Baccharis dracunculifolia* DC.; and i) fusiform gall with pupal exuvia on Flower-head of *Porophyllum ruderale* (Jacq.) Cass.

Table 1. Number of gall morphotypes by genera and species of Asteraceae, and the average number of gall per host plant species in each phytogeographic domain in Brazil.

Phytogeographic domain	Nº of galled plant genera	Nº of host species	Nº of morphotypes	Average number of gall/ host plant species
Atlantic Forest	29	87	200	2.3
Amazon	02	02	02	1.0
Caatinga-Cerrado	01	01	01	1.0
Cerrado	26	67	150	2.2
Pampa	03	04	05	1.25
Pantanal	02	02	03	1.5
No data	05	13	139	-

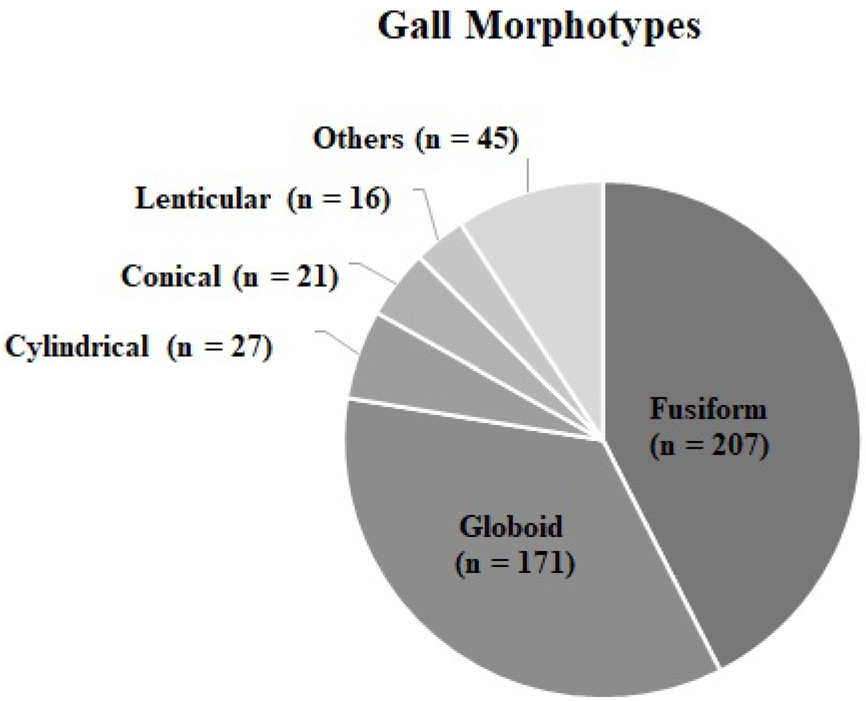

Figure 2. Number of gall morphotypes recorded on Asteraceae species in Brazil. Other morphotypes: rosette, marginal roll, amorphous, discoid*, claviform, ovoid*, spherical*, swelling* and not informed (* morphotypes with terminology not updated).

Table 2. Number of gall morphotypes per plant organ of Asteraceae species in each phytogeographic domain in Brazil.

Plant organs	Atlantic Forest	Cerrado	Pantanal	Pampa	Amazon	Caatinga-Cerrado
Stem	92	89	2	3	1	1
Leaf	84	51	1	2	1	-
Bud	35	20	-	-	-	-
Flower	3	2	-	-	-	-

them (n=12) are represented by a single identified species, except *Asphondylia* (n=4 species), *Liodiplosis* (n=3), and *Clinodiplosis* (n=2). Furthermore, nine genera include undetermined species, highlighting *Asphondylia* due to its highest richness. Tephritidae were registered in 14 gall morphotypes, with only one inducer identified in species, *Tomoplagia rudolphi* (Lutz & Lima, 1918) on *Vernonanthura polyanthes* (Sprengel) Vega & Dematteis.

Associated fauna were found in 43 gall morphotypes (8.8%) on 30 plant species of 15 genera (Table 4). *Mikania* and *Baccharis* comprised the highest number of morphotypes with associated fauna (14 and 9, respectively). Four guilds were represented: cecidophages, kleptoparasites, parasitoids, and successors. Among them, parasitoids were the most frequent, found in 29 gall morphotypes on 24 plant species, followed by cecidophages, found in 12 gall morphotypes on 11 plant species, and kleptoparasites in eight morphotypes on three plant species. Successors were the least frequent, being found in six gall morphotypes on six plant species.

Parasitoids were represented by Hymenoptera of six families, the most frequent being Eulophidae (n=9) and Eurytomidae (n=4). Multiparasitism was reported in six gall morphotypes. Cecidophages were represented by Diptera, Hemiptera, Coleoptera, Lepidoptera, and Thysanoptera. Diptera were the most frequent being found in seven gall morphotypes followed by Coleoptera (n=4), Lepidoptera (n=3), Hemiptera (n=2), and Thysanoptera (n=2). Kleptoparasites were represented by Cecidomyiidae (Diptera), while successors by Formicidae (Hymenoptera) and Collembola. Spiders were recorded in a single gall morphotype, but the authors did not include these organisms in any guild (Silva et al. 2018).

The four guilds were reported only in Atlantic Forest, while only kleptoparasites were not recorded in the Cerrado. In the Caatinga-Cerrado transition and Amazon Forest only one guild was reported, successors and kleptoparasites, respectively. Thirty gall morphotypes hosted associated fauna in the Atlantic Forest, 11 in the Cerrado, one in Amazon Forest, and one in the Caatinga-Cerrado transition.

Table 3. Richness of gall morphotypes per order and family of inducing insects.

Inducers		**Number of gall morphotypes**	**%**
Order	**Family**		
Diptera	Cecidomyiidae	301	61.8
	Tephritidae	14	2.9
Coleoptera		06	1.2
	Curculionidae	04	0.8
Hemiptera		05	1.0
	Psyllidae	11	2.3
Lepidoptera		29	6.0
Not determined		117	24.0

Table 4. Associated fauna of galls on Asteraceae species and their phytogeographic domains of occurrence. (1) Cecidophages, (2) Parasitoids, (3) Kleptoparasites, (4) Successors, (5) not determined guild.

Host plant species	**Gall morphotypes**	**Associated fauna**	**Phytogeographic domains**
Ageratum conyzoides L.	Bud / globoid	Aphidae (Hemiptera) (1) Muscomorpha (Diptera) (1)	Atlantic Forest
Baccharis bifrons Baker	Leaf / lenticular	Hymenoptera (2)	Atlantic Forest
Baccharis microcephala (Less.) DC.	Bud / globoid	Eulophidae (Hymenoptera) (2) *Galeopsomyia sp*. (Eulophidae) (2) *Eurytomasp.* (Eurytomidae, Hymenoptera) (2)	Atlantic Forest
	Leaf mid vein / fusiform	Eulophidae (Hymenoptera) (2) *Galeopsomyia* sp. (Eulophidae) (2) *Eurytoma* sp. (Eurytomidae, Hymenoptera) (2)	
Baccharis pedunculata (Mill.) Cabrera	Bud and stem / fusiform	Lepidoptera (1) Collembola (4) Platygastridae (Hymenoptera) (2)	Atlantic Forest
Baccharis pingraea (Lam.) Pers	Stem and leaf / globoid	Hymenoptera (2)	Cerrado
Baccharis reticularia DC.	Bud and stem / globoid	Sciaridae (Diptera) (1) Formicidae (Hymenoptera) (4)	Cerrado
	Leaf / marginal roll	Heteroptera (Hemiptera) (1)	
	Leaf petiole, leaf vein and stem / fusiform	Platygastridae (2)	
Baccharis singularis (Vell.) G. M. Barroso	Leaf vein and stem / fusiform	Thysanoptera (1)	Atlantic Forest
Calea pinnatifida (R. Br.) Less	Stem / fusiform	Hymenoptera (2)	Atlantic Forest
Chromolaena odorata (L.) R.M.King and H.Rob.	Stem / conical	*Trotteria lapalmae* Möhn, 1975 (Cecidomyiidae, Diptera) (3)	Amazon Forest
	Leaf vein / fusiform	Hymenoptera (2)	Atlantic Forest
Eremanthus capitatus (Spreng.) MacLeish	Stem / globoid	Formicidae (4)	Caatinga-Cerrado
Eremanthus polycephalus (DC.) MacLeish.	Stem / globoid	Formicidae (4)	Atlantic Forest
Graphistylis itatiaiae (Dusén) B.Nord.	Bud / fusiform	Hymenoptera (2)	Atlantic Forest
Grazielia gaudichaudeana (DC.) R.M.King and H.Rob.	Leaf / globoid	Lepidoptera (1)	Atlantic Forest
Lepidaploa rufogrisea	Stem / globoid	Sciaridae (1) Resseliella sp. (Cecidomyiidae) (3) Hymenoptera (2)	Atlantic Forest
Mikania argyreiae DC.	Leaf / globoid	Hymenoptera (2)	Atlantic Forest
Mikania glomerata Spreng.	Stem / conical	*Contarinia ubiquita* Gagné, 2001 (Cecidomyiidae) (3)	Atlantic Forest
	Leaf / globoid	*Contarinia* sp. (Cecidomyiidae) (3) *Clinodiplosis* sp. (Cecidomyiidae) (3) Coleoptera (1) Hymenoptera (2) Thysanoptera (4)	

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	Leaf / cylindrical	Hymenoptera (2)	
	Leaf petiole / fusiform	*Contarinia ubiquita* (3)	
	Stem / fusiform	*Clinodiplosis* sp. (3)	
	Bud / globoid	*Contarinia ubiquita* (3)	
Mikania hoehnei Robinson	Leaf / marginal roll	Hymenoptera (2) Eulophidae (2) Sciaridae (1)	Atlantic Forest
	Stem / globoid	*Dimeromicrus cecidomyiae* (Ashmead, 1887) (Torymidae, Hymenoptera) (2)	
Mikania involucrata Hook. &Arn.	Stem / amorphous	Torymidae (2)	Atlantic Forest
Mikania lasiandra DC.	Leaf and stem / globoid	Hymenoptera (2)	Atlantic Forest
Mikania lindbergii Baker	Stem / fusiform	Hymenoptera (2)	Cerrado
Mikania periplocifolia Hook. andArn.	Leaf vein / fusiform	Hymenoptera (2)	Atlantic Forest
Mikania pseudohoffmanniana G. M. Barroso	Stem / fusiform	Hymenoptera (2)	Atlantic Forest
Moquiniastrum paniculatum (Less.) G. Sancho	Leaf / globoid	Eulophidae (2) Eurytomidae (2)	Atlantic Forest Cerrado
Moquiniastrum pulchrum (Cabrera) G.Sancho	Leaf and bud / amorphous	Eulophidae (2)	Cerrado
Porophyllum ruderale (Jack.) Cass.	Stem / fusiform	Eulophidae (2) Eurytomidae (2)	Atlantic Forest Cerrado
	Inflorescence / fusiform	*Trypanea* sp. (Tephritidae, Diptera) (1)	Atlantic Forest
Pterocaulon virgatum (L.) DC.	Stem / globoid	Hymenoptera (2)	Atlantic Forest
Symphyopappus reticulatus Baker	Stem and leaf vein / fusiform	Muscomorpha cfr. (1) Hymenoptera (2)	Atlantic Forest
Verbesina macrophylla(Cass.) S.F.Blake	Bud / globoid	Araneae (5) Lepidoptera (5)	Cerrado
Vernonanthura beyrichii (Less.) H.Rob.	Bud and stem / fusiform	Sciaridae (1) Lepidoptera (1) Collembola (4)	Atlantic Forest
Vernonanthura membranacea (Gardner) H.Rob.	Bud / globoid	Hymenoptera (2)	Atlantic Forest
Vernonanthura polyanthes (Spreng) Vega & Dematteis	Stem and leaf bud / globoid and fusiform,	Braconidae (2) (Hymenoptera) Eulophidae (2)	Cerrado
	Leaf and stem / globoid	Torymidae (2) Chalcididae (2) (Hymenoptera)	Atlantic Forest Cerrado

5. *Origin, endemism and economical importance*

Regarding the origin of the host plant species, 96.8% (n=152) are native to Brazil, among them 58% (n=91) are endemic to Brazil, 25.3% (n=23) being endemic to the Atlantic Forest, 30.8% to Cerrado and 1.1% to Caatinga (Table 5). *Chromolaena ivifolia* (L.) R.M.King & Lamp; H.Rob. and *Mikania lindleyana* DC. were recorded in the Amazon rainforest and *Aspilia latissima* Malme and *Vernonanthura brasiliana* (L.) H.Rob. in the Pantanal, species that are native but not endemic. There was no occurrence of Asteraceae in the Caatinga, but one species was recorded in a transition area between Caatinga to Cerrado.

Concerning IUNC (2020) conservation categories, plant species were classified into: NE: 79.6% (n=125 plants; 392 morphotypes), LC: 7.6% (n=12; 43), NT: 4.5% (n=7; 13), VU: 3.8% (n=6; 30) and EN: 2.5% (n=4; 6). The Cerrado presented the largest number of threatened species (EN, NT and VU), 13, followed by the Atlantic Forest biomes with four (VU).

Among the 157 host plants, only 22 (14%) are economically useful, most being medicinal (59%), 36.3% have agroforestry use and only 18.8% are edible. Furthermore, 54.5% have other uses (Table 6). These plants host 98 gall inducing insects, most of them are Cecidomyiinae (65.3%), followed by Tephritidae (7.1%), Hemiptera (5.1%), Lepidoptera (4%) and Coleoptera (2%). In 16.3% the galling insects were not determined. Eighteen inducers were identified at species level and six at genera level.

Discussion

In this study we compiled 487 gall morphotypes on Asteraceae, a higher number than compiled on Fabaceae (n=437) by Santos-Silva & Araújo (2020), showing that Asteraceae are the main insect gall hosts in Brazil. Nevertheless, the number of galled species is higher in Fabaceae (n=178) than in Asteraceae (n=157). Although the Cerrado has the largest number of Asteraceae species (n=1,238) (Flora do Brasil 2020), the Atlantic Forest presents the highest number of galled plant species, gall morphotypes and articles reporting galls. In a similar study with Fabaceae, Santos-Silva & Araújo (2020) reported the greatest gall richness in the Cerrado, while the Atlantic Forest occupied the second place. The highest gall richness of Atlantic Forest and Cerrado can be explained by the fact that these biomes have been more intensely surveyed and additionally they housed the first research centers focusing on gall-inducing insects in Brazil (Universidade Federal de Minas Gerais and Universidade Federal do Rio de Janeiro, respectively) (Araújo et al. 2019).

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Table 5. Origin of Asteraceae host species and number of gall morphotypes in Brazil.

Origin	Number of host plants	Number of galls
Native	152	482
No data	05	05
Total	**157**	**487**
Endemic to Brazil	39	145
Endemic to Atlantic Forest	23	50
Endemic to Cerrado	28	74
Endemic to Caatinga	01	01
Not Endemic	59	205
No data	07	12
Total	**157**	**487**

Table 6. The Asteraceae species hosting insect galls in Brazil, their economic importance, and the number of gall inducer morphospecies by host plant.

Host Plant	Economic Importance				Number of gall inducer morphospecies
	Medicinal	Edible uses	Agroforestry	Other uses	
Achyrocline satureioides (Lam.) DC	x	-	-	-	01
Ageratum conyzoides L.	x	x	-	x	05
Aspilia foliosa (Gardner) Benth. & Hook.	x	-	-	-	02
Austroeupatorium inulaefolium (Kunth) R.M.King&H.Rob.	-	-	x	-	01
Baccharis dracunculifolia DC.	-	-	-	x	17
Baccharis reticularia DC.	-	-	-	x	17
Chromolaena odorata (L.) R.M.King and H.Rob.	x	x	x	x	05
Conyza canadensis (L.) Cronquist	x	x	x	x	01
Dasyphyllum brasiliense (Spreng.) Cabrera	-	-	-	x	02
Dasyphyllum spinescens (Less.) Cabrera	-	-	x	x	03
Eremanthus erythropappus (DC.) MacLeish	-	-	x	x	08
Lychnophora ericoides Mart.	x	-	-	-	02
Lychnophora pinaster Mart.	x	-	-	-	02
Mikania glomerata Spreng.	x	-	-	-	10
Mikania laevigata Sch.Bip. ex Baker	x	-	-	-	03
Mikania micrantha Kunth	x	-	x	-	08
Piptocarpha axillaris (Less.) Baker	-	-	-	x	01
Piptocarpha rotundifolia (Less.) Baker	x	-	x	x	02
Porophyllum ruderale (Jack.) Cass.	x	x	-	x	01
Pseudobrickellia brasiliensis (Spreng.) R.M.King	x	-	-	-	01
Vernonanthura brasiliana (L.) H.Rob.	-	-	-	x	02
Vernonanthura polyanthes (Sprengel) Vega & Dematteis	-	-	x	-	04

The genera with the largest number of galled species and gall morphotypes were *Baccharis* and *Mikania*. A large number of galls induced by insects have been studied on many species of *Baccharis* and *Mikania* (Fernandes et al. 2014). Both these genera are widely distributed throughout Brazil and they are the richer in species within the Asteraceae family, favoring their association with gall-inducing insects. The hypothesis of geographic area (Southwood 1960) and hypothesis of taxon size (Fernandes 1992) could perhaps help explain the greater insect galls richness on *Baccharis* and *Mikania*. They predict that taxa with wider geographic distribution and greater species richness have potentially a greater number of associated galling insects than taxa with more restricted distribution and lower species richness. The presence of super-hosts species is common in the Neotropical region; hence contributing to the increase of the local and regional fauna of gall-inducers in the communities (Fernandes et al. 2014). *Baccharis dracunculifolia* and *B. concinna* were also reported by Fernandes et al. (1996) as super-hosts, as well as *Mikania glomerata* in different inventories in Brazil (Maia 2013, Maia & Proença 2016).

The highest frequency of the fusiform galls in this study differs from the pattern observed in the Neotropical region where conical galls are the most common (Isaias et al. 2014). Santos-Silva & Araújo (2020) reported a predominance of globoid galls in Fabaceae. The highest number of fusiform galls in our study can be related to the high number of gall on stems. When compared to leaves, stems are less plastic organs, perhaps not allowing many variations in the gall shape (Valladares et al. 2006). The predominance of stem galls differ from the pattern of all zoogeographic regions, where most galls are on leaves (Mani, 1964). However, the higher number of stem galls in Asteraceae is a pattern already known for some gall-inducing taxa, as Tephritidae (Friedberg 1984), Chloropidae (Foote et al. 1993), and Lepidoptera (Maia 2006). In the present study, the last pattern is suggested for gall midges too.

Cecidomyiidae were the most frequent gall-inducers. In fact, they represent the most diverse gall-inducing family in the whole world. They comprise about 6,590 species, about 70% of them being gall-inducing (Gagné & Jaschhof 2021). The *Asphondylia* was the most diverse gall-inducing genus in our study. This genus is speciose, cosmopolitan and easily recognizable and these features could explain its richness (Gagné & Jaschhof 2021). Other Diptera families include gall-inducers, as Tephritidae, but in this family, only a small percentage of species (about 10%) exhibits this habit. Nevertheless, most Tephritidae species induce galls on Asteraceae and this is recognised as a worldwide pattern (Freidberg 1984). Most described species of Cecidomyiidae induce galls on Myrtaceae in Brazil (Maia 2019). Galls of Lepidoptera, Coleoptera and Hemiptera are less frequent than those of Cecidomyiidae, nevertheless, these orders are frequently reported (*e.g.* Gonçalves-Alvim & Fernandes 2001, Maia et al. 2008, Malves & Frieiro-Costa 2012), but always responsible for few gall morphotypes.

In this study parasitoids were the most frequent guild as in several Brazilian inventories, being represented exclusively by Hymenoptera (*e.g.* Carvalho & Mota 2018, Silva et al. 2018, Maia & Siqueira 2020). In fact, they are considered the most important natural enemies of the gall-inducing insects, not only in Brazil, but also in the world (Gagné 1994). Eulophidae and Eurytomidae are families usually cited as parasitoids of gall-inducers in Brazil (*e.g.* Maia 2001, Carvalho-Fernandes et al. 2016, Ribeiro et al. 2019). Cecidophages, kleptoparasites and successors are infrequent in inventories, and the insect taxa included in these guilds have been found in Asteraceae as well as in other plant families (*e.g.* Maia 2001, Carvalho-Fernandes et al. 2016, Maia & Siqueira 2020).

Few studies refer to the origin, endemism and threat category of host plant species associated with galling insects. Among the 88 scientific publications studied, only three presented this information (Maia & Mascarenhas 2017, Maia & Siqueira 2020, Santos-Silva & Araújo 2020). In our study 50% are endemic and this value is higher when compared to that of Santos-Silva & Araújo (2020) for Fabaceae (29%). Furthermore, 17 botanical species are under a threatened category of conservation. Given the accepted high levels of host-inducer specificity (Carneiro et al. 2009), we suggest that gall inducers associated exclusively with endemic hosts are endemic and those associated with threatened plants are also threatened.

Despite the low percentage of useful plants observed in this study, it is important to quantify the diversity of gall inducers, because their presence can cause several damage, such as, plant growth reduction, lower photosynthesis rate and reduced fruits, resulting in economic losses (McCrea et al. 1985, Fernandes 1987, Souza et al. 1998). These losses can be increased when more than one galling species occur on the same host, as we observed in 77.2% of the useful species of Asteraceae. Once again, based on species-specificity, we suggest that all Cecidomyiidae related to useful plants can be considered at least potentially of economic importance.

Conclusions

We can conclude that Asteraceae is the main host family of insect galls in Brazil and most morphotypes were reported in the Atlantic Forest and Cerrado. The richest genera in gall morphotypes were also the most widely distributed around Brazil and one of the best represented in number of species. The most attacked plant organ and the most frequent gall morphotype on Asteraceae do not corroborate the pattern observed for the Neotropical region.

The composition of gall inducing insects and fauna associated followed what is already known for the Neotropical region. Due to Cecidomyiidae high level of specificity, they can be considered endemic, useful, vulnerable and/or threatened as well as their host plants.

Finally, this study provides important data on the presence of gall inducing insects in one of the richest families of Angiosperms in Brazil. Such studies are rare in Brazil important to consolidate the current knowledge, and to show the diversity and frequency of these insects in Brazil. They also provide subsidies to other studies such as biogeography and conservation.

Supplementary Material

The following online material is available for this article:

Table - Insect galls on Asteraceae species occurring in Brazil.

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Conflicts of Interest

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

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Point-of-view

Remote spatial analysis lacking ethnographic grounding mischaracterizes sustainability of Indigenous burning regime

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Abstract: Scientific research that purports to evaluate Indigenous fire regimes in the absence of ethnographically contextualized ecological data runs the risk of exacerbating the fire blame game and providing evidence to support distorted narratives advanced by anti-Indigenous advocates. Spatial analysis of fire scars in Indigenous territories can be an effective tool for characterizing cultural fire regimes in terms of distribution and frequency, especially when qualified by linkages to different local ecosystems. A recently published article drew on fire scar mapping from satellite imagery to assess anthropogenic fire distribution and frequency in the Pimentel Barbosa Indigenous Land, Central Brazil. The authors use their findings to characterize A'uwẽ (Xavante) use of fire as unmanaged and a model of unsustainable use of cerrado resources. In this article, we discuss Aguiar & Martins's recent paper in light of our long-term research on A'uwẽ hunting with fire in the Pimentel Barbosa Indigenous Land, arguing that A'uwẽ hunters do burn according to established cultural protocols, manage their use of fire for conservationist purposes, and do not cause environmental degradation by burning.
Keywords: Spatial analysis, fire management, fire regimes, Indigenous fire use, cerrado.

Análise espacial remota sem embasamento etnográfico descaracteriza a sustentabilidade do regime de queimada indígena

Resumo: A pesquisa científica que pretende avaliar regimes indígenas de queimadas na ausência de dados ecológicos contextualizados etnograficamente corre o risco de exacerbar o jogo de culpabilização do fogo, fornecendo evidências para apoiar narrativas distorcidas apresentadas por militantes anti-indígenas. A análise espacial de cicatrizes de fogo em territórios indígenas pode ser uma ferramenta eficaz para caracterizar regimes culturais de fogo em termos de distribuição e frequência, especialmente quando qualificada por ligações a diferentes ecossistemas locais. Um artigo publicado recentemente se baseou no mapeamento de cicatrizes de fogo a partir de imagens de satélite para avaliar a distribuição e frequência antropogênica de fogo na Terra Indígena Pimentel Barbosa, Brasil Central. Os autores usam seus resultados para caracterizar o uso do fogo pelos A'uwẽ (Xavante) como não manejado e um modelo insustentável de uso de recursos do cerrado. Neste artigo, discutimos o artigo recente de Aguiar & Martins à luz de nossa pesquisa de longa duração sobre a caçada com fogo praticada pelos A'uwẽ na Terra Indígena Pimentel Barbosa, argumentando que os caçadores A'uwẽ queimam de acordo com protocolos culturais estabelecidos, manejam o fogo de maneira conservacionista e não causam degradação ambiental pela queimada.
Palavras-chave: Análise espacial, manejo do fogo, regimes de fogo, uso indígena do fogo, cerrado.

Introduction

Indigenous and traditional peoples who ignite landscape vegetation for subsistence purposes are often inappropriately blamed for purported increases in land degradation and carbon emissions even though agribusiness and other forms of non-traditional occupation contribute to these problems many times over in comparison (Fowler & Welch 2018). Subsistence burning accompanied by traditional cultural knowledge of appropriate fire regimes in fire-prone landscapes is well documented in diverse world contexts to increase biodiversity, promote vegetative regeneration, and reduce wildfire occurrence (Bird et al. 2016, Kelly et al. 2020, Kimmerer & Lake 2001, Trauernicht et al. 2015, Welch et al. 2018). Scientific research that purports to evaluate Indigenous fire regimes in the absence of ethnographically contextualized ecological data runs the risk of exacerbating the fire blame game and providing evidence to support distorted narratives advanced by anti-Indigenous advocates. As observed by Arruda et al (2018, p. 5), "evidence from the literature has shown that the response of biodiversity to fire is context-dependent."

Spatial analysis of fire scars in Indigenous territories can be an effective tool for characterizing cultural fire regimes in terms of distribution and frequency, especially when qualified by linkages to different local ecosystems. Much of this research provides evidence of patchy distribution and varied frequencies according to local ecosystems (Price et al. 2005, Russell-Smith et al. 1997, Vigilante et al. 2004). Studies that benefit from remote sensing analysis of vegetation cover change over time tend to show land cover stability or reforestation under Indigenous management, even when burn frequency in some ecosystems approaches annual (Mistry et al. 2016, Welch et al. 2013a). Indigenous burning influences ecosystem structure and plant diversity profiles, favoring some taxa over others, as has been reported in historical documents and ethnographic studies for decades to centuries, depending on the location (Anderson 2005, Bowman & Prior 2004, Pascoe 2014).

A recently published article drew on fire scar mapping from satellite imagery to assess anthropogenic fire distribution and frequency in the Pimentel Barbosa Indigenous Land, Central Brazil (Aguiar & Martins 2020). This particular territory, inhabited by A'uwẽ (Xavante) people, is well known in the literature as a rare contemporary example of an Indigenous society that continues to burn the cerrado landscape during large group hunts according to long established cultural protocols. A'uwẽ hunters aim to simultaneously elevate hunting yields and preserve the non-human landscape (flora and fauna) in such a manner as to ensure its productivity in the long term (Melo & Saito 2013, 2011, Welch 2015, 2014, Welch et al. 2013a, Welch & Coimbra Jr. 2019). However, the article by Aguiar & Martins paints a very different picture, characterizing A'uwẽ burning during hunting events as "indiscriminate fire use" contributing to "a burning regime without clear control of frequency or size" (2020, p. 16). According to the authors, the disjunction and reduction of their territory and integration of their Indigenous culture into the Brazilian national culture caused them to undergo a process of cultural fragmentation and abandonment of their traditional burning models. Thus, the authors argue, the burn pattern became more frequent and transformed into a model of unsustainable resource exploitation.

Our ethnographic and ecological experience accompanying A'uwẽ burning during group hunts over the last 17 years suggests that these characterizations are not only ethnographically incomplete, considering that the authors did no field work, but are also dangerous distortions of fact that contribute to a biased and unsubstantiated characterization of an Indigenous people as wanton ecological destroyers. Considering the larger regional frame of expansive non-Indigenous agribusiness vegetation clearing for cattle pasture and monoculture crops, the authors' characterization of A'uwẽ burning as unsustainable is based on speculation, ignores the Pimentel Barbosa Indigenous Land's important role as a green island amid veritable ecological desert, and appears to unfairly single out the A'uwẽ due to their cultural identity. In this article, we discuss Aguiar & Martins's arguments in light of our long-term research on A'uwẽ hunting with fire in the Pimentel Barbosa Indigenous Land, arguing that A'uwẽ hunters do burn according to established cultural protocols, manage their use of fire for conservationist purposes, and do not cause environmental degradation by burning.

Mapping of Indigenous fire regime with boots off the ground

The core methodology of the Aguiar & Martins (2020) study was to measure the distribution (area) and frequency (repetition in the same locations) of anthropogenic fires within the Pimentel Barbosa Indigenous Land during the dry season months of May through October from 1984 to 2018. A total of 153 satellite images were used to measure fire scars using polygons generated with the GIMP tool Fuzzy Select. Polygons created from multiple images for the same year were joined to create annual binary images distinguishing burned and unburned areas. These images were combined to create a composite image showing overall burn frequencies within the boundaries of the Indigenous land. The colors chosen for illustrating the frequency scale ranges from a warm yellow signifying fire in just one out of 34 years to a menacing black for fire in all 34 years. In between these extremes, fire in just five of 34 years was represented by a flame-like shade of orange. These color choices conspire to produce a burn frequency map that looks well charred even in areas that were burned during less than half of the evaluated years, which corresponds with the authors' own recommendation of burning no more frequently than every two years in savanna vegetation types.

Burn frequencies were also calculated according to six categories: five types of vegetation and one land use type (agriculture). These vegetation/land use frequencies were classified by the number of years out of the total of 34 that showed burn scars: Low Frequency (burns registered in 1 to 8 years), Medium Frequency (in 9 to 16 years), and High Frequency (in 17 to 34 years). No justification for these cutoff points was provided.

The authors characterized the resulting scar pattern as "an intense fire regime" (Aguiar & Martins, 2020, p. 7) based on the observation that fire was detected somewhere within the Indigenous land in all years. The burning regime was also somewhat mysteriously described in terms of the total area burned in all 34 years in hectares, presented as a percentage of the area of the Indigenous land (1.534,39%). The relevance and comparability of this awkward measure for evaluating fire regime intensity is unclear.

According to the authors, these yardsticks provide evidence of supposedly excessive burning that should be considered harmful to the landscape. However, they are measures without meaning based on arbitrary cutoff points, inconsistent criteria, and nonsensical calculations. We argue that the measures used by Aguiar & Martins serve more to obscure than to clarify the nature of the A'uwẽ anthropogenic fire regime in the Pimentel Barbosa Indigenous Land.

Several methodological and interpretive problems deriving from the study's lack of ethnographic data are apparent. For example, the vegetation/land use base map used was produced for an unpublished environmental impact report (Azanha 2013) contracted by a governmental railroad corporation, for the purpose of authorizing a cargo railway planned to cross the A'uwẽ region, intended for internal circulation and not subject to peer review. Consequently, Aguiar & Martins repeated Azanha's error of labeling areas as under active agricultural use when they are in fact former cattle pastures from the 1970s, when ranches occupied lands that were subsequently included within the borders of the Pimentel Barbosa Indigenous Land and are now under Indigenous management. At the time of their use, these areas were planted with fodder plants, generally introduced African grasses (e.g., *Hyparrhenia rufa* (Nees) Stapf, *Melinis minutiflora* P. Beauv., *Andropogon gayanus* Kunth, *Megathyrsus maximus* (Jacq.) B. K. Simon & S. W. L. Jacobs, and *Urochloa* spp.). These invasive taxa continue to dominate in the areas they were once planted and contribute to wildfires due to their higher fuel potential and burning temperatures (Hoffmann et al. 2004, Pivello et al. 1999, Rossi et al. 2014). This ecological history is necessary for interpreting the finding that 99,08% of "agricultural" areas was burned during the study period.

Another example is evident in the discussion of results, in which the authors cite sparse literature to assert that traditional burning models have been abandoned and current use of fire is irrational and repetitive (Aguiar & Martins 2020). However, the sources cited (Fragoso et al. 2000, Leeuwenberg 1994) do not argue for or present evidence in support of such extreme positions. It would seem the authors introduced bias in their characterizations of these references in order to sustain the characterization that culturally informed and managed burning is a thing of the past, having been replaced by uncontrolled pyromania unanchored from tradition.

The authors then delve into the speculative endeavor of guessing what undesirable environmental impacts this supposed uncontrolled burning is having. For example, they postulate that "indiscriminate" (Aguiar & Martins 2020, p. 14) burning may reduce biodiversity and affect climatic processes. They also construct the argument that native grasslands are particularly flammable, which could lead to a feedback loop whereby fire reduces tree cover, favoring the expansion of grasses, thereby increasing the flammability of the land cover, and ultimately encouraging the occurrence of new fires. Additionally, they argue that A'uwẽ burning could reduce plant biomass and litter, altering the ecosystemic flows of energy, nutrients, and water. These proposed outcomes are unverified for the Pimentel Barbosa Indigenous Land and depend on the mistaken ethnographic presumption that A'uwẽ hunters are reckless and unstoppable igniters of the tropical savanna vegetation.

Ethnographic context of managed burning at Pimentel Barbosa

A'uwẽ hunters are deliberative people. Whether or not fire is to be used, group hunts are planned, discussed, and debated for hours, days, months, and even years before they occur (Welch 2014). In the past, before the population was settled within the boundaries of a relatively small Indigenous land, which occurred in the 1970s (Welch et al. 2013b), they deliberated extensively about where and when to hunt for what purposes. Among their considerations were where desired large game animals were believed to be abundant, when they would be fat from eating seasonally available foods, and when ceremonial occasions involving gifts of game meat would be held. Such ceremonial events included weddings and rites of passage into adulthood. According to elders, at that time, they were free to hunt anywhere within an enormous traditional territory and therefore did not need to factor conservation of limited hunting grounds into their discussions. Considering that they often hunted while on trek away from the main village, they could distribute their hunting activities far and wide and thereby not cause over taxation of any particular place.

Now as then, hunting with fire is popular because it is an efficient means of acquiring large quantities of game meat in a short period of time and because it is a festive social occasion that hunters of all ages enjoy. It is also a ritual occasion, with representatives of opposite exogamous moieties competing in a foot race that involves running along opposite semicircular paths towards a predesignated ending point some four to seven kilometers distant (Welch 2014). These runners ignite the landscape vegetation as they go while exerting themselves to arrive first at the finishing point and thereby earn bragging rights. This race produces a temporary ring of fire, within which other hunters walk while igniting vegetation in such a manner as to produce an irregular mosaic of burnt, burning, and unburnt spaces. The fire serves to flush out game (not entrap it within a circular fire), which is dispatched as it runs through open spaces in search of cover.

A'uwẽ hunters are keenly aware of the potential ecological pressures that burning within their limited contemporary Indigenous land might cause. It is for this reason that they first sought to collaborate with ecologist Frans Leeuwenberg in studying the ecological impacts of their hunting practices and consider measures to mitigate against over hunting or excessive use of fire (Leeuwenberg 1997, Leeuwenberg & Robinson 2000). The results of that study are evident today as A'uwẽ hunters continue to deliberate based on its recommendations that hunting fires be distributed temporally and spatially by rotating hunting grounds and avoiding repetitive burns close to villages. Since that first study, they have collaborated with numerous other ecologists to evaluate the impacts of their hunting practices, none of which documented decreased fauna or loss of land cover as a result of their managed burning practices within the Indigenous land (Briani et al. 2004, Prada 2001, Villalobos 2002, Welch et al. 2013a).

When contemporary hunters deliberate hunting with fire, conservation issues are at the top of their list of considerations thanks in part to increased awareness generated through their collaborations with ecologists. Besides rotating hunting grounds, as recommended by Leeuwenberg, they also consider traditional ecological knowledge about when and how often each specific location should be burned. As elders tell younger hunters, according to traditional ecological knowledge and burning protocols, each location has its own proper fire regime, including when in the yearly cycle it should be ignited and with what frequency it should be burned to allow for regeneration and accumulation of adequate quantities of dead biomass. Some taller vegetation types burned in the late dry season require three to four-year intervals, while open grasslands burned at the beginning of the dry season may require only one or two years. They also consider ambient moisture, weather, wind, and natural fire barriers to help avoid fires getting out of control. A well-managed hunting fire burns fast due to adequate fuel but mostly remains cool and restricted to the understory. Also, a well-managed fire is considered to be one that was planned and executed according to traditional fire ecology knowledge.

These ideals are not always attained, especially as younger and less experienced hunters assume responsibility for planning hunting fires. Occasionally a fire will escape their control and continue burning from one day to the next, sometimes incinerating hunting grounds that had been reserved for future use. This is most common in the late dry season when the vegetation is highly combustible. When this happens, elders call meetings to discuss what went wrong and how to prevent it from occurring again in the future. The solution may involve a combination of insights from traditional ecological knowledge and from contemporary knowledge co-constructed with their ecologist partners. A'uwẽ hunters are responsive to all sources of information available to them and utilize this knowledge for conservation purposes, including burning for the long-term sustainability and productivity of the landscape and its non-human life. Elder and youth hunters express concern about the possibility of burning too frequently, but in our experience premature burning result in unproductive hunts, as there is not enough ground fuel to sustain an effective fire. When this happens, elders educate their younger hunting companions about the importance of burning less frequently in that particular location or vegetation type. Burning errors are always discussed at length and remembered into the future so they are not repeated.

The ecological results of these conservation efforts are striking. The Pimentel Barbosa Indigenous Land does not suffer from loss of vegetation cover. Comparing 1973 and 2010, deforestation within the boundaries of the land remained unchanged at 0.6% (Welch et al. 2013a). During the intervening years, the boundaries were expanded to include former cattle ranches with pastures planted with invasive African grasses. These adjustments resulted in temporarily elevated proportions of deforested area, reaching 1.9% in 2000, which declined again after these lands previously degraded by agribusiness were returned to Indigenous management with fire. Our analysis of burning patterns from 2007 to 2010 revealed that vegetation maintenance or recuperation occurred even in areas of high fire periodicity, including locations that burned in three or more consecutive years.

Conclusion: Dangers of misleading scientific publications

There is a danger in exaggerating or mischaracterizing the ethnographic context of anthropogenic burning by Indigenous peoples and smallholders. They may be blamed for problems that do not exist or are overwhelmingly caused by others. In the case of the A'uwẽ of the Pimentel Barbosa Indigenous Land, they have long been blamed for causing deforestation by burning while hunting in groups in the absence of any supporting data whatsoever and in denial of the real source of environmental degradation in the cerrado, which is agribusiness (Welch et al. 2013a). It is incumbent upon scientists to avoid playing into these biased blame games and refrain from repeating unsubstantiated rumors. Abundant scientific research has shown that contemporary A'uwẽ anthropogenic fire regimes within the Pimentel Barbosa Indigenous Land has not caused game animal depopulation since Leeuwenberg warned of over hunting based on data collected in 1991 and 1992 (Leeuwenberg 1997, Leeuwenberg & Robinson 2000) and has not caused deforestation since 1973 (Welch et al. 2013a).

By misconstruing the A'uwẽ as reckless burners unhinged from tradition, Aguiar & Martins (2020) risk providing the anti-Indigenous cause with apparent evidence that Indigenous peoples are irresponsible caretakers of their lands and therefore undeserving of them. This is no small thing considering that the current presidential administration in Brazil has expressed interest in reducing Indigenous lands in number and size. Nonscientific methods including the use of obscuring measures of burning frequency and intensity and ethnographic mischaracterization can only serve to punish a minority subsistence-based population for ecological crimes it has not committed.

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James R. Welch: Conceptualization, Investigation, Writing - Original draft preparation, Writing – Review & editing.

Eduardo S. Brondizio: Conceptualization, Investigation, Writing - Original draft preparation, Writing – Review & editing.

Carlos E. A. Coimbra Jr.: Conceptualization, Investigation, Writing - Original draft preparation, Writing – Review & editing.

Conflicts of interest

None declared.

Ethics

No permissions required.

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Article

Conservation of the Black-collared Swallow, *Pygochelidon melanoleuca* (Wied, 1820) (Aves: Hirundinidae) in Brazil: potential negative impacts of hydropower plants

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Abstract: We analyzed the overlap of the range of *Pygochelidon melanoleuca* in Brazil with active and planned hydropower plants in the country (current and future scenarios). We used the Random Forest, Maxent and Support Vector Machine algorithms to model the potential range of the species, which we then overlapped with the locations of active and planned hydropower plants in order to calculate how much the potential area of this species is and will be affected by them. Approximately 35% of active hydropower plants currently overlap with the potential distribution area of *P. melanoleuca*, and 44% of planned hydropower plants also coincide with this area. If the implementation of the planned hydropower plants occurs, the suitable habitat necessary for nesting and foraging of *P. melanoleuca* will be severely compromised.

Keywords: *Amazon; Aquatic Ecosystems; Species Distribution Modelling; Neotropics.*

Conservação da andorinha-de-coleira, *Pygochelidon melanoleuca* (Wied, 1820) (Aves: Hirundinidae) no Brasil: potenciais impactos negativos das hidrelétricas

Resumo: Analisamos a sobreposição da distribuição de *Pygochelidon melanoleuca* no Brasil com hidrelétricas ativas e planejadas no país (cenário atual e futuro). Utilizamos os algoritmos Random Forest, Maxent e Support Vector Machine para modelar a distribuição potencial da espécie, então sobrepomos com os locais das usinas hidrelétricas ativas e planejadas para calcular o quanto a área potencial desta espécie é e será afetada por elas. Aproximadamente 35% das hidrelétricas ativas estão sobrepostas com a área de distribuição potencial de *P. melanoleuca* e 44% das hidrelétricas planejadas coincidem com sua área. Se a implementação das hidrelétricas planejadas ocorrer, o habitat necessário para nidificação e forrageamento de *P. melanoleuca* estarão severamente comprometidos.

Palavras-chave: *Amazônia; Ecossistemas Aquáticos; Modelagem de Distribuição de Espécies, Neotrópico.*

Introduction

Aquatic ecosystems are among the most vulnerable to the impact of anthropogenic activities (Dudgeon et al. 2006). The installation of hydropower plants is considered one of the main threats to freshwater biodiversity by drastically changing the landscape, river flow and water temperature, reducing sediment transportation, and hindering or even stopping organisms from moving freely through watercourses (Winemiller et al. 2016, Zarf et al. 2015). The rise in energy demand, associated with a rich and unexplored hydrographic potential, has resulted in an increase of hydroelectric development in the Neotropical Region (Finer & Jenkins 2012). Brazil is among the top five countries with greatest hydropower cumulative potential in the world (IEA 2017), and the installation of approximately 1680 hydropower plants is currently planned for the country (ANEEL 2018).

The Amazonian region is currently one of the most targeted for the implementation of hydroelectric projects in Brazil due to its potential for hydroelectric exploration and the near exhaustion of hydroelectric potential in other regions of the country (Choueri & Azevedo 2017). Indeed, the Brazilian Amazon holds some of the greatest hydropower potential in the world owed to its extensive hydrographic network and topographic variation (Fearnside 2015). Small- and large-scale reservoir projects have already been proposed for the Amazon, with three out of ten mega-reservoirs proposed already completed (e.g., *Belo Monte*, *Santo Antônio*, and *Jirau*), and seven others in the planning stage (Latrubesse et al. 2017). The impacts of such a scaling in hydroelectric development could greatly reduce or even extinguish populations of species such as *Pygochelidon melanoleuca*, which are dependent on fluvial rocky outcrops (Lees et al. 2016).

The Black-collared Swallow, *Pygochelidon melanoleuca* (Wied, 1820) (Aves, Hirundinidae) is associated with rapids and rocky outcrops stretches of medium and large sized rivers (Cherie 1916, Ridgely & Tudor 1989, Hilty 2002, Turner 2020). These rapids are the species main foraging areas with the rocky outcrops serving as its nesting sites during reproductive season (Haverschmidt 1968, Hilty 2002, Barros 2008, Lopes et al. 2013, Lees et al. 2016). The distribution of *P. melanoleuca* extends throughout South America, from southeastern Colombia, southeastern and eastern Venezuela, Guyana, Suriname, French Guyana, Brazil, Bolivia, Paraguay, and northeastern Argentina (Birdlife International 2017). In Brazil, the species is common in the Amazon region at the Negro and Amapá rivers, and along the Madeira, Tapajós, Xingú and Tocantins river basins. Scattered records can also be found in the states of Pernambuco, Bahia, Goiás, Minas Gerais and Paraná (Sick 1997, Straube et al. 2004, Silva et al. 2017).

The global conservation status of *P. melanoleuca* is classified as being of “Least Concern” (BirdLife International 2017), since it has a large range, and an apparently stable population size above the thresholds for the “Vulnerable” category. In Brazil, however, the species was classified as “Near Threatened” (ICMBio 2018), with certain states, like Minas Gerais, considering the species as “Critically Endangered” due to a highly probable population reduction over the next 100 years (Drummond et al. 2008). The main threat to *P. melanoleuca* in Brazil is the loss of these unique habitats due to the installation of hydropower plants (Drummond et al. 2008, Silva et al. 2017). In fact, the implementation of two hydroelectric dams on the Araguari River, Minas Gerais, lead to a decline in populations of this species soon after its discovery in the state (Biovet 2012).

In face of the recent escalation of hydroelectric power development in Brazil, it is imperative to identify suitable areas and potential threats for *P. melanoleuca* populations. This would contribute to more efficient conservation strategies focused on reducing the negative impacts of these enterprises on the species. An efficient way to identify these areas and threats is through predictive species distribution models which are an important tool for biodiversity conservation (Guisan et al. 2013). Such models allow for the identification of priority areas for conservation, and/or areas where species are more vulnerable to anthropic activities. These can then be used by decision-makers to elaborate and implement more effective species conservation planning (Villero et al. 2016).

Although *P. melanoleuca* is not considered an aquatic bird, it relies on aquatic environments for nesting and foraging. Hence, it is also important to consider aquatic ecosystems when planning conservation measures for the species. In the present study we use predictive distribution modeling to (1) provide a potential distribution for *P. melanoleuca* in Brazil; (2) analyze the overlap between active hydropower plants and the potential occurrence areas for the species (current scenario); and (3) analyze the overlap between planned hydropower plants and the potential occurrence areas for the species (future scenario).

Material and Methods

1. *Study species*

Adults of *Pygochelidon melanoleuca* are approximatelly 14 cm in length and weigh between 10–12 g (Figure 1). The species is commonly found in large lowland rivers with rocky outcrops, preferring more wide and open stretches with exposed stones which it uses for reproduction and nesting during low-water periods (Turner and Rose 1989, Ridgely & Tudor 1989). These areas are currently threatened by the installation of hydropower plants which are predicted to severely compromise these microhabitats in most rivers of the Brazilian and Guiana shields (Lees et al. 2016). The dependence of *P. melanoleuca* on these particular habitats and the lack of recent records in areas where it once occurred (i.e. the Atlantic Forest), has shown that several populations of this species may be endangered (Moreira-Lima 2013).

2. *Species occurrence and environmental data*

Occurrence data was obtained from three different sources: (1) zoological collections of the Museu Paraense Emílio Goeldi (MPEG), Museu de Zoologia da Universidade de São Paulo (MZUSP), Instituto Nacional de Pesquisas da Amazônia (INPA) and Departamento de Zoologia da Universidade Federal de Minas Gerais (DZUFMG); (2) online databases, such as Global Biodiversity Information Facility (GBIF) (www.gbif.org) and Wikiaves community (http://www.wikiaves.com.br/); and (3) personal sightings and records by different ornithologists. Records without geographical coordinates or with inaccurate coordinates (e.g., coordinates to the municipality of the record) were not included in the analyses. We obtained 237 records of *P. melanoleuca*, of which 87 were excluded for not meeting the requirements for the models.

Figure 1. Juvenile (A) and adult (B) of the Black-collared Swallow (*Pygochelidon melanoleuca*) (Photos: Luiz Alberto 2019).

In order to model the species distribution, we obtained 19 climatic variables representing annual trends, seasonality, and extreme or limiting environmental factors in the WorldClim database (Fick & Hijmans 2017) all of them on a 5 arc-min resolution (~10 km grids). Two topographic variables (terrain slope and altitude) were also obtained from the Hydro-1K global digital elevation model (www.usgs.gov). To reduce data multicollinearity, we performed a Pearson correlation analysis with a matrix containing all variables. Out of 21 variables, 13 were correlated (correlation >70%) and were thus excluded. Models, therefore, were created using the two topographic variables and six climatic variables: maximum temperature of warmest month (Bio5), minimum temperature of coldest month (Bio6), precipitation of wettest month (Bio13), precipitation of the driest month (Bio14), precipitation of the wettest quarter (Bio16), and precipitation of driest quarter (Bio17). After combining the sampling points, we used Moran's I to test for spatial autocorrelation.

3. Model construction and evaluation

Different algorithms were used to minimize uncertainty of generated models. Distribution models were built in R 3.4 (R Development Core Team 2012) using the Random Forest (RF) algorithm from the 'randomForest' package (Liaw & Wiener 2002), and the Maxent and Support Vector Machine (SVM) algorithms from the 'dismo' package (Hijmans et al. 2017). A10-km pixel resolution was used for variables in the model building process, with a single record per pixel in order to avoid spatial autocorrelation. We generated 10 partials models for each algorithm. The original occurrence points were split in a way that 20% (test points) were used to evaluate the model and 80% (training points) to build the model, all of them adjusted with the ecological space. Models were evaluated using the TSS (True Skill Statistics) (Allouche et al. 2006) and the AUC (Area Under the Curve) (Fielding & Bell 1997). TSS models were considered useful when presenting a value between 0.5-0.8, and good when above 0.8. Likewise, AUC between 0.7-0.9 indicated useful models, and values above 0.9 indicated good models. Therefore, the final model was obtained from partial models with AUC $\geq$ 0.7 and TSS $\geq$ 0.5.

To generate the final model, we calculated the mean of the AUC and TSS values for each of the partial models obtained from each algorithm by using the 'ensemble' function of the 'sdm' package (Naimi & Araújo 2016). Next, we used the 'ensemble forecast' function to group the partial models (following Araújo & New 2007). This method considers that different errors affect each model differently, so it evaluates all models, reducing errors and producing a more reliable solution (Diniz-Filho et al. 2010). The final potential distribution model for *P. melanoleuca* was cut to the Brazilian territory and overlapped with hydrography to refine the model in light of species dependency on waterbodies (Nori & Rojas-Soto 2019) (Figure 2). Only data from third-order streams was selected, as the species does not occur in small streams (Schauensee & Phelps 1978, Hilty & Brown 1986, Turner 2016). For this purpose, we plotted the Brazilian hydrography using 3 arc-sec resolution files of flow accumulation and flow direction available on the HydroSHEDS database (https://hydrosheds.cr.usgs.gov/hydro.php). We then ordered rivers following the Strahler (1957) classification and added a 10-km buffer around the watercourses. Hydrography was divided in hydrographic regions (Amazon, *Marajó* Atlantic Coast, Northeast Atlantic Coast, *Tocantins, Paraná,* East Atlantic Coast) according to the Level 1 Otto-Codification methodology from the Agência Nacional das Águas (ANA), since these regions are used to guide the planning and management of hydric resources (CNRH 2003).

4. Overlap with hydropower plants and statistical analyses

To calculate the percentage of active (current scenario) and planned (future scenario) hydropower plants overlapping the potential distribution area of the species we created a 10-km buffer for each plant. We then transformed the final model into a binary model and extracted the total amount of pixels representing the hydropower plants that overlapped the potential distribution area. Data on the functioning and planned hydropower plants in Brazil was obtained in the Georeferenced Information System of the Electric Sector (ANEEL 2018).

The overlap between hydropower plants and the potential range of *P. melanoleuca* was evaluated with two-way ANOVA in two distinct scenarios: functioning hydropower plants (current scenario) and planned hydropower plants (future scenario). Hydropower plants were the predictor variable and the potential of occurrence (pixel-values in potential occurrence areas) the response variable, with hydropower plants and hydrographic regions as covariates. The two-way ANOVA evaluated the impact of hydropower plants and hydrographic regions on the potential occurrence of the species in each scenario and checked for interactions between both predictors over the response variable. To do so, we extracted the pixel-values from the potential distribution areas with and without hydropower plants.

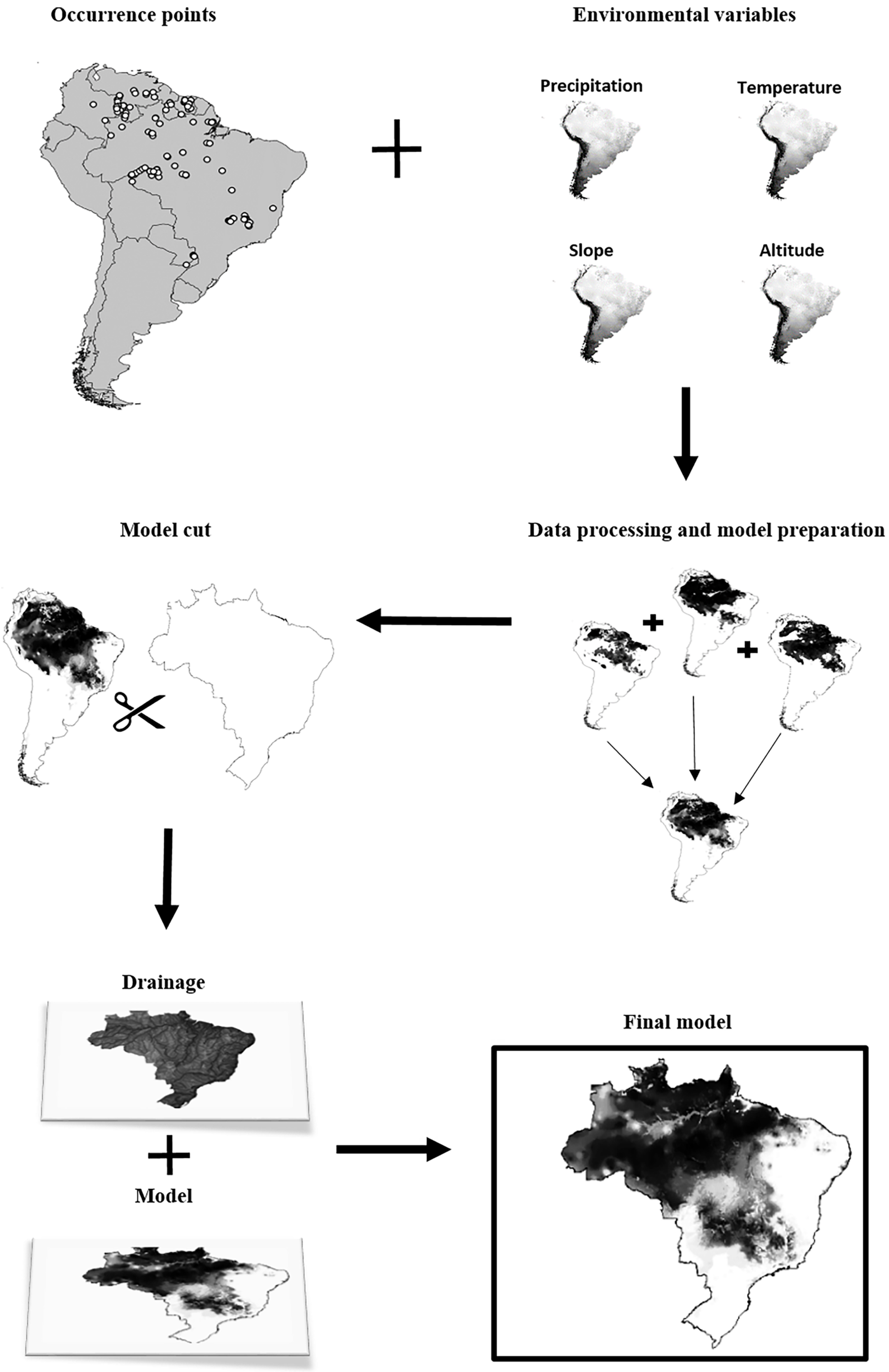

Figure 2. Schematic representation of the model preparation process.

Results

All generated models indicated a higher occurrence probability for *P. melanoleuca* in the Amazon, *Marajó* Atlantic Coast, Northeast Atlantic Coast and *Tocantins* regions, while at the same time, indicating the *Paraná* and East Atlantic Coast regions as having low occurrence probability. The final potential distribution model for the species showed good predictive capacity (TSS = 0.62 ± 0.08; AUC = 0.82 ± 0.07). The partial models generated by Maxent produced models with lower TSS values. The partial models generated by Random Forest and Support Vector Machine indicated good predictive performance (Table S1).

There are currently 653 active hydropower plants in Brazil and plans for the installation of almost 1680 more. Over 80% of active facilities and nearly 80% of planned facilities are located in the *Paraná* and East Atlantic Coast basins. However, most facilities in the *Paraná* and East Atlantic Coast are in areas of low habitat suitability for the species, and areas with greatest occurrence potential for *P. melanoleuca* in these regions have fewer active and planned hydropower plants. The hydropower plants in the Amazon and *Marajó* Atlantic Coast are in areas of high habitat suitability for *P. melanoleuca*. (Table 1).

Approximately 35% of active hydropower plants are in potential distribution areas for the *P. melanoleuca* (Figure 3A), varying according to each hydrographic region (F = 7.58; G.L.= 4; p < 0.01).

Table 1. Quantity of functioning and planned hydropower plants in Brazil according to the classes of habitat suitability for the occurrence of *Pygochelidon melanoleuca*. Very low 0.0-0.2; Low 0.2-0.4; Average 0.4-0.6; High 0.6-0.8; Very high 0.8-1.0; NA, Unsampled.

Hydrographic regions	**Category**	**Hydropower plants**		**Total**
		Active	**Planned**	
Amazon	Very low	0	1	**1**
	Low	3	15	**18**
	Average	4	13	**17**
	High	28	81	**109**
	Very high	34	83	**117**
	NA	1	0	**1**
Tocantins	Very low	1	6	**7**
	Low	7	25	**32**
	Average	18	42	**60**
	High	8	48	**56**
	Very high	5	23	**28**
	NA	0	4	**4**
Marajó Atlantic Coast	Very low	0	0	**0**
	Low	0	0	**0**
	Average	0	1	**1**
	High	0	0	**0**
	Very high	3	5	**8**
	NA	0	0	**0**
Northeast Atlantic Coast	Very low	0	0	**0**
	Low	0	0	**0**
	Average	0	0	**0**
	High	0	0	**0**
	Very high	0	0	**0**
	NA	0	0	**0**
East Atlantic Coast	Very low	189	343	**532**
	Low	25	40	**65**
	Average	12	41	**53**
	High	15	49	**64**
	Very high	2	8	**10**
	NA	6	1	**7**
Paraná	Very low	181	510	**691**
	Low	24	59	**83**
	Average	22	59	**81**
	High	34	101	**135**
	Very high	24	116	**140**
	NA	7	5	**12**
Total		653	1679	2.332

The *Paraná* and East Atlantic Coast regions have 30.49% and 12.89% of their active facilities within potential distribution areas for the species, respectively (Figure 4A, 4B). In contrast, 96.64% and 100% of active hydropower plants in the Amazon and *Marajó* Atlantic Coast, respectively, are in the species potential distribution area (Figure 4C, 4D).

Over 43% of planned hydropower plants were found to be in potential distribution areas for *P. melanoleuca* (Figure 3B). This overlap with the potential distribution for the species area varied with the geographic region (F = 18.82; G.L.= 4; p < 0.01). Should all planned hydropower plants be installed, the *Paraná* and East Atlantic Coast regions might respectively have 35.17% and 21.82% of installations within the potential range for the species (Figure 5A, 5B). The same scenario indicates that this overlap can reach 92.33% and 100% in the Amazon and *Marajó* Atlantic Coast regions respectively (Figure 5C, 5D).

Discussion

This study is one of the first Brazil-wide examinations of the overlap between active and planned hydropower plants and the potential occurrence areas of a bird species highly dependent on aquatic ecosystems. This overlap varied with each geographic region, due to the different number of hydropower plants and potential areas for the species. Since the total area affected by each hydropower plant is not available, it is noteworthy that the percentage of suitable area loss for the species could be greater than the one observed herein.

The largest potential distribution areas for *P. melanoleuca* are in the Amazon and *Marajó* Atlantic Coast regions, in which 96.64% and 100% of active hydropower plants, respectively, overlap with potential areas for the species. The impact of these projects on local populations of *P. melanoleuca* must be considered for this region, since most will be located directly over areas with high habitat suitability for the species.

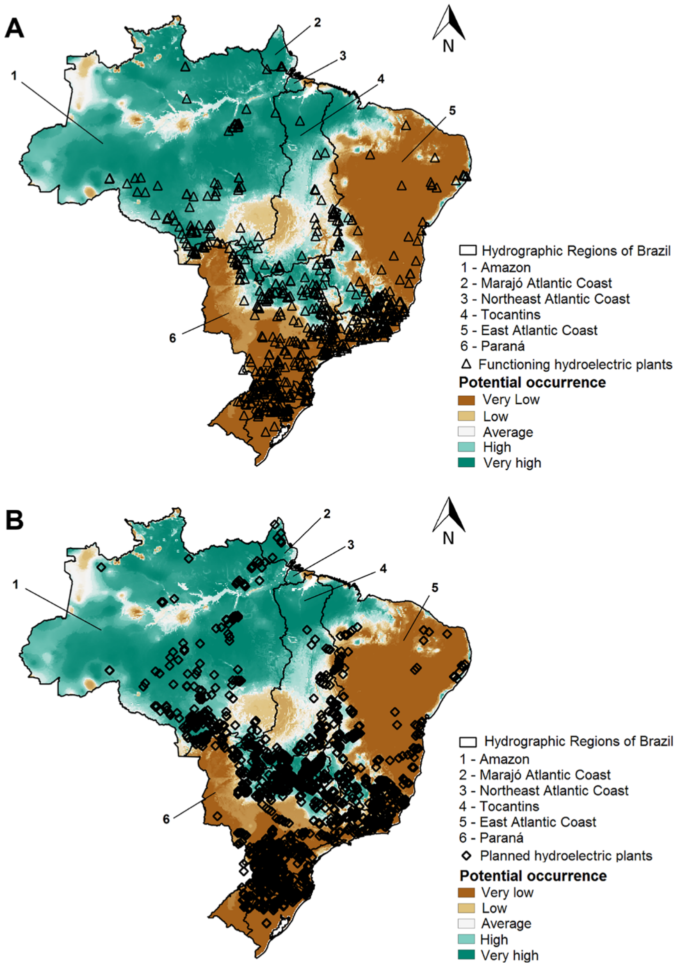

Figure 3. Active and planned hydropower plants (A and B, respectively) on potential occurrence areas of *Pygochelidon melanoleuca* in Brazil.

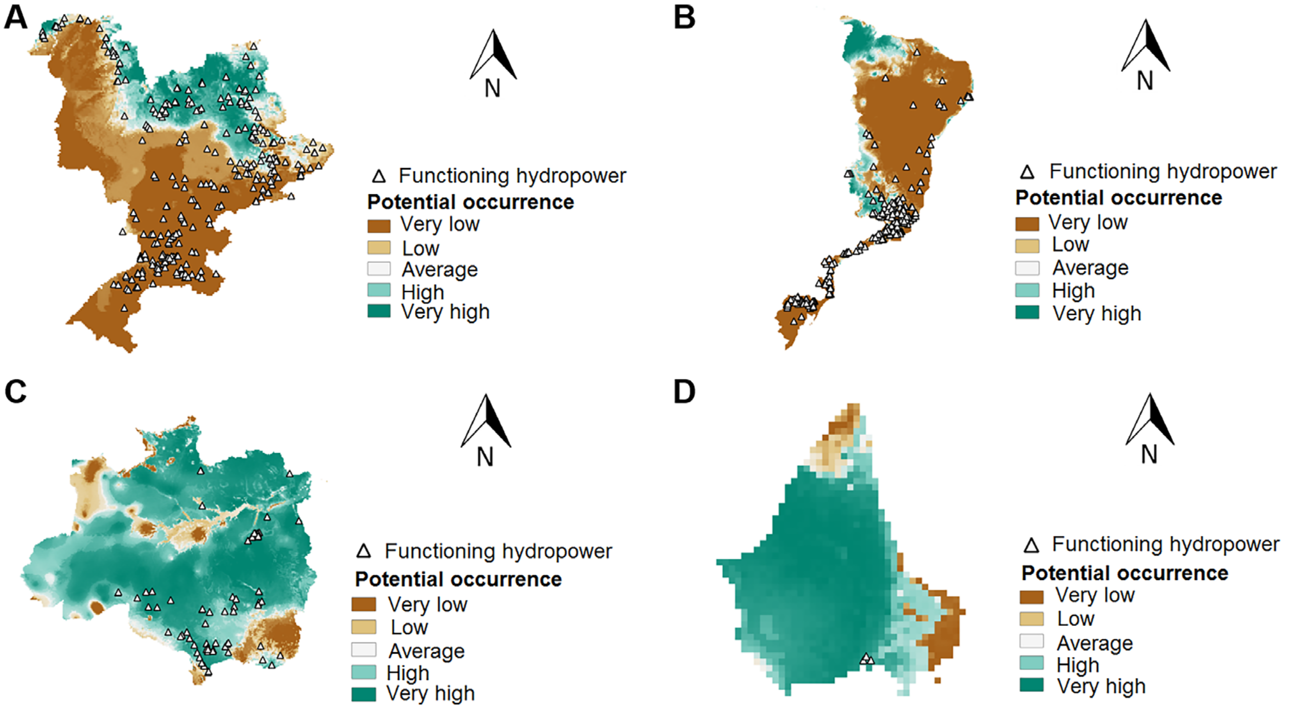

Figure 4. Active hydropower plants within potential occurrence areas of *Pygochelidon melanoleuca* in each hydrographic region: *Paraná* (A), East Atlantic Coast (B), Amazon (C) and *Marajó* Atlantic Coast (D).

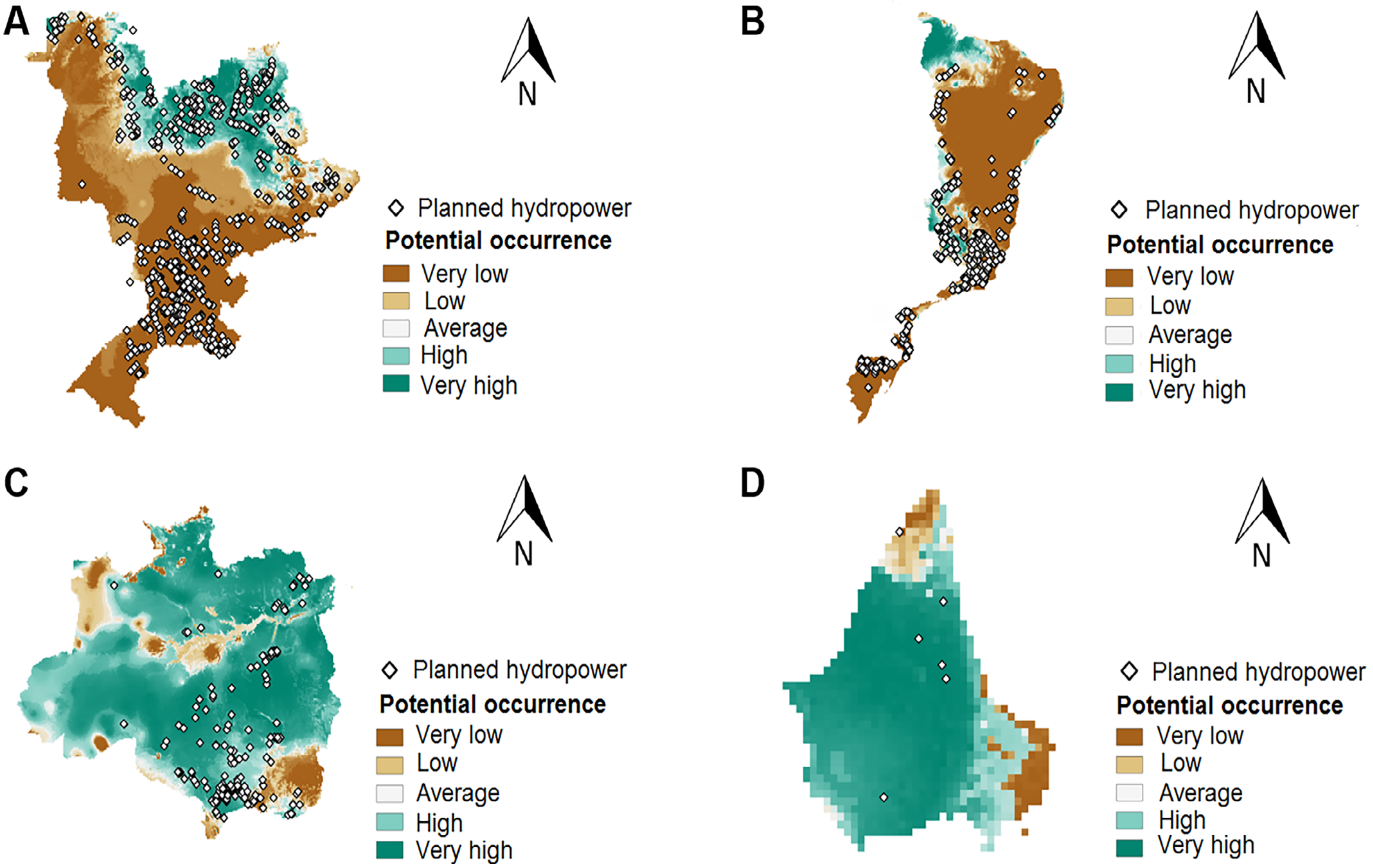

Figure 5. Planned hydropower plants within potential occurrence areas of *Pygochelidon melanoleuca* in each hydrographic region: *Paraná* (A), East Atlantic Coast (B), Amazon (C) and *Marajó* Atlantic Coast (D).

This highlights the need for careful assessment of the impacts caused by these ventures, since decision-making processes tend to underestimate these impacts while overestimating potential benefits (Fearnside 2005).

The distribution of *P. melanoleuca* appears to be more restricted in the *Paraná* basin, southern limit of its range, when compared to its wider distribution in areas such as the Amazon basin. Although our models indicate the *Paraná* basin as having low suitability for *P. melanoleuca*, 30.49% of its hydropower plants are located within potential areas for the species. The *Paraná* hydrographic region holds the largest urban areas in Brazil, and provides around 70% of the electricity produced in the country (Agostinho et al. 2007). The economic development in Brazil in the early 20th century, especially in the *Paraná* hydrographic region, combined with a high availability of water resource and foreign investments, turned hydropower plants into the most suitable means to meet energy demands (Valêncio et al. 1999). Approximately 850 hydropower plants are currently planned for this region (ANEEL 2018), a concerning scenario, since active plants might already have reduced suitable habitats for *P. melanoleuca* in the region. Should new hydropower plants be implemented in the *Paraná* hydrogeographic region, this species might lose a crucial microhabitat for reproduction and foraging, and could even become locally extinct.

Hydropower plants affect biodiversity and compromise ecosystem functioning (Couto & Olden 2018), their operational guidelines for optimizing energy production failing to meet the ecological needs of the biota associated with these ecosystems (Lees et al. 2016). Strategies aiming to reduce the impact of hydropower plants on biodiversity have already been proposed (e.g. Kitzes & Shirley 2015, Kang et al. 2016): controlling water level in reservoirs according to the ecological needs of aquatic birds (Zhang et al. 2016); elaborating an "Adaptive Management Plan" to evaluate the impacts of dam operation on watercourses (Lovich & Melis 2007); including hydrological models to help predict flood and drought patterns that might be linked to biological cycles and ecological processes (Kingsford 2000); researching the impact of reservoir installations on bird populations (e.g., distribution, survival, and reproductive success) (Claassen 2004); and, establishing river sections free of hydropower plants in order to minimize their impact on species populations (Silva et al. 2017). Hydropower plants are an important factor to be considered when planning the conservation of *P. melanoleuca*, since freshwater environments are crucial for maintaining their populations (Silva et al. 2017). The data presented here constitutes only an estimate of the extent to which hydropower plants overlap with the potential distribution areas of *P. melanoleuca* in Brazil, presently and in the future.

In this study, we observed that *P. melanoleuca* is widely distributed in the Amazon, *Marajó* Atlantic Coast, *Tocantins* and Northeast Atlantic Coast hydrographic regions, with a more restricted distribution in the *Paraná* and East Atlantic Coast regions. We also found that the overlap of potential areas of occurrence for the species with hydropower plants in current and future scenarios varied with region, the Amazon and *Marajó* Atlantic Coast regions presenting the highest overlap. In addition, we showed how the overlap between hydropower plants and the potential distribution area of *P. melanoleuca* can indicate a likely reduction of suitable habitat needed for the species to persist.

Supplementary Material

The following online material is available for this article:

Table S1 - Result of the partial distribuion models generated for *Pygochelidon melanoleuca* with the AUC (Area Under Curve) and TSS (True Skill Statistic) values. RF, Random Forest; SVM, Support Vector Machine.

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Associate Editor

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Author Contributions

Gabriele Andreia da Silva: concept and design of the study; data collection; data analysis and interpretation; manuscript preparation; critical revision, adding intelectual content.

Renata Guimarães Frederico: concept and design of the study; data collection; data analysis and interpretation; manuscript preparation; critical revision, adding intelectual content.

Sara Miranda Almeida: concept and design of the study; manuscript preparation; critical revision, adding intelectual content.

Gilberto Nepomuceno Salvador: concept and design of the study; manuscript preparation; critical revision, adding intelectual content.

Gustavo Bernardino Malacco: concept and design of the study; manuscript preparation; critical revision, adding intelectual content.

Celine de Melo: manuscript preparation; 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

Anuran species composition and density estimates from an Atlantic Forest area within the APA Serra da Mantiqueira, Rio de Janeiro state, Brazil

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Abstract: Among amphibian species from high elevation areas in the Brazilian Atlantic Forest there is a high percentage of threatened and endemic species, but there is still a relative scarcity of local inventories for these organisms. Here, we present data on anuran composition, relative abundance and estimated densities for leaf-litter frogs from an Atlantic Forest area within the APA Serra da Mantiqueira, in Rio de Janeiro state, Brazil, based on results of a short-term survey carried out at altitudes of 1,350-1,750 m, in November 2005 (with additional records from surveys made in 2010 and 2011). Three sampling methods were used during the 2005 survey: plot sampling, visual encounter surveys (VES; performed during the day, at the dusk, and at night), and pitfall traps with drift fences; only non-standardized visual searches were employed during the 2010 and 2011 surveys. We recorded 24 species, with the direct-developer *Ischnocnema* sp. (gr. *lactea*) being the most abundant. Most anurans (90% of all individuals) sampled by VES were captured during the crepuscular and nocturnal periods. The estimated density of the local leaf-litter frog assemblage based on plot sampling was 18.4 ind/100 m^2, which is one of the highest values currently reported for Atlantic Rainforest areas. This is the first study analyzing the anuran fauna composition of an Atlantic Forest area within the APA Serra da Mantiqueira and adds to the body of knowledge on the fauna of the southern region of Rio de Janeiro state.
Keywords: *amphibians, endemism, inventories, montane forest, southeastern Brazil.*

Composição de espécies de anuros e estimativa de densidade em uma área de Floresta Atlântica dentro da APA Serra da Mantiqueira, estado do Rio de Janeiro, Brasil

Resumo: Entre as espécies de anfíbios de áreas de altas altitudes da Mata Atlântica brasileira há uma elevada porcentagem de espécies ameaçadas e endêmicas, mas ainda há relativa escassez de inventários locais desses organismos. Aqui, apresentamos dados sobre a composição de espécies, abundância relativa e densidade estimada para anfíbios anuros de serapilheira de área de Mata Atlântica na APA Serra da Mantiqueira, estado do Rio de Janeiro, Brasil, com base em resultados de estudo de curto prazo realizado a altitudes de 1.350-1.750 m, em novembro de 2005 (incluindo registros adicionais de coletas feitas em 2010 e 2011). Três métodos de amostragem foram utilizados na campanha de 2005: amostragem em parcelas, encontros visuais (realizados durante o dia, no crepúsculo e à noite) e armadilhas de queda; apenas procuras visuais não padronizadas foram usadas nas coletas de 2010 e 2011. Foram registradas 24 espécies, sendo a mais abundante *Ischnocnema* sp. (gr. *lactea*), uma forma com desenvolvimento direto. A maioria dos anuros (90% de todos os indivíduos) amostrados pelo método de encontros visuais foi capturada durante os períodos crepuscular e noturno. A densidade estimada para anuros de serapilheira com base nas amostragens em parcelas foi de 18,4 ind/100 m2, um dos valores mais altos registrados até o momento para áreas de Mata Atlântica. Este é o primeiro estudo que analisa a composição da fauna de anuros de área de Mata Atlântica da APA Serra da Mantiqueira e contribui para o conhecimento da fauna da região sul do estado do Rio de Janeiro.
Palavras-chave: *anfíbios, endemismo, floresta serrana, inventários, sudeste do Brasil.*

Introduction

Like many other animals (e.g., Kattan & Franco 2001, McCain 2004, Liew et al. 2010), anuran amphibians typically present comparatively lower species richness at higher altitudes (Duellman 1988; Zancolli et al. 2014, Siqueira et al. 2021). On the other hand, highland habitats are often important centers of endemism for these organisms, especially in the tropics, with many species being restricted to these areas (e.g., Fu et al. 2006, Cruz & Feio 2007, Bernal & Lynch 2008).

Recent surveys have provided lists of amphibian species, mostly preliminary, for some high montane Atlantic Forest areas (i.e., above 1,000 m) in southeast Brazil (Cruz et al. 2009, Siqueira et al. 2011a,b, Garey et al. 2014, 2016, Folly et al. 2016, Lima et al. 2021). These habitats are known for their high rates of endemism of anurans, particularly in portions of the Serra do Mar (Siqueira et al. 2011b, Carvalho-e-Silva et al. 2020) and Serra da Mantiqueira regions (Neves et al. 2018, Silva et al. 2018).

The mountain complex of Serra da Mantiqueira contains the highest areas of the Atlantic Forest domain (reaching nearly 2,900 m of altitude), harboring at least 234 amphibian species, 88 of them endemic to the region (Silva et al. 2018). In the Serra da Mantiqueira complex, the Serra do Itatiaia region is reported as the area with the highest amphibian richness, with 61 species (nine endemic), many of them originally described from the Parque Nacional de Itatiaia (Neves et al. 2018). However, there is still no published list of amphibian species for the region of Itatiaia since Neves et al. (2018) presented the species richness value but listed only the endemic species. Adjacent to the Parque Nacional de Itatiaia, there is another Conservation Unit, the Área de Proteção Ambiental Serra da Mantiqueira (APA Serra da Mantiqueira). In spite of covering more than 400,000 ha and encompassing portions of three Brazilian states (MMA 2018), published data on the amphibian fauna from this Conservation Unit is, to our knowledge, available for only one locality in the state of Rio de Janeiro. Information on the anuran species occurring in that locality is presently restricted to studies reporting an extension of the geographical distribution of *Paratelmatobius mantiqueira* (Vrcibradic et al. 2010), some ecological aspects of *Proceratophrys mantiqueira* (Almeida-Gomes et al. 2007, Almeida-Santos et al. 2017), and a record of snake predation on *Scinax cardosoi* (Dorigo et al. 2014). Another study presented data on sampling efficiency during different periods of the day and the number of species sampled at the area but did not provide information on the local species composition (Rocha et al. 2015).

The state of Rio de Janeiro, whose territory is entirely included within the Atlantic Forest domain, was recently reported to harbor 201 amphibian species (Dorigo et al. 2018), and new species with occurrence in the state are still being steadily described (e.g., Folly et al. 2018, Pereira Silva et al. 2018, Taucce et al. 2018, Cruz et al. 2019, Silva et al. 2020, Nunes et al. 2021). In recent years, lists of species of amphibians have been compiled or updated for several forested areas in the state of Rio de Janeiro (e.g., Almeida-Gomes et al. 2014, Martins et al. 2014, Caram et al. 2016, Rocha et al. 2018, Carvalho-e-Silva et al. 2020, Dorigo et al. 2021), contributing considerably to the knowledge of its amphibian fauna. However, gaps of knowledge for areas at high altitudes still remain, not only for the state of Rio de Janeiro, but for the Atlantic Forest biome as a whole (Lima et al. 2021).

In this study, we address some parameters of the amphibian assemblage from a locality within the APA Serra da Mantiqueira for which preliminary data on the anuran fauna has been previously published, as mentioned above (Almeida-Gomes et al. 2007, Vrcibradic et al. 2010, Dorigo et al. 2014, Rocha et al. 2015, Almeida-Santos et al. 2017). We present data on species composition, richness and relative abundances of anuran amphibians in that locality, as well as density estimates (individuals per 100 m^2) for frogs inhabiting the forest floor leaf litter.

Material and Methods

1. *Study area*

The study was carried out in a locality within the APA Serra da Mantiqueira, a Conservation Unit encompassing parts of the states of Minas Gerais, Rio de Janeiro and São Paulo, southeastern Brazil. Created in 1985, the APA Serra da Mantiqueira (437,525 ha) comprises an area varying from 458 m up to 2,798 m of elevation, and covers, totally or partially, approximately 50 Conservation Units (MMA 2018). This study's surveys were carried out within a private property, the Marimbondo Farm (22°21'50" S, 44°35'25" W; 34,475 ha), whose area is contained within both Resende and Itatiaia municipalities, state of Rio de Janeiro (Figure 1). Vegetation in the study area is characterized by Upper Montane Rain Forest (*sensu* Oliveira-Filho & Fontes 2000; Figure 2). Mean annual temperature in the region is 14.9° C, and mean annual precipitation is 1,813 mm (Attias et al. 2009).

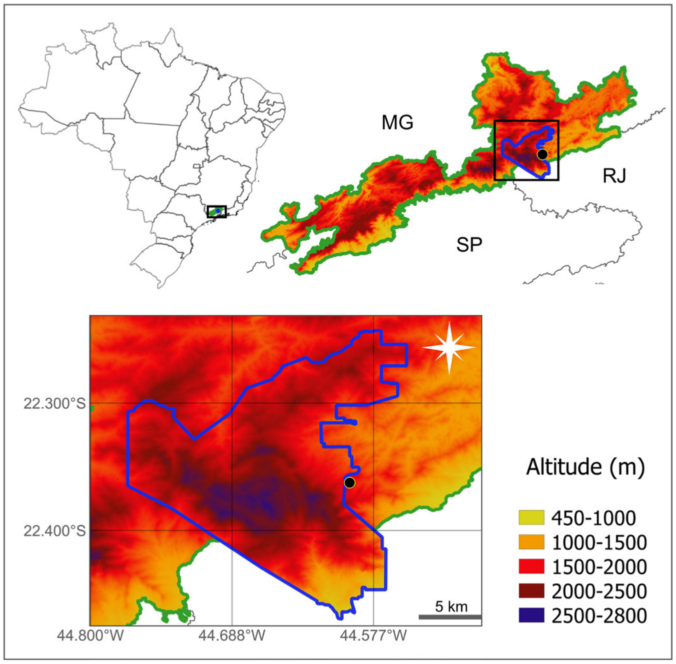

Figure 1. Map of Brazil (upper left) showing the region of the APA Serra da Mantiqueira and the Parque Nacional de Itatiaia (rectangle). The other maps (upper right and bottom) show the location of the study site (black dot), with the limits of the APA Serra da Mantiqueira outlined in green and those of the Parque Nacional de Itatiaia outlined in blue. State codes: MG - Minas Gerais; RJ - Rio de Janeiro; SP - São Paulo.

2. *Research design, field methods and data analysis*

Fieldwork was conducted at sites between 1,350 m and 1,750 m of elevation, in three different years: 2005, 2010, and 2011. In November 2005, to obtain a representative dataset of the anuran fauna for the study area (including both qualitative and quantitative data) we used three sampling methods. Two of them, visual encounter surveys (Crump & Scott 1994) and plot sampling (Jaeger & Inger 1994), were performed from 14-18 November 2005. The third method, pitfall traps with drift fences (Corn 1994), was employed from 05-11 and from 14-19 November 2005. Additional sampling was carried out from 18-20 December 2010 and from 13-15 November 2011 using non-standardized methodology.

Figure 2. View of the montane Atlantic Forest (A) and large rocky stream within the forest (B) in the Marimbondo Farm, within the APA Serra da Mantiqueira, state of Rio de Janeiro, Brazil. Photos by D. Vrcibradic (A) and C.V. Ariani (B).

For visual encounter surveys (VES), 150 time-constrained searching bouts of 30 minutes each were carried out daily by ten people wearing headlamps, totaling 75 hours of sampling effort. Equal numbers of transects (50) were surveyed during the diurnal (11:00-16:30h), crepuscular (17:30-18:30h) and nocturnal (19:30-22:00h) periods. During transect sampling, each observer moved at a slow walking pace, carefully searching all types of potential microhabitats for anurans (e.g., leaf litter, water bodies, bromeliads, fallen logs, tree trunks, shrubs, rocks).

For the plot method, 30 quadrats of 5 x 5 m were established on the forest floor during the afternoon, totaling 750 m^2 of area sampled. The corners of each plot were marked with wooden stakes and the area inside was enclosed with a 50 cm high soft plastic fence, whose base was buried or attached to the ground. Soon after sunset, each plot was carefully searched for about half an hour by a crew of five persons using headlamps, moving on hands and knees, side-by-side. During the searches the leaf-litter was stirred with hand rakes, and leaves, stones and fallen branches were overturned; rock crevices and fissures among tree roots were also checked for anurans. Plot sampling was performed during five consecutive days, with six quadrats surveyed per night.

Three pitfall trap systems were established within the forest and remained open for a total of 11 days. Each system consisted of ten 30-liter buckets buried on the ground up to their rims, each bucket set *ca*. 5 m apart from the nearest one, with soft plastic drift fences about 50 cm high extended between them. Six buckets were set in line and the other four were placed at opposite ends of the fence, perpendicular to the main axis. Pitfalls were checked once per day for captured animals, always in the morning.

All anurans found by the three sampling methods were collected and identified (whenever possible). Estimates of leaf litter frog density (ind/100 m^2) were produced based on the data obtained through plot method. An evaluation of sampling effectiveness was undertaken by cumulative and individual-based rarefaction curves performing 1,000 randomizations without replacement, using the program EstimateS 9.1.0 (Colwell 2013). We carried out this procedure for VES and plot methods separately, using abundance data.

Further excursions for sampling the same area occurred in December 2010 and in November 2011 for collection of additional specimens. In these excursions, a three-person team performed haphazard visual searches (not standardized by time, unlike those of 2005) between 17:00 and 22:00h, at altitudes of 1,450-1,550 m, during two consecutive nights. Additionally, in 2011, funnel traps (made with five-liter plastic bottles) baited with ham were set at the bottom of a stream to capture tadpoles. Records obtained by these collections were incorporated into the local species list but were not used in quantitative analyses.

3. *Taxonomic issues*

Voucher specimens of anuran species collected during the study were deposited at the amphibian collection of the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), in the municipality of Rio de Janeiro (see Appendix).

Bokermannohyla circumdata can be potentially confused with *B. luctuosa* (Pombal & Haddad, 1993) (which also occurs in the region of Itatiaia; see Silva et al. 2018) since the taxonomic limits between these two species may be considered problematic (M. F. Napoli, pers. comm.). The larger *Bokermannohyla* individuals from the Marimbondo Farm were assigned to *B. circumdata* due to the presence (albeit not in all specimens) of bifid distal subarticular tubercles on fingers III and IV (see Napoli 2000). Also, Silva et al. (2018) cited our specimens as *B. circumdata* in their list of material examined, which indicates that those authors have seen that material and confirmed its identification. A second, smaller form of *Bokermannohyla* recorded during our surveys could not be positively identified to any currently described species in the genus and is treated herein as *Bokermannohyla* sp.

Phantasmarana (formerly *Megaelosia*) *lutzae* remained unrecorded for decades since the types and other associated specimens were collected in the Parque Nacional de Itatiaia (Izecksohn & Gouvêa 1987). Even though only tadpoles were recorded in our surveys, we felt confident in assigning them to *P. lutzae* as this is the only species of large-bodied hylodid currently known from the region of Itatiaia and its surroundings (Vittorazzi et al. 2021). Moreover, some traits of the tadpoles, such as the presence of distinct dark blotches on the tail and the small marginal papillae (compared to those of *Megaelosia goeldii* tadpoles) match the brief description given by Izecksohn & Gouvêa (1987).

Results

A total of 24 anuran species belonging to eight families were recorded, of which six were registered only during samplings carried out in 2010 and 2011 (Table 1, Figures 3 and 4). For two species, *Phantasmarana lutzae* and *Phasmahyla cochranae*, only tadpoles were recorded. The anuran assemblage was dominated by species of the family Hylidae (ten species).

The VES method yielded records of 132 individuals from 17 anuran species, with *Proceratophrys mantiqueira* (N = 28, or 21.1% of all individuals found), *Ischnocnema* sp. (gr. *lactea*) (N = 21, or 15.9%), and *Bokermannohyla circumdata* (N = 18, or 13.6%) being the ones most frequently sampled (Table 1). Most anuran species recorded during transects were found during the nocturnal (N = 14 species) and crepuscular periods (N = 9), with eight species being recorded only at night and three species found only during crepuscular samplings (Table 1). Four species were recorded both during crepuscular and nocturnal periods, and only the two most abundant species, *P. mantiqueira* and *Ischnocnema* sp. (gr. *lactea*), were recorded on all three sampling periods (Table 1). Moreover, most individuals (regardless of the species) were found during the nocturnal (N = 78, or 59.1%) and crepuscular (N = 41, or 31.1%) transect searches.

Table 1. Anuran species recorded at the Atlantic Rainforest of the Marimbondo Farm, APA Serra da Mantiqueira, state of Rio de Janeiro, Brazil. Number of individuals is presented for each species recorded by visual encounter surveys (VES) during diurnal (D), crepuscular (C) and nocturnal (N) periods, plot and pitfall trap sampling. Species added to the list in 2010 (*) and 2011 (**) were not quantified. T = species recorded only as tadpoles.

Species	**VES**			**Plot**	**Pitfall**	**Total**
	D	**C**	**N**			
Brachycephalidae						
Brachycephalus rotenbergae Nunes, Guimarães, Moura, Pedrozo, Moroti, Castro, Stuginski & Muscat, 2021						*
Ischnocnema aff. *guentheri*		2	8	1	1	12
Ischnocnema juipoca (Sazima & Cardoso, 1978)			1			1
Ischnocnema sp. (gr. *lactea*)	3	15	3	121	1	143
Bufonidae						
Rhinella icterica (Spix, 1824)		3	5	1		9
Centrolenidae						
Vitreorana uranoscopa (Müller, 1924)		3				3
Hylidae						
Aplastodiscus arildae (Cruz & Peixoto, 1987)		2				2
Aplastodiscus leucopygius (Cruz & Peixoto, 1985)			4			4
Boana pardalis (Spix, 1824)						*
Boana polytaenia (Cope, 1870)			8			8
Bokermannohyla circumdata (Cope, 1871)		1	17			18
Bokermannohyla sp. (gr. *circumdata*)		1	5			6
Dendropsophus minutus (Peters, 1872)						*
Scinax cardosoi (Carvalho-e-Silva & Peixoto, 1991)			13			13
Scinax dolloi (Werner, 1903)			5			5
Scinax flavoguttatus (Lutz & Lutz, 1939)		1				1
Hylodidae						
Phantasmarana lutzae (Izecksohn & Gouvêa, 1987) (T)						**
Leptodactylidae						
Leptodactylus latrans (Steffen, 1815)						**
Paratelmatobius mantiqueira Pombal & Haddad, 1999			1			1
Physalaemus cuvieri Fitzinger, 1826						*
Odontophrynidae						
Odontophrynus americanus (Duméril & Bibron, 1841)			1			1
Proceratophrys boiei (Wied-Neuwied, 1824)			2			2
Proceratophrys mantiqueira Mângia, Santana, Cruz & Feio, 2014	10	13	5	15	7	50
Phyllomedusidae						
Phasmahyla cochranae (Bokermann, 1966) (T)						*
Total	13	41	78	138	9	279

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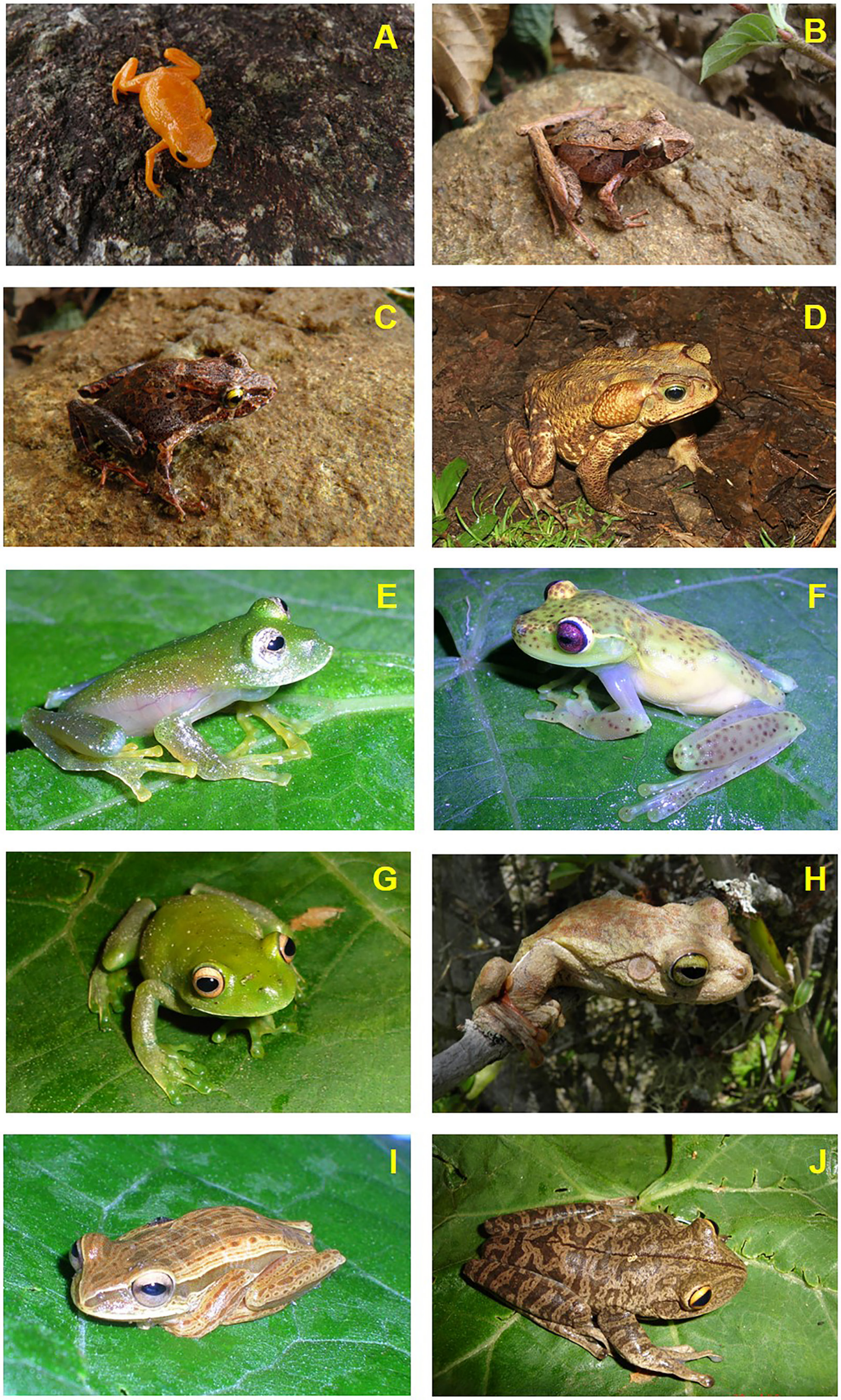

Figure 3. Some anurans recorded in an Atlantic Rainforest area within the APA Serra da Mantiqueira, state of Rio de Janeiro, Brazil: (A) *Brachycephalus rotenbergae*; (B) *Ischnocnema* aff. *guentheri*; (C) *Ischnocnema* sp. (gr. *lactea*); (D) *Rhinella icterica*; (E) *Vitreorana uranoscopa*; (F) *Aplastodiscus arildae*; (G) *Aplastodiscus leucopygius*; (H) *Boana pardalis*; (I) *Boana polytaenia*; (J) *Bokermannohyla circumdata*. Photos by D. Vrcibradic (A, C, D, H), M. Targino (B), T. Klaion (E, F, I) and V.N.T. Borges-Júnior (G, J).

Figure 4. Some anurans recorded in an Atlantic Rainforest area within the APA Serra da Mantiqueira, state of Rio de Janeiro, Brazil: (A) *Bokermannohyla* sp. (gr. *circumdata*); (B) *Dendropsophus minutus*; (C) *Scinax cardosoi*; (D) *Scinax dolloi*; (E) *Scinax flavoguttatus*; (F) *Paratelmatobius mantiqueira*; (G) *Physalaemus cuvieri*; (H) *Odontophrynus americanus*; (I) *Proceratophrys boiei*; (J) *Proceratophrys mantiqueira*. Photos by C.V. Ariani (A, D, F, J), M. Targino (B), V.N.T. Borges-Júnior (C, E) and D. Vrcibradic (G, H, I).

A total of 138 individuals belonging to four anuran species were found during plot sampling (Table 1). The number of anurans per plot ranged from zero (7/30 or 23.3% of all plots) to 25 (1/30 or 3.3% of all plots). The estimated overall density of the local leaf litter frog assemblage was 18.4 ind/100 m^2. The highest density was that of *Ischnocnema* sp. (gr. *lactea*) (16.1 ind/100 m^2), followed by *Proceratophrys mantiqueira* (2.0 ind/100 m^2), and those two species together comprised about 98.5% of individuals found in plots. Both *Ischnocnema* aff. *guentheri* and *Rhinella icterica* had an estimated density of 0.1 ind/100 m^2.

Nine individuals of three species were captured in pitfall traps, with *Proceratophrys mantiqueira* (N = 7) being the most frequent (Table 1). Overall (i.e., including all methodologies pooled) the most abundant species recorded during the study were *Ischnocnema* sp. (gr. *lactea*) and *P. mantiqueira* (comprising 51.3% and 17.9% of all individuals collected, respectively).

Cumulative and individual-based species-rarefaction curves showed an asymptotic shape for VES, but it was non-asymptotic for plot-sampling data (Figure 5).

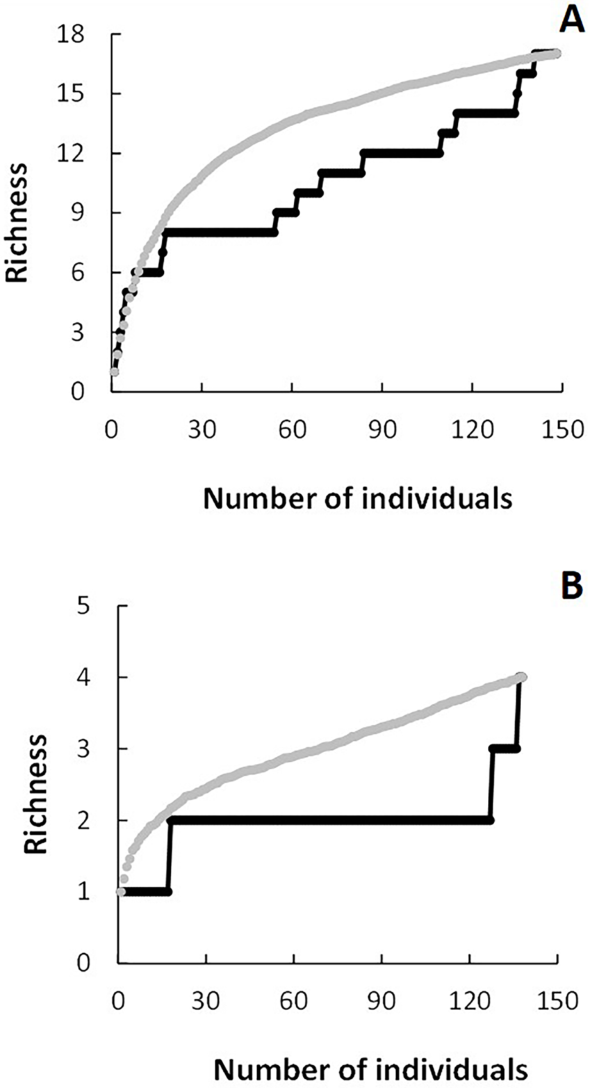

Figure 5. Cumulative (black) and rarefaction (gray) curves of species sampled of anurans recorded using VES (A) and plot (B) methods, in an Atlantic Rainforest area within the APA da Mantiqueira, in Rio de Janeiro state, Brazil.

Discussion

This is the first study to provide an approach of species composition, richness and estimated density (on the forest floor) of amphibians for any area within the APA Serra da Mantiqueira, reporting a total of 24 anuran species for the local assemblage. The anuran assemblage in the study area was dominated by species of the family Hylidae, which is the richest anuran family in the Atlantic Forest domain (Rossa-Feres et al. 2017). Most of the hylids here recorded were found in disturbed areas, and many individuals in five species (*Boana polytaenia*, *Bokermannohyla circumdata*, *Bokermannohyla* sp., *Scinax cardosoi*, and *S. dolloi*) were collected in a single marsh. The higher species richness of hylids found in tropical regions of the Americas, compared to that of temperate regions, may reflect the fact that these anurans originated in tropical South America and there has been a long time for species to accumulate through in-situ speciation (Wiens et al. 2006).

Some of the anurans recorded in this study represent new or undetermined species. The anuran most frequently found during our fieldwork was an undetermined species of *Ischnocnema* of the *lactea* group (currently in the process of being formally described) that superficially resembles other members of that group such as *I. melanopygia* Targino, Costa & Carvalho-e-Silva, 2009 and *I. concolor* Targino, Costa & Carvalho-e-Silva, 2009 (which also occur in the Itatiaia region, but at higher altitudes; Targino et al. 2009). We also recorded members of the *Ischnocnema guentheri* species complex (see Gehara et al. 2013), which also likely represents an undescribed taxonomic entity. Gehara et al. (2013) restricted *I. guentheri sensu stricto* to the Parque Nacional da Tijuca, municipality of Rio de Janeiro, based on molecular and bioacoustic data. The specimens reported herein may belong to the *Ischnocnema* aff. *guentheri* lineage CS3 of Gehara et al. (2013), since the samples from Itatiaia used in their study fell within that lineage in their phylogenetic tree. *Bokermannohyla* sp. (gr. *circumdata*) may also represent an undescribed taxon (A.C.C. Lourenço, *pers. comm.*).

Two other species recorded during our surveys have only been described within the last ten years, being previously confused with other taxa: *Proceratophrys mantiqueira* (split off from *P. melanopogon* by Mângia et al. 2014) and *Brachycephalus rotenbergae* (split off from *B. ephippium* by Nunes et al. 2021). *Paratelmatobius mantiqueira* was also described relatively recently, based on specimens collected in 1953 (Pombal & Haddad 1999), and remained unrecorded in the wild for more than 50 years until one specimen was collected during our 2005 surveys at the studied site in the APA Serra da Mantiqueira (Vrcibradic et al. 2010). Finally, *Scinax dolloi* is a taxon that remained in obscurity for more than a century, with no natural populations being associated with this name since its description by Werner (1903), until its "rediscovery" in 2016 (Santos et al. 2021). The latter authors reported a population of the species from its type locality in Vila de Maringá, municipality of Itatiaia (see Caramaschi et al. 2013), some 2.6 km in straight line from the locality where the present study was carried out. These cases, together with the aforementioned undescribed/undetermined taxa, highlight how incomplete the knowledge of the Atlantic Forest anuran fauna still is, particularly in the Serra da Mantiqueira region.

The genus *Megaelosia* has recently been rendered monotypic (for *M. goeldii*) due to non-monophyly, and a new genus (*Phantasmarana*) was erected to accommodate the other species, including *M. lutzae* (Vittorazzi et al. 2021).

Phantasmarana lutzae was described (as *Megaelosia lutzae*) by Izecksohn & Gouvêa (1987) based on a few adult and juvenile specimens collected between 1949 and 1964 and some tadpoles (not included in the type series) without collection date. This species remained unrecorded in the wild for more than five decades until Vittorazzi et al. (2021) reported five tadpoles collected at the type locality (Parque Nacional de Itatiaia), which they used in a molecular analysis. Rosa et al. (2003) referred some tadpoles from the municipality of Pindamonhangaba, São Paulo state (ca. 100 km westward from Parque Nacional de Itatiaia), to *Megaelosia lutzae*, but Vittorazzi et al. (2021) re-identified those specimens as *Phantasmarana jordanensis* (Heyer 1983) based on analyses of DNA samples. Thus, *P. lutzae* is currently known only from the type locality and its surroundings. Our record of *P. lutzae* tadpoles for the APA Serra da Mantiqueira represents the second confirmed report of the species since its description (but it does not constitute an actual range extension, as our study site is located adjacent to and just outside the limits of the Parque Nacional de Itatiaia). Although no adults were found, the fact that several tadpoles were collected in 2011 in spite of relatively little sampling effort suggests that *P. lutzae* may not be an uncommon species, at least in our study site. Further sampling is required to gather information on this poorly known species.

The treefrog *Scinax cardosoi* is another species that remains poorly known (Kirchmeyer et al. 2019), being currently reported from a few isolated localities in the states of Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo, in southeastern Brazil (Frost 2021). Studies updating the geographic distribution of this species (Pinto et al. 2009, Linares et al. 2011, Moroti et al. 2017) have omitted its presence in Visconde de Mauá, in the municipality of Resende, state of Rio de Janeiro, apparently overlooking the fact that one of its paratypes came from that locality (Carvalho-e-Silva & Peixoto 1991). The village of Visconde de Mauá is about 6 km distant from our study site. More recently, Kirchmeyer et al. (2019) reported five newly metamorphosed individuals from the Parque Nacional de Itatiaia. Those records, together with those of our study, confirm the presence of *S. cardosoi* in the region of Itatiaia and its occurrence in other areas within the state of Rio de Janeiro besides its type locality in Teresópolis municipality (Carvalho-e-Silva & Peixoto 1991).

The estimated overall density of the local leaf litter frog assemblage was high (18.1 ind/100 m^2) when compared to those found for most other sites sampled (also with large quadrats) within the Atlantic Forest in southeastern and southern Brazil (1.4-13.2 ind/100 m^2; Giaretta et al. 1997, 1999, Rocha et al. 2001, 2007, 2011, 2013, Almeida-Gomes et al. 2008, 2010, Santos-Pereira et al. 2011, Siqueira et al. 2011a, Oliveira et al. 2013). Exceptions are two localities in the municipality of Cachoeiras de Macacu, state of Rio de Janeiro, that yielded comparable (17.1 ind/100 m^2; Siqueira et al. 2009) or higher estimates (24.6 ind/100 m^2; Siqueira et al. 2014). However, the high frog density observed in this study reflects the high local abundance of *Ischnocnema* sp. (gr. *lactea*), since 88% of the frogs found in plots represented that species. Indeed, this small direct-developing anuran numerically dominated the anuran assemblage at the studied site in the APA Serra da Mantiqueira (as also evidenced by the VES methodology). Several other plot-sampling studies in Atlantic Forest areas have also evidenced a clear dominance of species with direct development of terrestrial eggs in the local leaf-litter frog assemblages (e.g., Giaretta et al. 1997, 1999, Rocha et al. 2001, 2007, 2013, Almeida-Gomes et al. 2008, 2010, Siqueira et al. 2009, 2011a, 2014), and this has also been observed in Amazonian (e.g., Rodriguez 1992, Gascon 1996) and Central American forest areas (e.g., Scott 1976, Toft 1981, Liebermann 1986, Fauth et al. 1989, Hofer & Bersier 2001). Direct-developing anurans may be more widely distributed within the forest due to their independence of water bodies for reproduction (Haddad & Prado 2005), though other factors such as rates of dehydration/rehydration (Dabés et al. 2012) and a generalist diet (Alves-dos-Santos et al. 2021) may also play a role.

Cumulative and rarefaction curves using data from VES method indicated that sampling effort was almost enough to obtain a representative sample of the local anuran fauna, with a tendency toward stabilization, despite plot methodology falling short of reaching the asymptote. Plot sampling is useful for sampling anurans typical of forest floor leaf litter in Atlantic Forest areas (Giaretta et al. 1997, 1999, Rocha et al. 2001, 2007, 2013, Siqueira et al. 2009, 2011a) and, despite our effort being not enough using this method, members of that guild of anurans were also recorded by VES, adding four leaf litter species to our list that were not recorded in plots (e.g., *Ischnocnema juipoca*, *Paratelmatobius mantiqueira*, *Odontophrynus americanus* and *Proceratophrys boiei*). Moreover, our additional effort with non-standardized samplings in 2010 and 2011, added two additional species that live in the forest floor (*Brachycephalus rotenbergae* and *Physalaemus cuvieri*), besides five more anuran taxa with arboreal or semi-aquatic habits (*Boana pardalis*, *Dendropsophus minutus*, *Leptodactylus latrans*, *Phantasmarana lutzae* and *Phasmahyla cochranae*). The exception is *P. lutzae*, which was sampled only as tadpoles, through the use of funnel traps (the only other species recorded only in larval stage, *Phasmahyla cochranae*, was sampled as a tadpole found during haphazard visual searching). Thus, our data showed that visual searches were sufficient to produce a representative sample of the anuran fauna of our study site, with pitfall traps and plot sampling providing no additional species records. However, in some other short-term inventories employing multiple sampling methods in tropical areas, plot and/or pitfall trap sampling added anuran species that had not been recorded by standardized visual searches or occasional encounters (Almeida-Gomes et al. 2008, Ribeiro-Júnior et al. 2008, Siqueira et al. 2009, Rocha et al. 2011). Nevertheless, pitfall traps are generally considered to have low cost-benefit for simple short-term surveys of tropical amphibians (Cechin & Martins 2000, Rödel & Ernst 2004), which is supported by the results of our study.

Phantasmarana lutzae and *Scinax dolloi* are the only species recorded in our study that are currently known only from the state of Rio de Janeiro and are possibly endemic to the Itatiaia region (Frost 2021, Vittorazzi et al. 2021), though Santos et al. (2021) suggested that *S. dolloi* may occur more widely. Neves et al. (2018) considered the Serra do Itatiaia as the area of highest amphibian endemicity rate within the Mantiqueira/Espinhaço/Canastra mountain complex in southeastern Brazil, with nine endemic anuran species (*Scinax dolloi* was not mentioned, possibly because it was a species of problematic identity at the time). Among the undescribed species recorded in the APA Serra da Mantiqueira survey, at least one, *Ischnocnema* sp. (gr. *lactea*), may be endemic to the Itatiaia region, and it is possible that the degree of anuran endemicity in the area is still underestimated.

Regarding their conservation status, most species from the APA Serra da Mantiqueira are listed under "Least Concern" (LC) by the IUCN (2021), though the status of *Brachycephalus rotenbergae*, *Proceratophrys mantiqueira*, *Scinax dolloi*, and the undescribed species have not been assessed.

Phantasmarana lutzae (Carvalho-e-Silva & Carvalho-e-Silva 2004) and *Paratelmatobius mantiqueira* (Nascimento & Garcia 2004) are both classified as "Data Deficient" (DD) with population trends classified as "unknown" and "decreasing", respectively (IUCN 2021). None of the species found in the present survey is listed as threatened in the Red Book of Threatened Brazilian Fauna (ICMBio/MMA, 2018) either. Threatened species are usually the focus of conservation efforts and funding programs, but little attention is given to species considered "Data-Deficient", contradicting the IUCN's recommendation to give them the same degree of attention as threatened taxa (Pimenta et al. 2005, Siqueira et al. 2011b).

Lists of species present in a given area constitute a fundamental tool for the development and management of effective conservation interventions. Our inventory at an area within the APA Serra da Mantiqueira adds to the body of knowledge on the amphibian diversity of high-elevation forests in an Atlantic Forest area, which is still insufficiently known.

Supplementary Material

The following online material is available for this article:

Appendix I - Voucher specimens of species collected in the present study deposited at the Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro municipality, Rio de Janeiro state, Brazil.

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Conflicts of Interest

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

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Article

Predicting potential distribution and evaluating biotic interactions of threatened species: a case study of *Discocactus ferricola* (Cactaceae)

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Abstract: Information on distribution, number of populations, and biotic interactions are essential for assessing the threat status of species and to establish more effective conservation initiatives. Ecological niche modeling have been successfully applied to identify the potential distribution, even for rare species that have few recorded occurrence points. In this study, we evaluated the potential distribution and additionally generated the first data on the reproductive biology of *Discocactus ferricola*, due to its degree of threat and the absence of ecological data for that species. The potential distribution map highlighted areas with higher probability of occurrence of *D. ferricola* on the Residual Plateau of Maciço do Urucum located in Mato Grosso do Sul, Brazil. The occurrence of *D. ferricola* populations was limited to outcrops of flat ironstone (cangas) distributed in patches across the landscape, increasing the chances of serious threats, such as habitat loss due to mining and species extraction. We also found that *D. ferricola* is xenogamous. Therefore, *in situ* conservation actions must prioritize the maintenance of interactions with pollinators by preserving the flora and fauna of rocky outcrops and adjacent forests in areas of greater environmental suitability for *D. ferricola*. Our study highlights the use of ecological niche modeling and data on biotic interactions to evaluate species potential distribution, to guide new sampling efforts, and to assist conservation and management initiatives.

Keywords: *Conservation; ecological niche modeling; pollinator dependence; outcrop; restrict distribution.*

Prevendo a distribuição potencial e avaliando interações bióticas de espécies ameaçadas: um estudo de caso de *Discocactus ferricola* (Cactaceae)

Resumo: Informações sobre distribuição, número de populações e interações bióticas são essenciais para avaliar o status de ameaça das espécies e estabelecer iniciativas de conservação mais eficazes. A modelagem de nicho ecológico tem sido aplicada com sucesso para identificar a distribuição potencial, mesmo para espécies raras que possuem poucos pontos de ocorrência registrados. Neste estudo, avaliamos a distribuição potencial e adicionalmente geramos os primeiros dados sobre a biologia reprodutiva de *Discocactus ferricola*, devido ao seu grau de ameaça e à ausência de dados ecológicos para essa espécie. O mapa de distribuição potencial destacou áreas com maior probabilidade de ocorrência de *D. ferricola* no Planalto Residual do Maciço do Urucum localizado em Mato Grosso do Sul, Brasil. A ocorrência de populações de *D. ferricola* foi limitada aos afloramentos ferruginosos planos (cangas) que são distribuídos em manchas pela paisagem, aumentando as chances de ameaças graves, como perda de habitat devido à mineração e extração da espécie. Também descobrimos que *D. ferricola* é xenogâmica. Portanto, ações de conservação *in situ* devem priorizar a manutenção das interações com os polinizadores através da preservação da flora e da fauna nos afloramentos rochosos e florestas adjacentes nas áreas de maior adequabilidade ambiental para *D. ferricola*. Nesse estudo, nós destacamos o uso da modelagem de nicho ecológico e de dados sobre interações bióticas para avaliar a distribuição potencial de espécies, orientar novos esforços de amostragem e auxiliar iniciativas de conservação e manejo.

Palavras-chave: *afloramentos rochosos; conservação; dependência de polinizador; distribuição restrita; modelagem de nicho ecológico.*

Introduction

The occurrence points of a species carry information about its distribution, which is a complex expression of its ecology and evolutionary history (Brown 1995). Each point reflects part of realized niche and, therefore, contains information on abiotic conditions, biotic interactions, and the dispersion capacity that are required for the occurrence of species (Soberón & Peterson 2005). Thus, the points of occurrence can be used to construct ecological niche models (ENM) also known as species distribution models (SDM) (Soberón & Peterson 2005, Peterson et al. 2011, Peterson & Soberón 2012). This approach is considered of low cost and has been applied to several researches, especially for the conservation of species and biomes (Le Lay et al. 2010, Werneck et al. 2012, Sobral-Souza et al. 2018, Adhikari et al. 2019, Kolanowska & Jakubska-Busse 2020). Specifically, ENM has been used to identify likely areas for invasive species (Qiao et al. 2017, Zhu et al. 2017), in paleoecological studies (Lima-Ribeiro & Diniz-Filho 2013), to highlight areas of potential distribution (McCune 2016, Fois et al. 2018), to indicate areas for species reintroduction (Martínez-Meyer et al. 2006), among others.

Noteworthy, ecological niche modeling have been successfully applied to provide information on potential distribution of species even with few recorded occurrence points (Le Lay et al. 2010, McCune 2016, Fois et al. 2018). Distribution data is important for the assessment of the threat status of these species, since the extent of occurrence and number of populations are criteria used to define threat categories of the International Union for Conservation of Nature (IUCN) (Martinelli & Moraes 2013). After identifying the threat level, the next step is to implement *in situ* and or *ex situ* management actions, when necessary. For the correct management of species, information about their ecology, including reproductive aspects and biotic interactions, are required (Scheele et al. 2018). The lack of these information weaken management and conservation strategies, prevent adequate actions for population growth, and obscure the construction of more assertive hypotheses about the evolution and dispersion of species.

Basic information on distribution and reproductive biology are crucial for assessments of the current threat status of species and for the establishment of more accurate conservation plans, especially for rare and endangered species such as *Discocactus ferricola* Buining & Brederoo (Machado 2004, Ribeiro-Silva et al. 2011). This species is currently categorized as endangered on the Red List of Threatened Species of IUCN, mainly due to its small area of occurrence and small number of known populations (Braun 2013). In this study, we used the ecological niche modeling approach to assess the potential distribution and additionally to generate the first ecological data on reproductive biology of *D. ferricola*. The general aim of this study was to provide ecological data of *D. ferricola* to assist in conservation initiatives and to guide new sampling efforts. Specifically, we aimed to answer the following questions: (1) What is the current potential distribution of *D. ferricola*? (2) Does *D. ferricola* depend on pollinators for reproduction? (3) What species visit *D. ferricola* flowers?

Material and Methods

1. *Study area*

The present study was carried out on eight farms, a settlement and a municipal park, all located in the mountainous complex of the Residual Plateau of Maciço do Urucum (RPMU), in the municipalities of Corumbá and Ladário in Mato Grosso do Sul state, MS, Brazil (Tab. 1). According to Köppen's classification, the climate of the region is Aw megathermal, with dry winters and rainy summers (Soriano 2000). The average annual temperature is 25.1°C, with maximum temperatures reaching 40°C and minimum temperatures close to 0°C (Soriano 2000). The average annual precipitation is 1070 mm, and the average annual relative humidity is 75% (Soriano 2000).

2. *Studied species*

Discocactus ferricola has a flattened globular shape with a pale to dark green stem measuring 8-9 cm high and 20-25 cm in diameter (Anderson 2001). The number of ribs is 14, and they form tubercles (Anderson 2001). The 5-8 spines, arranged radially, are 4.5-5 cm and brown, becoming gray with age. The central spines are mostly absent but may present as one spine at 2-2.5 cm (Anderson 2001). The cephalium measures 7 cm high and 6.5 cm in diameter and has white wool with dark gray bristles that can be 5 cm (Anderson 2001). The flowers are white and tubular and measure 5.5 cm long (Figure 1A). The fruits are elongate to club shaped, measure 3-4 cm long, and are greenish cream to white. Seeds are broadly oval to subglobose and are shiny black color, with numerous papillae or tubercles, and they measure 2-2.5 mm long (Anderson 2001).

Discocactus ferricola is endemic to the mountainous complex of the RPMU on the border of Brazil and Bolivia (Braun 2013, Takahasi & Meirelles 2014). The RPMU is known as the oldest rock formation in the world, with intact exposed rocks that are approximately 70 million years old (Vasconcelos et al. 2019). In the RPMU, *D. ferricola* occurs on flat ironstone outcrops, locally known as "bancadas lateríticas" or "cangas", formed by the deposition of iron and manganese laterites in the drainage areas of the hills and slopes (Braun 2013, Takahasi & Meirelles 2014). *Discocactus ferricola* have clonal reproduction and is abundant locally, forming large aggregates (Figure 1B).

Currently, the main threats to *D. ferricola* are habitat loss due to mining (especially in Bolivia), human occupation, and species extraction, as this species is consumed as food, used as herbal medicine by traditional communities, and has ornamental potential (Lüthy 2001, Ribeiro-Silva et al. 2011, Braun 2013). The total area of occurrence of *D. ferricola* is approximately 20 km^2 patchily distributed on three outcrops of iron ore and manganese surrounded by Cerrado vegetation and forests (Braun 2013). Such habitats are threatened by mining and urbanization, which has led to a 30% population decline of *D. ferricola* over the last 30 years (Braun 2013). The continuous loss of adult individuals, its occurrence out of protected areas, and its generation time of 10 years also justify the endangered status of *D. ferricola* (Braun 2013).

3. *Species distribution modeling*

To identify areas of greatest climatic suitability for *D. ferricola*, we initially performed a search on the Global Biodiversity Information Facility (GBIF) and Species Link platforms to access all recorded occurrences. In addition, we actively searched for *D. ferricola* populations in the RPMU. The occurrence points were used to construct *D. ferricola* occurrence map. After removing uncertain, redundant, and historical data, we had 11 occurrence points of *D. ferricola* covering the entire known distribution of the species (Tab. 1). To remove environmentally autocorrelated points, a rarefaction analysis for environmental heterogeneity was carried out in ArcGIS v.10.3 (Anderson & Gonzalez 2011, ESRI 2014, Varela et al. 2014). The remaining 10 occurrence points were used to model the potential niche of the species (Tab. 1). The area included in the model extended approximately 140000 km^2 and contained the main rock outcrops of the region: 1- Rincon del Tigre in Bolivia, 2 - Serra do Amolar, 3 - Residual Plateau of Maciço do Urucum, and 4 - Serra da Bodoquena in Brazil.

Table 1. Occurrence sites of *Discocactus ferricola* on the Residual Plateau of Maciço do Urucum, Mato Grosso do Sul state, Brazil.

Population	Latitude	Longitude	Origin	City
Vale do Paraíso	19° 10' S	57° 33' W	GBIF	Ladário
Estrada Parque Pantanal	19° 05' S	57° 36' W	Species Link	Ladário
Monjolinho	19° 16' S	57° 31' W	new record	Corumbá
Parque Piraputangas I	19° 14' S	57° 38' W	new record	Corumbá
Parque Piraputangas II	19° 17' S	57° 37' W	new record	Corumbá
São João	19° 10' S	57° 32' W	new record	Ladário
Mutum	19° 12' S	57° 51' W	new record	Corumbá
Carandá	19° 06' S	57° 31' W	new record	Ladário
Banda Alta	19° 09' S	57° 34' W	new record	Ladário
Rabichão	19° 09' S	57° 31' W	new record	Ladário

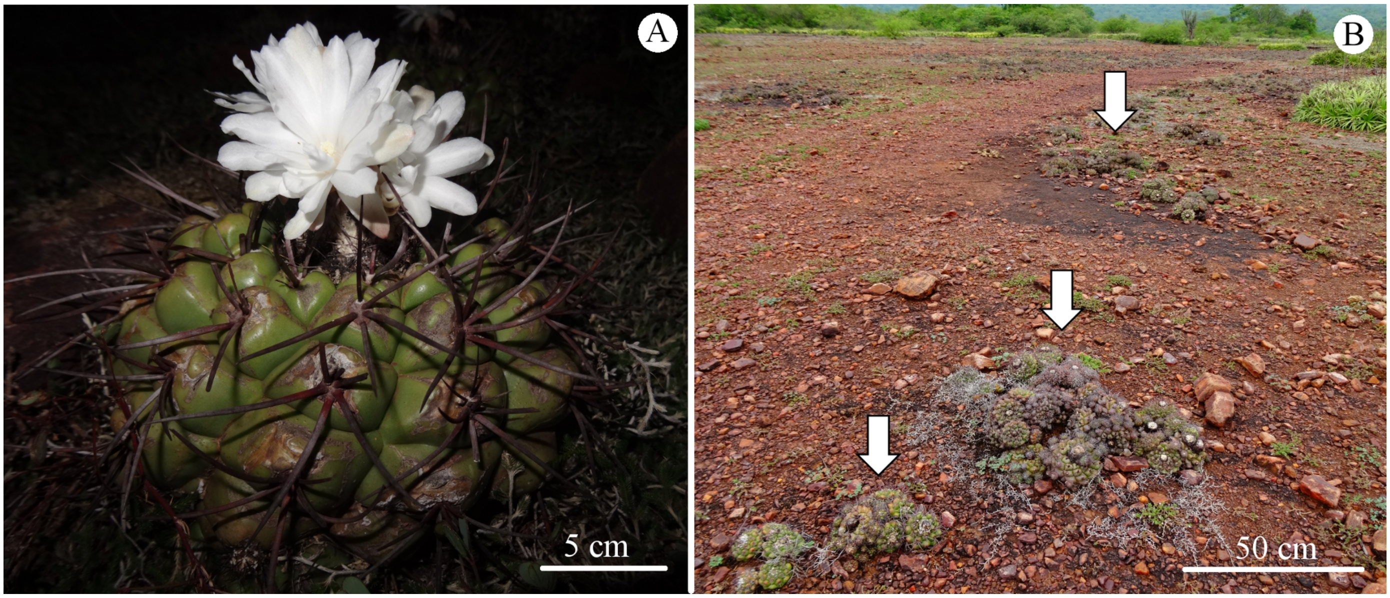

Figure 1. *Discocactus ferricola*. **A.** A closer look at one individual with opened nocturnal flowers. **B.** Flat ironstone outcrop area on the Vale do Paraíso farm showing the locally abundant population of *D. ferricola.* The arrows show aggregate individuals forming large clumps. The reddish color of the soil is typical of ironstone outcrops.

We used bioclimatic variables from the WorldClim database at a resolution of 30 arc sec (~1 km^2) (www.worldclim.org, Fick & Hijmans 2017) and the maximum entropy algorithm (MaxEnt v.3.4.1, Phillips et al. 2006) to model the potential niche distribution of *D. ferricola*. We used the area under the curve (AUC) receiver operating characteristics (ROC) to validate the output model from MaxEnt. We then used the maximum threshold value to produce a binary map and True Skill Statistics to evaluate its reliability (Allouche et al. 2006). Thus, we generated a final map that narrowed the searches for new populations in areas where *D. ferricola* would most likely occur. Four variables were used in the modeling: mean diurnal range (BIO2, mean monthly temperature (max temp - min temp)), mean temperature of the warmest quarter (BIO10), annual precipitation (BIO12), and precipitation of driest quarter (BIO17). The four variables were selected after a principal axis factor analysis with varimax rotation in a data set consisting of 19 bioclimatic variables, an elevation raster available from the database USGS (hydrosheds.cr.usgs.gov), and the Harmonized World Soil raster (www.fao.org, Fischer et al. 2008). We conducted analyses in R v.3.5.2 (R Core Team 2018) using the packages "vegan" (Oksanen et al. 2018), "raster" (Hijmans 2018), and "rgdal" (Nenzén & Araújo 2011, Porfirio et al. 2014, Bivand et al. 2018).

4. *Pollinator dependence*

We conducted four treatments on 46 flowers from 25 individuals to determine if *D. ferricola* depends on a pollinator for reproduction: (1) unmanipulated self-pollination (*n* = nine flowers) - the intact flower buds were bagged, and there was no additional manipulation; (2) manual self-pollination (*n* = 10 flowers) - intact flower buds were bagged, and as soon as the flowers opened, they were manually pollinated with their own pollen; (3) manual cross-pollination (*n* = 13 flowers) - intact flower buds were bagged, and as soon as the flowers opened, they were emasculated and pollinated with pollen grains from different individuals; and (4) natural pollination (*n* = 14 flowers) - flowers accessible to pollinators were marked as a control. Treatments were conducted on individuals from Vale do Paraíso Farm from November 2017 to February 2018.

We used generalized linear models (GLMs) with binomial and Poisson distributions to look for treatment effects regarding the probabilities of fruit formation and number of seeds per fruit, respectively. We determined overdispersion and model fit through residual analyses (Zuur et al. 2007, Mazerolle 2019). Because we found overdispersion in the GLM with a Poisson distribution, standard errors were corrected using the dispersion parameter in a quasi-GLM. We formulated two competitive models for each response variable (Tab. 2). We fitted models to the data and ranked them according to Akaike's information criterion with a second-order bias adjustment (AICc, Burnham & Anderson 2002, Anderson 2008). We used ΔAICc, the 95% confidence interval of the regressor, Akaike's weights (AICcW), and the evidence ratio to compare the competitive models (Burnham & Anderson 2002, Anderson 2008). We conducted analyses in R 3.5.2 (R Development Core Team) using the packages AICcmodavg (Mazerolle 2019), binom (Dorai-Raj 2015), bbmle (Bolker et al. 2020), and stats (Bolar 2019).

5. *Floral visitors*

We recorded floral visitors during field observations in the mornings (7 h-10 h; *n* = four days), afternoons (15 h-18 h; *n* = three days), and evenings (18 h-21 h; *n* = three days) for two populations: Vale do Paraíso and São João farms. We categorized species as floral visitors if they touched the reproductive parts of the flowers. Floral visitors were photographed and, whenever possible, were collected, preserved in 70% alcohol, and sent to specialists for identification. We also used a camera trap (Reconyx©) to record nocturnal pollinators. The camera was placed in two different locations on the Vale do Paraíso Farm for six days, totaling 144 hours of sampling effort.

Results

1. *Species distribution modeling*

We generated a map that shows areas of high climatic suitability for *D. ferricola* based on niche modeling through the maximum entropy algorithm (AUC = 0.97; Figure 2). The TSS on the maximum threshold indicated a good fit of the final model (TSS = 0.67). The model indicated areas with adequate climates for the occurrence of *D. ferricola* in small discontinuous areas of the Serra do Amolar and on some nearby hills, as well as in the Serra da Bodoquena (Figure 2A-B). However, the greatest areas of climatic suitability for *D. ferricola* were concentrated on the hills of RPMU, specifically on the Urucum, Grande, Rabichão, São Domingos, and Mutum hills (Figure 2B).

2. *Pollinator dependence*

Only the manual cross pollination and natural pollination treatments produced fruits and, therefore, were included in the statistical analyses. The mean probability of fruit formation for natural pollination was 57% (95% CI = 33% – 79%; *n* = 14 flowers resulting in eight fruits), while for manual cross pollination, it was 85% (95% CI = 57% – 87%; *n* = 13 flowers resulting in 11 fruits). Although the estimated probability of fruit formation varied according to treatments, our data did not allow us to distinguish between the null and the alternative hypothesis of a treatment effect. That result was clear from the small values of ΔAICc (< 2.0) and the evidence ratio being close to one (Tab. 2). Regarding the number of seeds produced per fruit, our data strongly supported the hypothesis that there were more seeds in fruits that were manually pollinized. Such a result was confirmed by the large ΔAICc of the constant model (>> 7.00) and the AICcW for the treatments model (Tab. 2). The estimated mean number of seeds per fruit through manual pollination was 43 (95% IC = 36 – 52; *n* = 11), which was 2.4 times the estimated mean number of 18 seeds produced per fruit through natural pollination (95% IC = 14 – 23; *n* = 8).

3. *Floral visitors*

During the mornings, we found small beetles from the Nitidulidae family covered in pollen inside closed flowers of *D. ferricola*, which had been open the night before (Figure 3A). During the afternoons, we observed that as soon as the flower buds of *D. ferricola* emerged from the cephalium, small beetles from the Nitidulidae family pierced and penetrated them (Figure 3B). *Discocactus ferricola* flowers opened at approximately 6 p.m. (*n* = seven days) and closed at approximately 2 a.m. (*n* = five days). During the nocturnal observations, we found the same beetle species as those observed during the mornings visiting opened flowers (Figure 3C). Moreover, we found larvae of the beetles on older flowers, i.e., those that had closed on the previous days. Other species of beetles belonging to the Chrysomelidae family were found visiting the flowers and mating inside them at night (Figure 3D). Finally, using camera traps, we recorded visits of moths to the *D. ferricola* flowers. We registered four moth visits between 7 p.m. and 1 a.m. on five nights of sampling (Figure 3E and 3F). Despite these attempts, it was not possible to capture the moth species during the observations.

Discussion

1. *Species distribution modeling*

In this study, we increased the number of known populations of *D. ferricola* from three to 11 populations. With the new occurrence sites, it was possible to construct a map of climatic suitability for the occurrence of *D. ferricola* based on ecological niche modeling. The area with the highest suitability identified by the model was concentrated mainly in the RPMU, which corroborates the high environmental specificity described for *Discocactus* species (Machado et al. 2005). Most of the area with high values of climatic suitability for *D. ferricola* in the RPMU is distributed continuously. Therefore, these areas of higher environmental suitability for *D. ferricola* may also represent possible dispersal routes among populations (Figure 2). We also observed small disconnected areas with climatic suitability for *D. ferricola* in the Serra do Amolar and on some nearby hills as well as in the Serra da Bodoquena. Currently, neither GBIF nor Species Link have records of *D. ferricola* at these localities, which may be the result of undersampling due to difficulty in accessing the areas of occurrence or even of a limited dispersion capacity of this species, preventing it from colonizing these regions even with an adequate climate for its occurrence. The potential distribution map highlighted areas with higher probability of occurrence of *D. ferricola* on the Residual Plateau of Maciço do Urucum, which concentrates the largest area with the highest climatic suitability among the analyzed rock formations, probably concentrating the largest number of populations. Due to the high environmental specificity of *D. ferricola*,

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Table 2. Model selection table for the probability of fruit formation and number of seeds per fruit in *Discocactus ferricola* for different reproduction treatments: natural pollination and manual cross-pollination. K: number of parameters; AICc: Akaike's information criterion with the second bias adjustment; AICcW: AICc weighted; *b* ± se: estimated beta ± standard deviations; 95% CI: 95% confidence interval for beta; ER: evidence ratio; ~ 1 (constant): model representing no differences between treatments; ~ Treatments: model representing the hypothesis of a treatment effect.

Response and models	K	ΔAICc	AICcW	*b* ± se	95% CI	ER
Fruit formation						
~ 1 (constant)	1	0.19	0.48	-	-	-
~ Treatments	2	0.00	0.52	-	-	1.1
Number of seeds*						
~ 1 (constant)	2	26.26	0.00	-	-	-
~ Treatments	3	0.00	1.00	0.89 ± 0.18	0.55 – 1.24	∞

* For the number of seeds, we used (Q)ΔAICc and (Q)AICcW. Therefore, we added one parameter (dispersion) to each model.

it is advisable that searches for new populations be carried out in areas of shallow soils, sandy or exposed rock within the areas described with relatively high values of climatic suitability to optimize time and resources (Machado et al. 2005).

Among the 11 populations sampled, only two populations of *D. ferricola* were found inside a protected area (Piraputanga Municipal Park), although the area has no maintenance, inspection or access restrictions. The other nine populations were on farms accessible to cattle and subject to fire or close to mining. Therefore, all known *D. ferricola* populations are exposed to threats like habitat loss, wildfires, and extraction for consumption as food or by collectors due to their ornamental potential. The available information about the distribution of *D. ferricola* in the Red List of Threatened Species states an area of occurrence of no more than 20 km^2 (Braun 2013). However, ironstone outcrops represent a total area of only 6.4 km^2 distributed irregularly on the RPMU (Pott et al. 2000). Thus, it is possible that the area of occurrence of *D. ferricola* is much less than 20 km^2 (Braun 2013).

2. *Pollinator dependence and floral visitors*

The absence of fruits in the self-pollination treatments may have been the result of a self-incompatible reproductive system or of extreme inbreeding depression (Mandujano et al. 2010). Despite of the cause, our results indicated that *D. ferricola* is obligatory xenogamic, and depends on the action of pollinators for effective pollination to occur. Reproductive mechanisms that act to prevent inbreeding and the consequent loss of genetic diversity are often found in species of the family Cactaceae (Mandujano et al. 2010). Xenogamy has been described for several species of cacti, including species that occur in rocky outcrops such as *Cipocereus minensis* (Werderm.) Ritter, *Uebelmannia buiningii* Donald, *Pilosocereus catingicola* (Gürke) Byles & Rowley, *P. chrysostele* (Vaupel) Byles & G.D.Rowley, and *P. pachycladus* F.Ritter (Martins et al. 2016, Teixeira et al. 2018, Rocha et al. 2020).

We found a lower number of seeds per fruit in the natural pollination treatment than in the manual pollination treatment, which suggests that the pollination function in *D. ferricola* may be inefficient due to a limitation in pollen deposition (Burd 1994, Larson & Barret 2000). This limitation could be caused by pollen of poor quality (flowers usually receive self-pollen, in addition to crossed pollen) or by the amount of pollen available (frequency of visits) (Aizen & Harder 2007). Pollen limitation is more frequent in self-incompatible species; in such cases, it is possible that the stochastic behavior of pollinators and local ecological conditions limit the activity of pollinators and are associated with reduced fertility (Burd 1994, Larson & Barret 2000). This may be the case of *D. ferricola* since we recorded only four moth visits, with flowers receiving no more than one visit, and because we found the species to be dependent on crossed pollen. Self-incompatibility and pollen limitation have also been described for *Discocactus pseudoinsignis* N.P.Taylor & Zappi and *Discocactus placentiformis* K.Schum., species that occur in Minas Gerais state, Brazil (Silveira 2015).

Flowers of *D. ferricola* were visited by two species of beetles, one from the Nitidulidae family and another from the Chrysomelidae family, and by moths from the Sphingidae family. The presence of beetles on flowers is often considered negative, as they usually act as pollen/nectar harvesters and as consumers of flowers (Pimienta-Barrios & del Castillo 2002, Martínez-Peralta & Mandujano 2011). Studies on other cactus species from outcrops, such as *Cipocereus laniflorus* N.P.Taylor & Zappi, *Pilosocereus catingicola* subsp. *salvadorensis* (Werderm.) Zappi, and *Micranthocereus purpureus* (Gürke) F.Ritter, have also found small beetles piercing flower buds serving as pollen robbers and consuming floral parts mainly during anthesis and postanthesis stages, as found for *D. ferricola* in this study (Locatelli et al. 1997, Pimienta-Barrios & Castillo 2002, Aona et al. 2006, Rego et al. 2012).

In this study, moths were the only registered nighttime visitors and they did it in a low frequency (four visits recorded from camera traps). Although we could confirm the introduction of month proboscis in the flowers, we could not affirm from photos if they touched the stigma. However, the moth likely touches the stigma due to its location within the tubular corolla and the sphingophilous syndrome (Pimienta-Barrios & del Castillo 2002, Machado et al. 2005). In addition, the time of anthesis in *D. ferricola* restricts visits from nocturnal pollinators, as the flowers are still closed during early evening. Some cacti that have nocturnal anthesis extend the anthesis period until the morning of the following day (Fleming et al. 2001), as observed by Silveira (2015) in *D. pseudoinsignis* and *D. placentiformis*, which keep flowers open until 11 a.m. of the next day, allowing visits from diurnal pollinators such as bees. Extending the anthesis period to diurnal hours increases the diversity of pollinators and may be related to greater reproductive success (Fleming et al. 2001).

Our study highlighted the benefits and practicalities of the use of ecological niche modeling and data on reproductive biology to guide new sampling efforts, identify threats, and to assist conservation and management initiatives of endangered species. Moreover, this

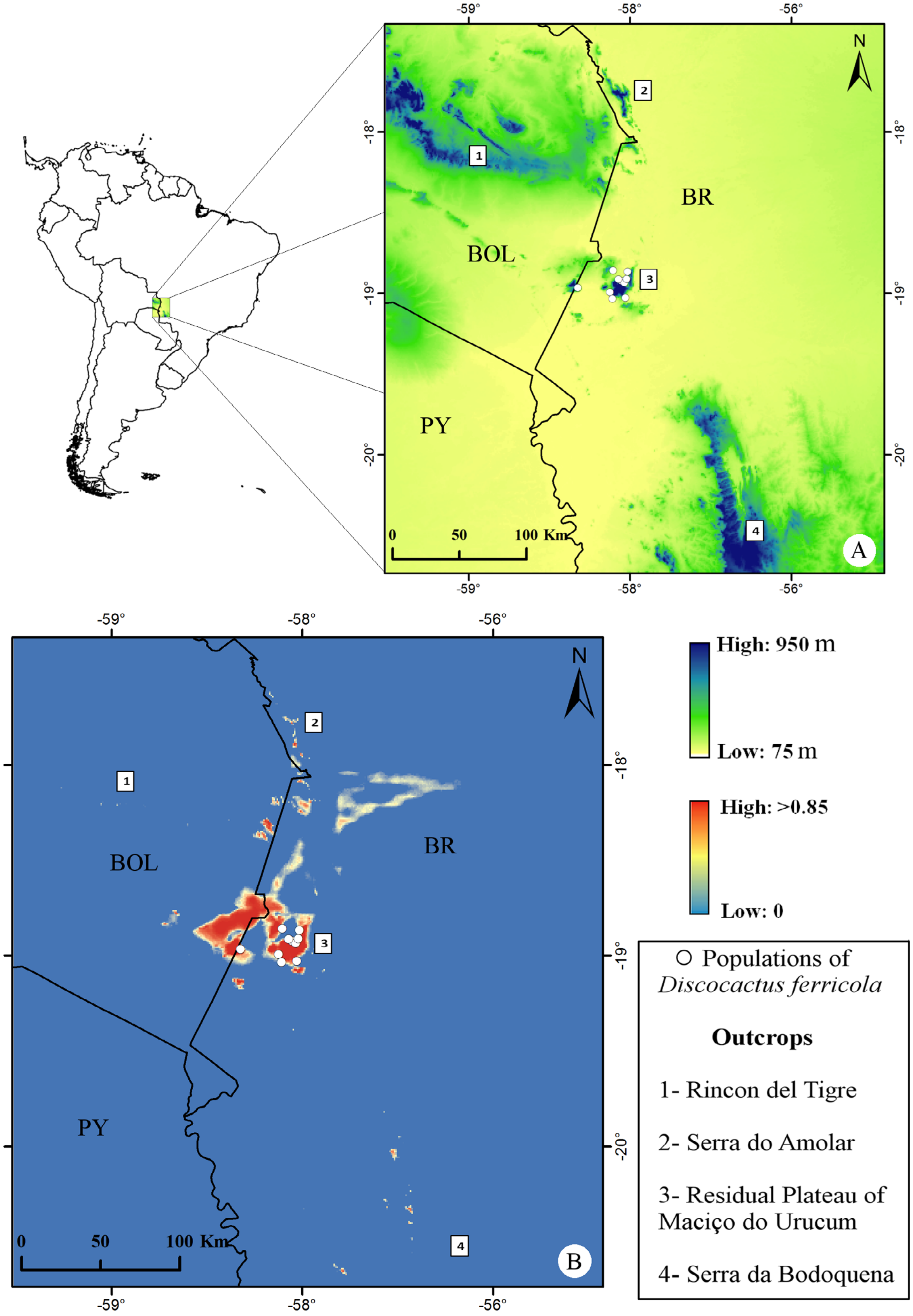

Figure 2. Geographic distribution of *Discocactus ferricola* populations. **A.** Elevation map of the area used in the modeling (background), showing the rocky outcrops of the region. **B.** Map of climatic suitability for *D. ferricola*.

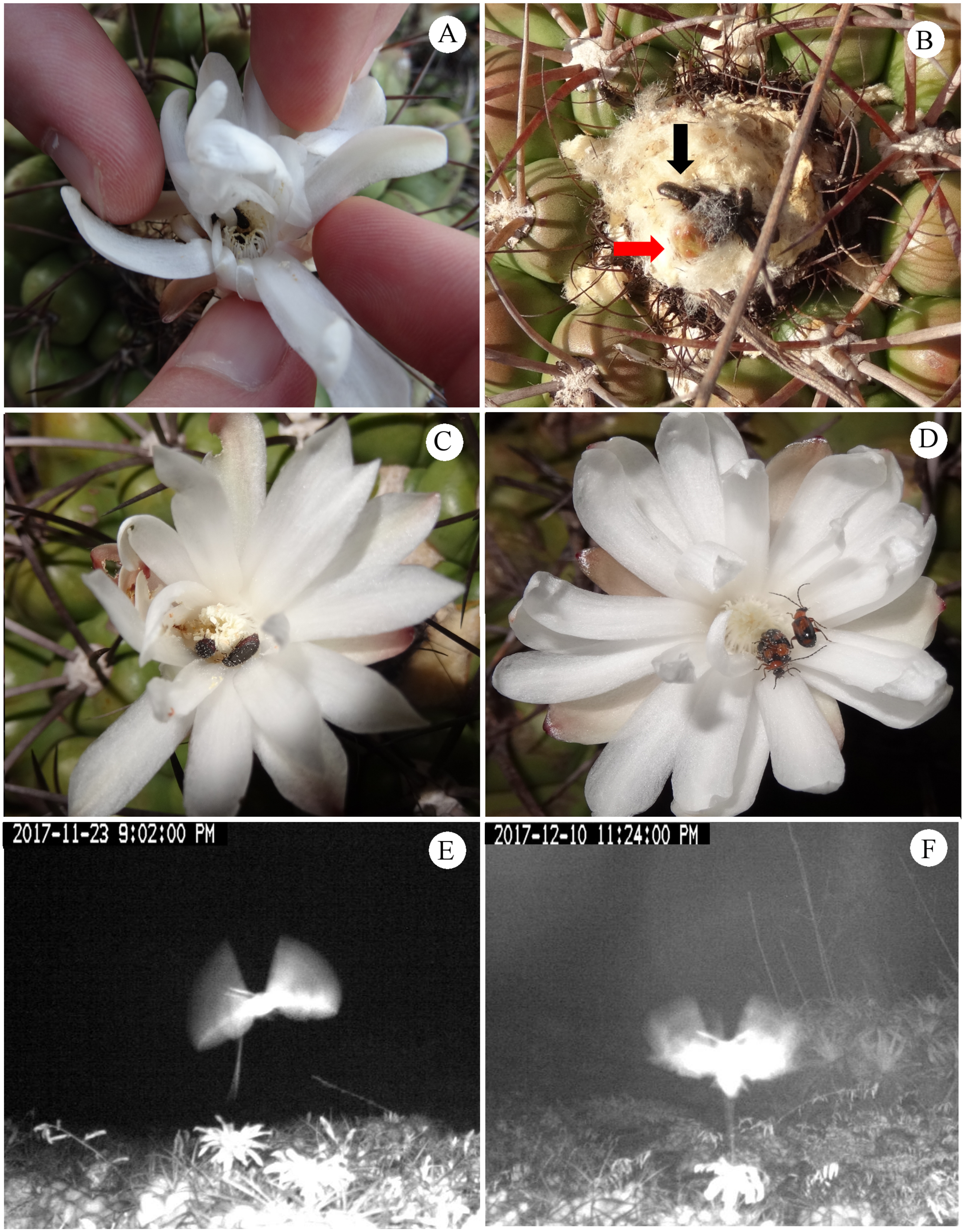

Figure 3. Pollinators recorded on *Discocactus ferricola* flowers. **A.** Beetles inside flowers after anthesis. **B.** Beetles piercing the flower buds. The black arrow points to the beetles, and the red arrow points to the floral bud starting to emerge from the cephalium. **C.** Beetles of the Nitidulidae family inside the open flowers. **D.** Beetles of the Chrysomelidae family inside the open flowers. **E-F.** Moths visiting flowers.

information allow the construction of more assertive hypotheses about the evolution and dispersion of species. The potential distribution map showed that the RPMU represents the largest and most important area of distribution of *D. ferricola*, concentrating the largest number of populations. We also found small disconnected areas with climatic suitability for *D. ferricola* in the Serra do Amolar and the Serra da Bodoquena, which represents potential areas for future sampling.

Strengthening public policies for *in situ* and *ex situ* conservation of threatened species of cactus should be in the spotlight of conservation plans. For *D. ferricola*, *in situ* conservation actions should prioritize the maintenance of interactions with pollinators by preserving flora and fauna of rocky outcrops and adjacent forests. For that, there is the need for revitalization and implementation of reserves, parks, and ecological corridors in areas of greater environmental suitability. As *ex situ* actions, we recommend the expansion of inspection activities by environmental authorities concerning the illegal trade of threatened and endemic species. Our approach and results may assist in the evaluation and implementation of more efficient conservation actions for rare and endangered species, especially for *D. ferricola*.

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Author Contributions

Luciana Vicente-Silva: Contributed equally to the concept and design of the study, manuscript preparation, data collection, data analysis, and interpretation.

Gabriel Paganini Faggioni: Contributed equally to data collection, critical review, data analysis, and interpretation.

Gecele Matos Paggi: Contributed equally to the concept and design of the study, critical revision, and 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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Article

Contribution of the Brazilian National Forest Inventory to the knowledge of Cerrado woody flora

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Abstract: The National Forest Inventory (Inventário Florestal Nacional-IFN) is a large initiative that uses standardised methods to survey Brazilian forestry resources. One target of the IFN is the Cerrado, which contains one of the richest floras in the world. The aim of this study was to assess the contribution of the IFN to the knowledge of Cerrado woody flora. We analysed data from field-collected vouchers sampled by the IFN Cerrado. We restricted our analyses to IFN collections of native trees and shrubs, including palms, which were identified at the species level. Habitat of each collection was obtained by overlaying specimens' geographic coordinates with land cover maps available in the Mapbiomas platform. Our final dataset comprised 28,602 specimens distributed in 2,779 sites (conglomerates) in Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo and Tocantins. Collections were located in the following habitats: savannas (40.5%), forests (30.2%), anthropic areas (25.6%), grasslands (3.5%), and water (0.2%). We recorded 1,822 species belonging to 543 genera and 105 families, representing 34% of Cerrado woody species recorded on Flora do Brasil 2020. Fabaceae had the largest number of species, while *Tapirira guianensis* and *Matayba guianensis* were the most collected species. We highlight 60 potentially new records of occurrence for several states and 64 new records for the Cerrado, primarily in riparian forests where species from other biomes occur. In addition, 232 recorded species are Cerrado endemics, while 36 are cited in the CNCFlora's red list as endangered. The systematic sampling carried out by the IFN enabled vegetation sampling in remote and poorly known areas, which expanded the geographic range of many woody species and contributed to the knowledge of plant diversity in the Cerrado.
Keywords: *conservation; endemism; plant diversity; sampling effort; survey; vegetation types.*

A contribuição do Inventário Florestal Nacional para o conhecimento da flora lenhosa do Cerrado

Resumo: O Inventário Florestal Nacional (IFN) é uma ampla iniciativa que emprega métodos padronizados para inventariar recursos florestais brasileiros. Um dos alvos do IFN é o Cerrado, o qual possui uma das floras mais ricas do mundo. O objetivo deste estudo foi avaliar a contribuição do IFN para o conhecimento da flora lenhosa do Cerrado. Nós analisamos dados de vouchers coletados em campo pelo IFN Cerrado. Nós restringimos nossas análises a coletas do IFN pertencentes a árvores e arbustos, incluindo palmeiras, identificadas ao nível de espécie. O habitat de cada coleta foi obtido pela intersecção entre as coordenadas geográficas dos espécimes com mapas de cobertura disponíveis na plataforma Mapbiomas. O conjunto final de dados foi composto por 28.602 coletas distribuídas em 2.779 sítios (conglomerados) localizados na Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo e Tocantins. As coletas foram realizadas nos seguintes habitats: savanas (40,5%), florestas (30,2%), áreas antrópicas (25,6%), campos (3,5%) e água (0,2%). Ao todo foram registradas 1.822 espécies pertencentes a 543 gêneros e 105 famílias, representando 34% das espécies lenhosas do Cerrado registradas na Flora do Brasil 2020. Fabaceae apresentou o maior número de espécies, enquanto que *Tapirira guianensis* e *Matayba guianensis* foram as espécies mais coletadas. Destacam-se possíveis novos registros de ocorrência de 60 espécies para diversos estados e de 64 espécies para o Cerrado, predominantemente nas florestas ripárias onde geralmente ocorrem espécies de outros biomas. Além disso, foram registradas 232 espécies endêmicas do Cerrado, bem como 36 espécies citadas na lista vermelha do CNCFlora como ameaçadas. A amostragem sistemática realizada pelo IFN permitiu o inventário da vegetação em áreas remotas e pouco coletadas, permitindo a expansão da distribuição geográfica de diversas espécies lenhosas, e contribuindo para o conhecimento da diversidade vegetal no Cerrado.

Palavras-chave: *conservação; endemismo; diversidade vegetal; esforço amostral; inventário; tipos de vegetação.*

Introduction

Brazil harbours one of the richest floras in the world, and it is home to a large number of endemics (BFG 2021). Recent advances took place after the compilation of an updated national checklist containing information on species descriptions and geographic distributions (Flora do Brasil 2020; http://floradobrasil.jbrj.gov.br). However, large gaps still exist in the documentation of plant diversity in Brazil. Such knowledge gaps are related to incomplete taxonomic knowledge ("Linnean shortfall"; Whittaker et al. 2005), as represented by a high number of new species being described every year in the country (BFG 2021). These gaps are also a product of unsatisfactory understanding of the geographic distribution of species ("Wallacean shortfall"), particularly in poorly sampled regions of the country (e.g., Sousa-Baena et al. 2014, Oliveira et al. 2016).

Since 2007, the Brazilian Forest Service (Serviço Florestal Brasileiro-SFB) has been coordinating a survey of Brazilian forest resources through the National Forest Inventory (Inventário Florestal Nacional-IFN). The IFN aims to provide data about forest structure, composition, vitality, biomass, wood and carbon stock (SFB 2020). This initiative seeks to support development policies and assist in the identification of strategies and opportunities for sustainable use, restoration and conservation of forest resources (SFB 2019, 2020). The IFN adopts a standardised sampling method applied to each Brazilian biome (Amazon, Atlantic Forest, Caatinga, Cerrado, Pantanal and Pampa; SFB 2017a). IFN systematic sampling generates a vast amount of scientific data, including thousands of plant occurrence records widely distributed across the country, mostly trees and shrubs. The compilation and analysis of such dataset would be a useful source of biodiversity information on Brazilian biomes that would help to increase floristic knowledge and support conservation planning. Although summary reports for some states have been published (https://www.florestal.gov.br/resultados), comprehensive analyses, including the large volume of plant diversity data sampled by the IFN surveys, are still missing for most states (but see Versieux et al. 2017 and Vibrans et al. 2020 for analyses of Rio Grande do Norte and Santa Catarina, respectively).

The Cerrado, which originally occupied around 23% of the Brazilian territory, is located in Central Brazil between two areas of wet forests, the Amazon and Atlantic Forest, and forms a dry corridor, together with the Caatinga, in the northeast and the Chaco in the southwest (Oliveira-Filho & Ratter 2002, Werneck et al. 2012). The Cerrado presents notable physiographic variation (e.g., Sano et al. 2019) and an associated number of vegetation types. These include grasslands, wetlands, savannas, and seasonally dry and wet forests (Ribeiro & Walter 2008), the occurrence of which depends on ecological factors at the local scale, such as soil fertility, water availability and fire regime (Bueno et al. 2018). Because of this complex mosaic of vegetation types, the Cerrado is a savanna-dominated biome with the richest flora in the world (Klink & Machado 2005). It has around 12,000 angiosperm species, including 40% endemics (Flora do Brasil 2020). However, the Cerrado has been highly threatened by deforestation, which has resulted in the loss of around 50% of its native vegetation (Alencar et al. 2020). Because of its species richness, high levels of endemism and anthropic pressure, the Cerrado is considered one of the 35 global biodiversity hotspots for conservation (Mittermeier et al. 2011). Its plant species extinctions are projected to increase as a consequence of habitat loss (Strassburg et al. 2017).

Considering the elevated rates of habitat loss and outstanding species richness, it is essential to increase knowledge about the Cerrado's rich and endangered flora through large-scale inventories. Such inventories are certain to result in new collections of herbarium specimens and occurrence data. This would, in turn, tackle both Linnean and Wallacean shortfalls (Whittaker et al. 2005) on plant diversity by increasing the number of specimens available for taxonomic work. It would also expand the geographic ranges of known species, particularly in poorly collected areas. Information provided by new inventories can help in the development of conservation strategies, identify priority areas for threatened species, and foster the discovery of new species.

Here, we carried out a data compilation of species occurrence records generated by the IFN Cerrado, seeking to assess the project's contribution to knowledge of the Cerrado's woody flora. It is expected that the systematic data survey and wide geographic breadth of the IFN will provide useful information on species geographical distribution in the Cerrado. Questions we intended to resolve herein are as follows: (1) How many species were recorded by the IFN? (2) What is the proportion of woody species surveyed by IFN compared to information available in the literature? (3) Were any species found and recorded for the first time for the Cerrado flora or for individual states? (4) How many endemic or endangered species were recorded?

Material and Methods

The Serviço Florestal Brasileiro provided the data collected by the IFN Cerrado, which followed a standardised methodology (SFB 2017b). Data were surveyed across most of the extension of the Cerrado biome (sensu Instituto Brasileiro de Geografia e Estatísticas; https://www.ibge.gov.br) in different habitats, except in indigenous lands, where plant collecting would demand special permissions. Surveys were carried out in sampling sites called conglomerates (0.4 ha) that were systematically distributed on a 20 km x 20 km grid that sampled different habitats, including various types of natural vegetation and also anthropic areas (e.g. pastures, agriculture). Each conglomerate was composed of four crosswise subunits formed by rectangles of 20 m x 50 m located 30 m from the conglomerate's centre. Sampling included all individuals with diameter at breast height (DBH) ≥ 10 cm, or diameter at 30 cm height from the soil (DSH) ≥ 10 cm in cases of adult individuals with height > 1.5 m, but DBH absent. Individuals with DBH ≥ 5 cm were measured in two smaller subplots (10 m x 10 m each). Individuals taller than 1.3 m, but with DBH/DSH < 5 cm, were sampled in two subplots (5 m x 5 m each). This sampling strategy was particularly designed for the IFN Cerrado in order to maximise sampling of savanna trees, which are normally stunted with twisted trunks. Individuals were assigned to morphotypes in the field and later received more accurate taxonomic identification. Detailed information on IFN Cerrado sampling methodology is provided in SFB (2017b).

The analyses presented here were based on the herbarium vouchers collected during field surveys carried out during the IFN Cerrado. Voucher specimens (sterile or fertile) representing species measured within conglomerates were collected in the field for identification in herbaria. Field teams were required to make a collection of any given species at least once every 15 conglomerates, including species readily identified in the field, as well as individuals with uncertain identification. Thus, specimens collected correspond to a subset of all individuals measured in IFN surveys, and are expected to comprise a representative sample of species surveyed. Conglomerates placed in areas harboring a richer flora (e.g., preserved forest) are expected to be more intensively collected than anthropic areas with fewer species (e.g., pastures). Extra collections not corresponding to sampled individuals within plots were also made. Appropriate information regarding collections, such as sampling unit, location, geographic coordinates, field name, habit and other observations, was annotated in a standardised form and later entered into a database. A total of 52,778 specimens collected were sent to the CEN, IBGE and UB herbaria for identification by generalist botanists and also by specialists in several families (Table S1, supplementary material). Specimens were databased, imaged, and finally incorporated into these collections. Duplicates were sent to other herbaria.

Our analysis included specimens collected in 2,779 conglomerates surveyed in ten states (Bahia, Distrito Federal, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Piauí, São Paulo and Tocantins) from 2011 to 2020. Field surveys and botanical identifications for Minas Gerais and São Paulo were not completed by the finalization of our analyses, and data for these states are partial. We restricted our analyses to only herbarium specimens (sterile and fertile) since these records are more reliable than unvouchered occurrence data that were also available from the IFN Cerrado dataset. We included only woody species, the main focus of the IFN, by retaining records belonging to species with life form reported as "shrub" or "tree", according to the Flora do Brasil 2020, including shrubby and arborescent palms. From the initial dataset of 52,778 specimens, we excluded 3071 records corresponding to herbs, subshrubs, lianas and bamboos. We also excluded 20,710 records identified only at family, genus or not determined, as well as those records identified at the species level, but with an ambiguous identification (indicated by "cf." or "aff."). Also, 323 records corresponding to exotic or introduced species, according to the Flora do Brasil 2020, were excluded. Infraspecific categories were treated at the species level. After this filtering process, the final dataset totalled 28,602 records of native woody plants identified at the species level (Table S2, supplementary material).

Species names were checked in the RStudio program, version 3.6.3 (RStudio Team 2020), by using the flora package (Carvalho 2017), the underlying database of which is the Flora do Brasil 2020. By the end of name checking, species names identified as synonyms were replaced by their respective accepted names. The geographic distribution of each species was verified based on information provided by Flora do Brasil 2020 (accessed through flora package) in order to identify possible new occurrence records for the Cerrado or for individual states. Species that only occur within the Cerrado in Flora do Brasil 2020 were listed here as Cerrado endemics. To identify endangered species collected by the IFN Cerrado, we consulted the CNCFlora Red list (National Centre for Plant Conservation; CNCFlora 2021).

To characterise the environmental variation of IFN Cerrado sampling sites, we analysed the different habitats in which each specimen was collected. The habitat of each collection was obtained by overlaying specimens' geographic coordinates with land cover maps available in the Mapbiomas platform (collection 6.0; Souza et al. 2020), which have a spatial resolution of 30 x 30 m that is compatible with the size of our sampling units (conglomerates). Original land use classes from Mapbiomes (see terms listed in parentheses) were merged into five major categories: grasslands ("formação campestre"), savannas ("formação savânica"), forests ("formação florestal"), anthropic areas ("pastagem", "agricultura", "mosaico de agricultura e pastagem", "silvicultura"), and water ("água"). A land use map for 2017 was downloaded from Mapbiomes as a shapefile, and habitat classes for each of the 28,602 occurrence records were retrieved using ArcGIS 10.3 (Environmental Systems Resource Institute).

We assessed the completeness of species richness recorded in the IFN Cerrado based on the sampling effort (Martins & Santos 1999). We verified sampling efficiency by using the rarefaction/extrapolation curve based on sample size (number of specimens) according to the Hill number (q=0) using the iNext package (Chao et al. 2014) in the RStudio program. We also compared the number of species in our dataset with the data available in Flora do Brasil 2020 to assess the representativeness of the woody flora sampled by the IFN Cerrado.

Results

Our clean dataset, composed of 28,602 specimens distributed in IFN's 2,779 conglomerates, covered most of the Cerrado (Figure 1). The number of specimens collected varied from one to 127 per conglomerate (average 10.3) with 68% of conglomerates presenting less than ten collections (Figure 2). The number of specimens was not equally distributed among states, with a higher incidence of collections in the central region of the Cerrado, especially in Goiás, and also in northern Maranhão (Figure 1). Collections were recorded in the following land use classes: savannas (40.5%), forests (30.2%), anthropic areas (25.6%), grasslands (3.5%), and water (0.2%).

We recorded a total of 1,822 woody species sampled by the IFN Cerrado. However, the rarefaction curve did not reach stability (Figure 3), suggesting that increasing sampling would result in recording additional species. Species numbers varied among states (Table 1) with the highest numbers in Goiás (735), followed by Mato Grosso (683), Maranhão (647), Tocantins (563), Bahia (513), Mato Grosso do Sul (382), São Paulo (314), Piauí (291), Distrito Federal (190), and Minas Gerais (132). Most species were found in savannas (1,204 species) and forests (1,200), followed by anthropic areas (891), grasslands (373), and water (130).

Species recorded belong to 543 genera and 105 families, including two *Podocarpus* gymnosperm species (see Table S3 for full species list, authorship, habit, number of collections and distribution). Among families collected, the top ten presenting the highest number of species were Fabaceae (336 species), Rubiaceae (85), Myrtaceae (78), Malvaceae (72), Melastomataceae (65), Euphorbiaceae (63), Asteraceae (60), Sapotaceae (49), Malpighiaceae (47) and Annonaceae (46). These families represented 47.2% of specimens and contributed to 49.6% of species.

The number of specimens collected per species varied widely with 29% of species (528) represented by a single collection (Figure 4). The most collected species were *Tapirira guianensis* (374 specimens), *Matayba guianensis* (305), *Vatairea macrocarpa* (290), *Myrcia splendens* (269), *Diospyros lasiocalyx* (249), *Machaerium acutifolium* (220), *Qualea parviflora* (215), *Terminalia argentea* (207), *Pouteria ramiflora* (206) and *Callisthene fasciculata* (204). All these species are typically found in savannas and forests within the Cerrado, including the widespread riparian forest species *Tapirira guianensis*. The IFN Cerrado contributed

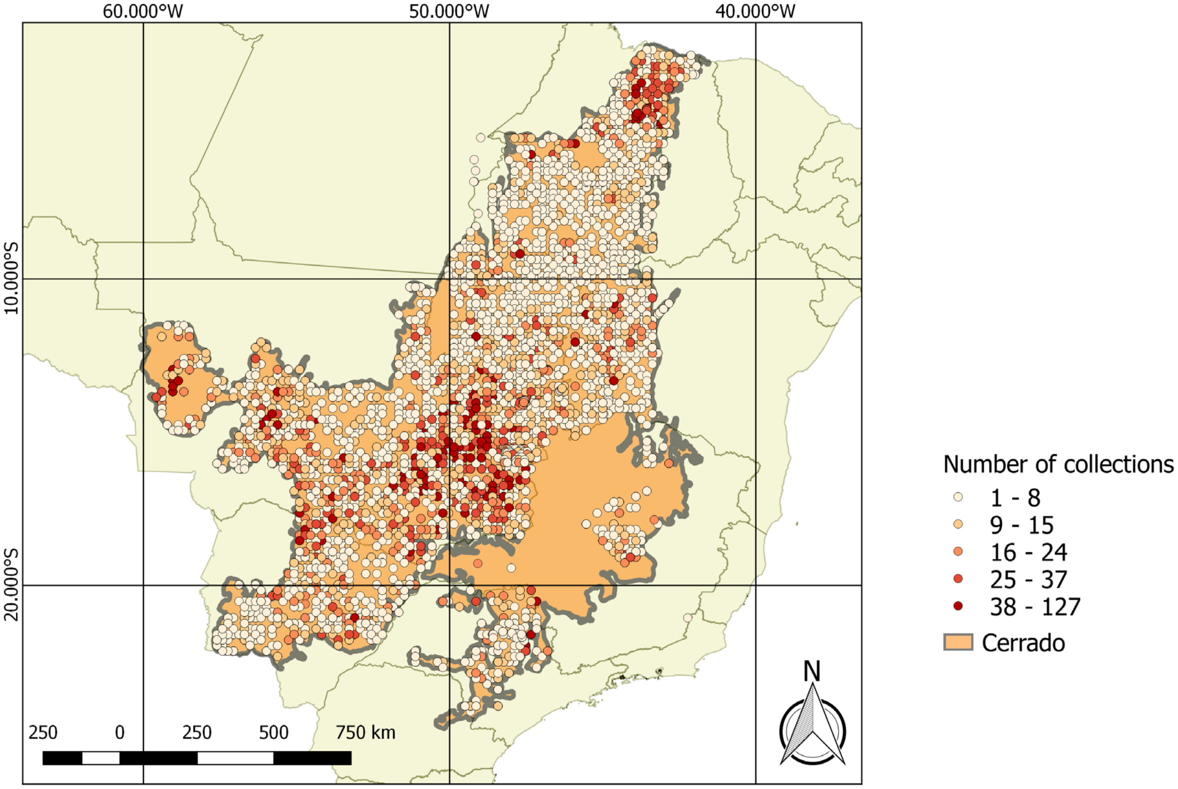

Figure 1. Geographic distribution of 2779 conglomerates (sampling sites) within the Cerrado showing the number of collections of woody plants identified at species level per conglomerate. The state of Minas Gerais was only partially sampled. Indigenous lands were not sampled, which correspond to large areas in Mato Grosso and Tocantins.

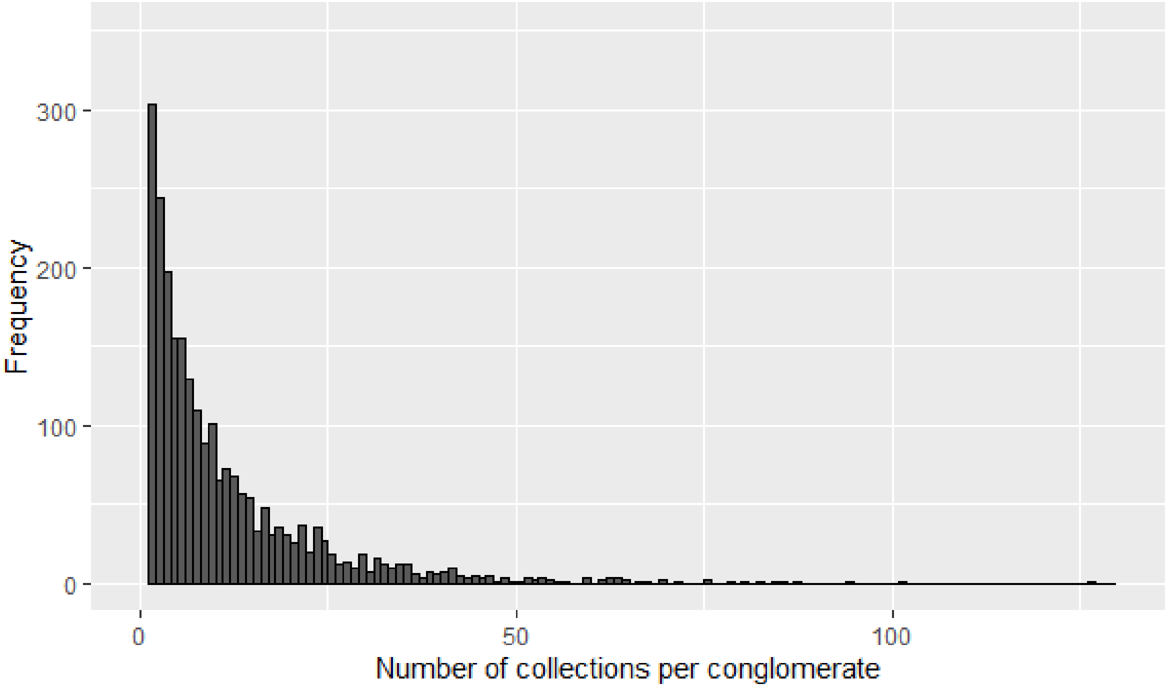

Figure 2. Histogram showing the number of collections of woody plants per sampling unit (conglomerate; 0.4 ha) in the IFN Cerrado. Among the 2779 conglomerates sampled, most were represented by less than ten collections.

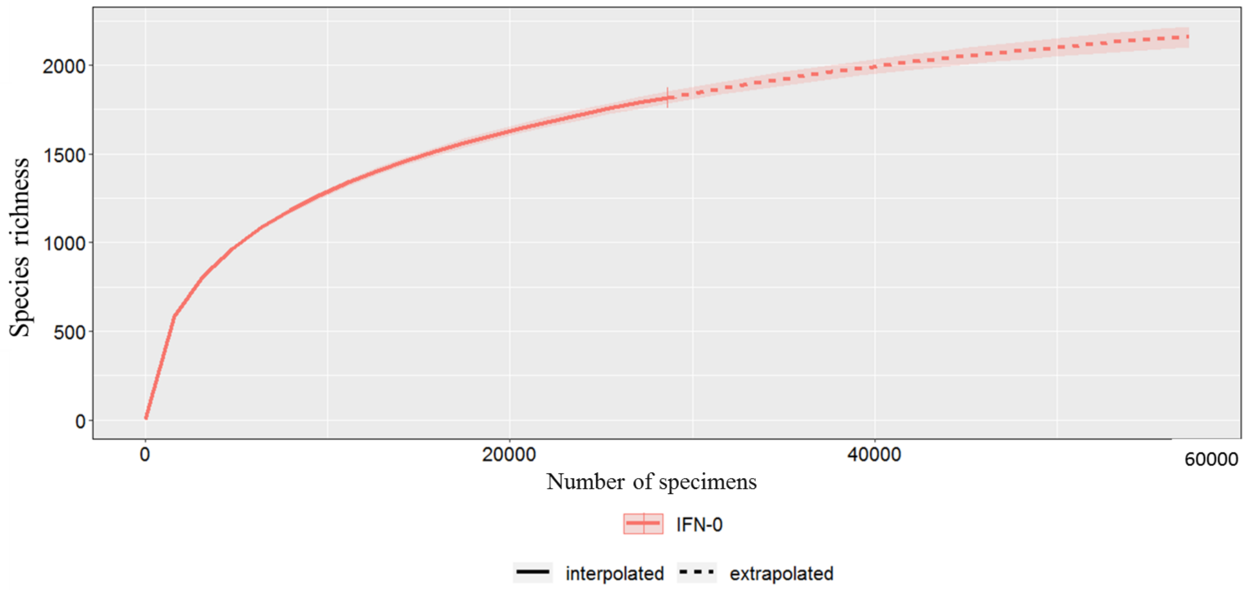

Figure 3. Rarefaction/extrapolation curve of richness of woody species based on the number of specimens (28,602) collected by the IFN Cerrado. Extrapolation of species richness is represented, considering a doubling of sampling effort.

Table 1. IFN Cerrado summary statistics by state. The total sampled area is the sum of sampled conglomerates (sampling units), each one with 0.4 ha. Figures are based on herbarium specimens belonging to woody species identified at the species level. The average of specimens is presented followed by its respective standard deviation. Habitat: G = grasslands, S = savannas, F = forests, A = anthropic areas, W = water.

State	Number of conglomerates	Total sampled area (ha)	Specimens collected	Average of specimens per conglomerate	Total number of species	Number of collected specimens per habitat (G/S/F/A/W)
Bahia	249	99.6	1,964	7.9 ± 8.2	513	122/1,551/137/100/54
Distrito Federal	34	13.6	275	8.1 ± 7.2	190	3/154/83/34/1
Goiás	641	256.4	10.618	16.6 ± 17.2	735	263/3,755/2,719/3,870/11
Maranhão	403	161.2	3,891	9.7 ± 11.2	647	80/1,276/2,157/336/42
Mato Grosso	446	178.4	4,625	10.4 ± 10.7	683	113/2,050/1,544/888/30
Mato Grosso do Sul	290	116.0	2,418	8.3 ± 9.5	382	57/519/993/841/8
Minas Gerais	56	22.4	419	7.5 ± 7.3	132	39/95/29/256/0
Piauí	148	59.2	751	5.1 ± 4.4	291	52/574/100/16/9
São Paulo	110	44.0	949	8.6 ± 9.4	314	14/49/393/465/28
Tocantins	402	160.8	2,692	6.7 ± 7.3	563	245/1,566/484/396/1
Total	2,779	1,111.6	28,602	10.3 ± 12.2	1,822	988/11,589/8,639/7,202/184

potentially new occurrence records for the Cerrado (64 species) and individual states (60), such as Tocantins (18), Mato Grosso do Sul (16), Goiás (11), and Maranhão (ten). Furthermore, 233 Cerrado endemics were recorded, as well as 36 endangered species (Table 2).

Discussion

Botanical collections made during IFN Cerrado surveys covered a broad geographic range that included poorly collected regions. However, the number of specimens collected varied greatly among sampling units (conglomerates) and states. The unbalanced number of collections per conglomerate could have been influenced by the number of species present within each conglomerate. For example, a conglomerate placed in a diverse, well-preserved forest is likely to produce more collections than a conglomerate situated in pastureland wherein only one or a few tree species occur. In addition, we observed that sampling effort varied among the field teams that conducted surveys in different regions within the Cerrado. Although all field teams are supposed to follow the same sampling protocol, we found that some teams were more likely to produce more collections per conglomerate than others. For example, sampling in Piauí averaged only 5.1 specimens per conglomerate, while in Goiás, this number was more than three times higher (16.6). Therefore, it is likely that differential sampling effort among regions may have biased our results. We recommend for future IFN surveys that field teams should increase the number of collections, assuring that a representative sample of the flora within each conglomerate is

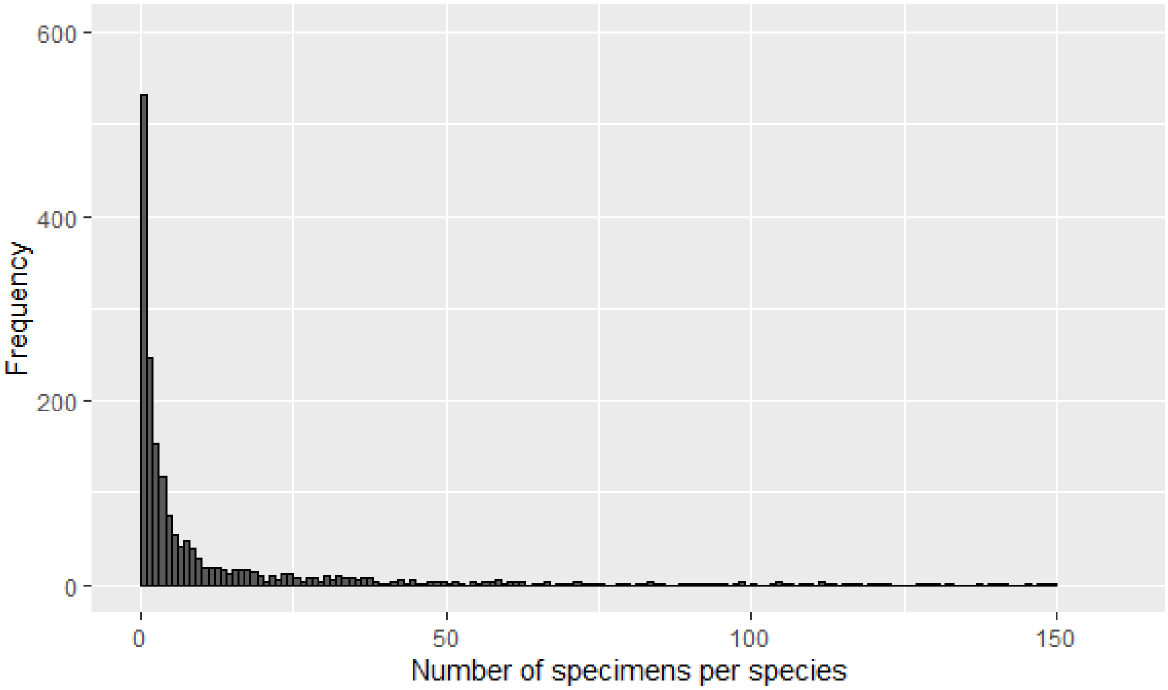

Figure 4. Histogram showing species frequency per range of number of collections made by the IFN Cerrado. Among the 1,822 species recorded, most were collected only once or twice, while 67 species are represented by more than 100 specimens.

represented by a set of herbarium specimens. This could be achieved by demanding field teams to collect a minimum number of vouchers per conglomerate, considering the peculiarities of each vegetation type. Such measures would reduce discrepancies in the number of specimens between conglomerates, resulting in a more even collecting effort across different regions. Increasing collecting effort would be particularly important for highly diverse sites where species identification tends to be more problematic (e.g., wet forests).

We listed a total of 1,822 woody species collected by the IFN Cerrado, which corresponds to 34% of the 5,373 woody species native to the Cerrado in the Flora do Brasil 2020 repository. These statistics indicate that the survey was not able to sample the rich Cerrado plant diversity in its entirety. It is likely that unsampled species are rare, i.e., having restricted geographic range and/or low abundance, since the most frequent species in the various habitats were sampled in the IFN Cerrado. The rarefaction curve clearly showed that additional collections would result in higher species richness. Doubling sampling effort would result in an estimated 2,380 species. However, we must recognise that a considerable number of collected specimens (20,806 or 39.4%) were not identified at species level. Improving the identification of these specimens would certainly result in an increased number of species sampled by the IFN Cerrado. Likewise, the completion of the survey in Minas Gerais would have also contributed to an increase in IFN Cerrado species numbers.

Accurate identification of specimens at the species level is clearly a challenge for many large and taxonomically complex plant families (e.g., Fabaceae, Lauraceae and Myrtaceae), and reliable identifications often require samples of fruits or flowers. The challenge of naming species is particularly difficult when identifications are based on sterile specimens, which make up 86.4% of the IFN Cerrado specimens analysed here. In many cases, we recognise that an accurate determination of sterile material at the species level is not possible, even for experienced taxonomists. Uncertainties underlying plant identifications pose a limitation to forest inventories in species-rich countries, and improving this situation demands innovative approaches (Drapper et al. 2020) that could be adopted by the IFN. Compared to our results, sampling efficiency of floristic diversity was apparently higher in the IFN survey carried out in Santa Catarina where 831 species of trees and shrubs were found (Vibrans et al. 2020). However, despite all collecting efforts, a considerable number (150 species) of trees and shrubs reported for Santa Catarina in previous studies remained unsampled by that survey.

In general, the most frequently collected species in the IFN Cerrado are among the most common species cited in the literature for the different vegetation types of the Cerrado. Therefore, we can assume that an elevated number of collections for a given species reflects its high natural abundance. For example, among the 38 species considered the most frequent (oligarchic) in the *cerrado sensu lato* (Ratter et al. 2003), all were recorded by the IFN Cerrado, including some highly collected (>100 records) species, such as *Agonandra brasiliensis, Bowdichia virgilioides, Byrsonima coccolobifolia, Connarus suberosus, Machaerium acutifolium, Myrcia splendens, Plathymenia reticulata, Pouteria ramiflora, Qualea grandiflora, Q. parviflora, Terminalia argentea* and *Vatairea macrocarpa*. Typical species of "*cerradão*", a forest formation composed of mostly savanna tree species, such as *Callisthene fasciculata, Emmotum nitens, Lafoensia pacari* and *Magonia pubescens* (Ribeiro & Walter 2008), were also highly collected in the IFN Cerrado.

Wet forests in the Cerrado are often associated with water courses. Among the species frequently found in Cerrado's riparian forests, several were highly collected in the IFN Cerrado, such as *Tapirira guianensis*, which was the most collected species in the whole survey, as well as *Calophylllum brasiliense, Chysophyllum marginatum,*

Table 2. Selected species collected by the IFN Cerrado highlighting those of high conservation value (threatened and endemics), as well as potential new occurrence records for states. Cerrado endemic species and new records of occurrence are based on information available in Flora do Brasil 2020. BA = Bahia, DF = Distrito Federal, GO = Goiás, MA = Maranhão, MG = Minas Gerais, MS = Mato Grosso do Sul, MT = Mato Grosso, PI = Piauí, SP = São Paulo, TO = Tocantins. Endangered species listed by the CNCFlora according to the categories of the International Union for Conservation of Nature (IUCN): CR = critically endangered, EN = endangered, VU = vulnerable.

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Acanthaceae				
Justicia nodicaulis (Nees) Leonard	X			
Annonaceae				
Duguetia calycina Benoist		X		
Guatteria rigida R.E.Fr.			MS	
Oxandra reticulata Maas			MS	
Trigynaea duckei (R.E.Fr.) R.E.Fr.		X		
Xylopia discreta (L.f.) Sprague		X		
Apocynaceae				
Aspidosperma dispermum Müll.Arg.	X			
Aspidosperma melanocalyx Müll.Arg.			MS	
Aspidosperma spruceanum Benth. ex Müll.Arg.		X		
Aspidosperma rizzoanum Scudeler & A.C.D. Castello	X			
Aspidosperma verbascifolium Müll.Arg.	X			
Rauvolfia weddelliana Müll.Arg.	X			
Araliaceae				
Dendropanax denticulatus Fiaschi		X		
Didymopanax macrocarpus (Cham. & Schltdl.) Seem.			MA, TO	
Didymopanax vinosus (Cham. & Schltdl.) Marchal			TO	
Arecaceae				
Euterpe edulis Mart.				VU
Asteraceae				
Acilepidopsis echitifolia (Mart. ex DC.) H.Rob.	X			
Chromolaena chaseae (B.L.Rob.) R.M.King & H.Rob.	X			
Chromolaena myriocephala (Gardner) R.M.King & H.Rob.	X			
Chromolaena pungens (Gardner) R.M.King & H.Rob.	X			
Eremanthus brasiliensis (Gardner) MacLeish	X			
Eremanthus glomerulatus Less.	X			
Eremanthus goyazensis (Gardner) Sch.Bip.	X			
Eremanthus mollis Sch.Bip.	X			
Eremanthus uniflorus MacLeish & H.Schumach.	X			
Lepidaploa muricata (DC.) H.Rob	X			
Lepidaploa remotiflora (Rich.) H.Rob.	X			
Lepidaploa rufogrisea (A.St.-Hil.) H.Rob.	X			
Lessingianthus brevipetiolatus (Sch.Bip. ex Baker) H.Rob.	X			
Lessingianthus floccosus (Gardner) H.Rob.	X			
Lessingianthus ligulifolius (Mart. ex DC.) H.Rob.	X			
Lessingianthus myrsinites H.Rob.	X			
Lessingianthus obscurus (Less.) H.Rob.	X			
Lessingianthus obtusatus (Less.) H.Rob.	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Lessingianthus zuccarinianus (Mart. ex DC.) H.Rob.	X			VU
Moquiniastrum barrosoae (Cabrera) G.Sancho	X			
Moquiniastrum blanchetianum (DC.) G.Sancho	X			
Moquiniastrum floribundum (Cabrera) G.Sancho	X			
Moquiniastrum paniculatum (Less.) G.Sancho	X			
Piptocarpha oblonga (Gardner) Baker	X		MS	
Piptocarpha rotundifolia (Less.) Baker	X			
Strophopappus glomeratus (Gardner) R.Esteves	X			
Vernonanthura ferruginea (Less.) H.Rob.	X			
Vernonanthura membranacea (Gardner) H.Rob.	X			
Wunderlichia crulsiana Taub.				EN
Bignoniaceae				
Anemopaegma arvense (Vell.) Stellfeld ex de Souza				EN
Fridericia cinerea (Bureau ex K.Schum.) L.G.Lohmann	X			
Handroanthus spongiosus (Rizzini) S.Grose				EN
Jacaranda copaia (Aubl.) D.Don		X		
Jacaranda grandifoliolata A.H.Gentry	X			EN
Jacaranda ulei Bureau & K.Schum.	X			
Xylophragma heterocalyx (Bureau & K.Schum.) A.H.Gentry	X			
Zeyheria tuberculosa (Vell.) Bureau ex Verl.				VU
Burseraceae				
Protium rhoifolium (Benth.) Byng & Christenh.		X	MA	
Calophyllaceae				
Kielmeyera grandiflora (Wawra) Saddi	X			
Kielmeyera lathrophyton Saddi			MA	
Kielmeyera neriifolia Cambess.	X			
Kielmeyera petiolaris Mart. & Zucc.	X			
Kielmeyera rubriflora Cambess.	X		PI	
Kielmeyera speciosa A.St.-Hil.	X			
Kielmeyera tomentosa Cambess.	X			
Cannabaceae				
Celtis fluminensis Carauta		X		
Caricaceae				
Jacaratia corumbensis Kuntze	X			
Caryocaraceae				
Caryocar brasiliense Cambess.	X			
Caryocar cuneatum Wittm.	X			
Celastraceae				
Monteverdia acanthophylla (Reissek) Biral				VU
Monteverdia gonoclada (Mart.) Biral			PI	
Monteverdia guyanensis (Klotzsch ex Reissek) Biral		X		
Salacia crassifolia (Mart. ex Schult.) G.Don	X			
Chrysobalanaceae				
Couepia ovalifolia (Schott) Benth. ex Hook.f.		X		
Exellodendron gardneri (Hook.f.) Prance	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Hirtella hoehnei Pilg.	X		TO	
Leptobalanus parvifolius (Huber) Sothers & Prance		X		
Licania canescens Benoist		X		
Licania coriacea Benth.		X		
Licania gracilipes Taub.		X		
Licania nitida Hook.f.			MS, TO	
Clusiaceae				
Clusia criuva Cambess.	X			
Clusia renggerioides Planch. & Triana		X		
Symphonia globulifera L.f.			GO	
Combretaceae				
Combretum pyramidatum Ham.			GO	
Terminalia phaeocarpa Eichler	X			
Connaraceae				
Connarus suberosus Planch.	X			
Rourea induta Planch.	X			
Cunoniaceae				
Lamanonia brasiliensis Zickel & Leitão	X		GO	EN
Dilleniaceae				
Davilla elliptica A.St.-Hil.	X			
Davilla grandiflora A.St.-Hil. & Tul.	X			
Davilla lacunosa Mart.	X			
Davilla villosa Eichler	X			
Ebenaceae				
Diospyros coccolobifolia Mart. ex Miq.	X			
Diospyros tetrandra Hiern		X		
Elaeocarpaceae				
Sloanea subsessilis D.Sampaio e V.C.Souza	X			
Erythroxylaceae				
Erythroxylum argentinum O.E.Schulz		X		
Erythroxylum ayrtonianum Loiola & M.F.Sales			GO	
Erythroxylum betulaceum Mart.			MS	
Erythroxylum tianguanum Plowman				CR
Erythroxylum tortuosum Mart.	X			
Erythroxylum umbu Costa-Lima		X		
Euphorbiaceae				
Bernardia gardneri Müll.Arg.	X			
Croton micans Sw.	X			
Manihot anomala Pohl	X			
Manihot caerulescens Pohl	X			
Manihot cecropiifolia Pohl	X			
Manihot tripartita (Spreng.) Müll.Arg.	X			
Manihot triphylla Pohl	X			
Sapium laurifolium (A.Rich.) Griseb.		X		
Fabaceae				

Continued...

Family/ species	Cerrado Endemic	New records (Cerrado)	New records (states)	IUCN category
Abarema cochliacarpos (Gomes) Barneby & J.W.Grimes			GO	
Andira cordata Arroyo ex R.T.Penn. & H.C.Lima	X		MS	
Andira inermis (W.Wright) DC.			TO	
Apuleia leiocarpa (Vogel) J.F.Macbr.				VU
Bauhinia dumosa Benth.	X			
Bauhinia holophylla (Bong.) Steud.	X			
Bauhinia membranacea Benth.	X			
Bauhinia rufa (Bong.) Steud.	X			
Calliandra dysantha Benth.	X			
Calliandra silvicola Taub.	X			
Cassia fastuosa Willd. ex Benth.		X		
Cenostigma bracteosum (Tul.) Gagnon & G.P.Lewis			MS	
Chamaecrista acosmifolia (Mart. ex Benth.) H.S.Irwin & Barneby	X			
Chamaecrista ciliolata (Benth.) H.S.Irwin & Barneby	X		BA	
Chamaecrista claussenii (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista conferta (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista coradinii H.S.Irwin & Barneby	X			VU
Chamaecrista crenulata (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista desvauxii (Collad.) Killip	X			
Chamaecrista geminata (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista isidorea (Benth.) H.S.Irwin & Barneby	X			
Chamaecrista ramosa (Vogel) H.S.Irwin & Barneby	X			
Copaifera depilis Dwyer	X			
Copaifera luetzelburgii Harms	X			
Copaifera magnifolia Dwyer	X			
Copaifera malmei Harms	X			
Copaifera oblongifolia Mart. ex Hayne	X			
Copaifera sabulicola J.Costa & L.P.Queiroz	X			
Cratylia mollis Mart. ex Benth.		X		
Dalbergia cuiabensis Benth.	X			
Dalbergia elegans A.M.Carvalho				VU
Dalbergia glandulosa Benth.	X			
Dalbergia miscolobium Benth.	X			
Dalbergia nigra (Vell.) Allemão ex Benth.				VU
Dimorphandra gardneriana Tul.			MS	
Dipteryx alata Vogel	X			
Diptychandra aurantiaca Tul.	X			
Enterolobium timbouva Mart.			TO	
Guibourtia chodatiana Hassl.	X			
Harpalyce brasiliana Benth.	X			
Harpalyce magnibracteata São -Mateus, D.B.O.S.Cardoso & L.P.Queiroz	X			
Harpalyce minor Benth.	X			
Hymenaea maranhensis Lee & Lang.	X			
Hymenaea parvifolia Huber				VU
Hymenolobium heringerianum Rizzini	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Leptolobium elegans Vogel	X		DF	
Luetzelburgia praecox (Harms) Harms	X			
Machaerium nigrum Vogel		X		
Machaerium opacum Vogel			MS	
Machaerium scleroxylon Tul.			TO	
Machaerium ternatum Kuhlm. & Hoehne		X		
Melanoxylon brauna Schott				VU
Mimosa claussenii Benth.	X			
Mimosa decorticans Barneby	X			
Mimosa densa Benth.	X			
Mimosa dichroa Barneby ex G.P.Lewis	X		MS	
Mimosa gardneri Benth.	X			
Mimosa gemmulata Barneby			MS	
Mimosa hapaloclada Malme	X			
Mimosa hebecarpa Benth.	X			
Mimosa hypoglauca Mart.	X			
Mimosa insignis (Hassl.) Barneby	X			
Mimosa interrupta Benth.	X			
Mimosa kalunga M.F.Simon & C.E.Hughes	X			
Mimosa laniceps Barneby	X			
Mimosa laticifera Rizzini & A.Mattos	X			
Mimosa melanocarpa Benth.	X			
Mimosa nitens Benth.	X			
Mimosa nothopteris Barneby		X		
Mimosa oedoclada Barneby	X			
Mimosa oligosperma Barneby	X			EN
Mimosa somnians Humb. & Bonpl. ex Willd.	X			
Mimosa xanthocentra Mart.	X			
Muellera montana (MJ.Silva & AMG.Azevedo) MJ.Silva & AMG. Azevedo	X			
Myrocarpus frondosus Allemão		X		
Ormosia coarctata Jacq.		X		
Peltogyne maranhensis Huber ex Ducke				VU
Schizolobium parahyba (Vell.) Blake		X		
Stryphnodendron fissuratum E.M.O.Martins	X			
Stryphnodendron polyphyllum Mart.	X			
Swartzia laurifolia Benth.		X		
Tachigali aurea Tul.	X			
Tachigali rubiginosa (Mart. ex Tul.) Oliveira-Filho	X			
Tachigali subvelutina (Benth.) Oliveira-Filho	X			
Zapoteca scutellifera (Benth.) H.M.Hern.			GO, TO	
Hypericaceae				
Vismia macrophylla Kunth		X		
Lacistemataceae				
Lacistema hasslerianum Chodat	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Lamiaceae				
Hypenia calycina (Pohl ex Benth.) Harley	X			
Hypenia macrosiphon (Briq.) Harley	X			
Hyptidendron arbusculum (Epling) Harley	X			
Hyptidendron canum (Pohl ex Benth.) Harley	X			
Hyptidendron caudatum (Epling & Játiva) Harley	X			
Hyptidendron conspersum (Benth.) Harley	X			EN
Hyptidendron leucophyllum (Pohl ex Benth.) Harley	X			
Hyptis lutescens Pohl ex Benth.	X			
Hyptis pachyphylla Epling	X			VU
Hyptis rubiginosa Benth.	X			
Hyptis saxatilis A.St.-Hil. ex Benth.	X			
Medusantha multiflora (Pohl ex Benth.) Harley & J.F.B.Pastore			MA	
Mesosphaerum pectinatum (L.) Kuntze			MA	
Vitex flavens Kunth	X			
Lauraceae				
Aiouea macedoana Vattimo-Gil	X			
Aniba hostmanniana (Nees) Mez		X		
Aniba williamsii O.C.Schmidt		X		
Dicypellium caryophyllaceum (Mart.) Nees,				CR
Endlicheria lhotzkyi (Nees) Mez	X			
Nectandra warmingii Meisn.	X			
Ocotea leucoxylon (Sw.) Laness.		X		
Persea splendens Meisn.	X			
Lecythidaceae				
Cariniana legalis (Mart.) Kuntze				EN
Couroupita guianensis Aubl.		X		
Eschweilera grandiflora (Aubl.) Sandwith		X		
Eschweilera parviflora (Aubl.) Miers		X		
Loganiaceae				
Antonia ovata Pohl			MS	
Lythraceae				
Diplusodon virgatus Pohl	X			
Lafoensia pacari A.St.-Hil.	X			
Malpighiaceae				
Banisteriopsis argyrophylla (A.Juss.) B.Gates	X			
Banisteriopsis latifolia (A.Juss.) B.Gates	X			
Banisteriopsis malifolia (Nees & Mart.) B.Gates	X			
Banisteriopsis megaphylla (A.Juss.) B.Gates	X			
Banisteriopsis variabilis B.Gates	X			
Byrsonima affinis W.R.Anderson	X			
Byrsonima basiloba A.Juss.	X			
Byrsonima clausseniana A.Juss.	X			
Byrsonima guilleminiana A.Juss.	X			
Byrsonima variabilis A.Juss.			DF	

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Heteropterys byrsonimifolia A.Juss.	X			
Heteropterys dumetorum (Griseb.) Nied.	X			
Heteropterys procoriacea Nied.	X			
Heteropterys rhopalifolia A.Juss.	X			
Peixotoa glabra A.Juss.	X		PI	
Peixotoa magnifica C.E.Anderson	X			
Malvaceae				
Byttneria glazioui Hochr.	X			
Ceiba samauma (Mart.) K.Schum.			GO	
Eriotheca pubescens (Mart. & Zucc.) Schott & Endl.	X			
Hibiscus capitalensis Krapov. & Fryxell	X			
Luehea crispa Krapov.			TO	
Mollia lepidota Spruce ex Benth.			GO	
Matayba peruviana Radlk.			MT	
Pavonia immitis Fryxell	X			
Pavonia pohlii Gürke	X			
Pseudobombax longiflorum (Mart.) A.Robyns	X			
Pseudobombax tomentosum (Mart.) A.Robyns	X			
Theobroma speciosum Willd. ex Spreng.		X		
Marcgraviaceae				
Schwartzia adamantium (Cambess.) Bedell ex Gir.-Cañas	X			
Melastomataceae				
Cambessedesia hilariana (Kunth) DC.	X			
Lavoisiera pohliana O.Berg ex Triana	X			
Leandra chaetodon (DC.) Cogn.		X		
Leandra deflexa (Triana) Cogn.	X			
Miconia abbreviata Markgr.		X		
Miconia affinis DC.			TO	
Miconia burchellii Triana	X			
Miconia eugenioides Triana		X		
Miconia herpetica DC.	X			
Miconia pepericarpa DC.	X			
Miconia sclerophylla Triana	X		SP	
Microlicia euphorbioides Mart.	X			
Mouriri elliptica Mart.	X			
Mouriri gardneri Triana	X		MA	
Ossaea congestiflora (Naudin) Cogn.	X			
Pleroma stenocarpum (Schrank et Mart. ex DC.) Triana	X			
Meliaceae				
Cedrela fissilis Vell.				VU
Cedrela odorata L.				VU
Trichilia elegans A.Juss.			TO	
Trichilia stellato-tomentosa Kuntze				VU
Moraceae				
Ficus maxima Mill.		X		

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Sorocea hilarii Gaudich.			PI	
Myristicaceae				
Virola subsessilis (Benth.) Warb.	X			
Virola surinamensis (Rol. ex Rottb.) Warb.				VU
Virola urbaniana Warb.	X		MS	
Myrtaceae				
Eugenia cupulata Amshoff		X		
Eugenia matogrossensis Sobral	X			
Eugenia megaflora Govaerts	X			
Eugenia pyrifera Faria & Proença	X			
Eugenia stipitata McVaugh		X		
Myrcia camapuanensis N.Silveira	X			
Myrcia myrtillifolia DC.	X			
Myrcia neorubella A.R.Lourenço & E.Lucas		X		
Myrcia tortuosa (O.Berg) N.Silveira	X			
Psidium oligospermum Mart. ex DC.			MS, TO	
Psidium salutare (Kunth) O.Berg	X			
Psidium sessiliflorum (Landrum) Proença & Tuler	X			
Siphoneugena densiflora O.Berg			TO	
Nyctaginaceae				
Guapira campestris (Netto) Lundell	X			
Guapira noxia (Netto) Lundell	X			
Neea floribunda Poepp. & Endl.		X		
Ochnaceae				
Elvasia calophyllea DC.		X		
Elvasia canescens (Tiegh.) Gilg		X		
Ouratea acicularis R.G.Chacon & K.Yamam.				EN
Ouratea cauliflora Fraga & Saavedra		X		
Ouratea paraensis Huber		X		
Olacaceae				
Dulacia egleri (Bastos) Sleumer			GO	
Oxalidaceae				
Oxalis goyazensis Turcz.	X			
Peraceae				
Pera anisotricha Müll.Arg.			MA	
Polygalaceae				
Moutabea excoriata Mart. ex Miq.	X			
Polygonaceae				
Coccoloba brasiliensis Nees & Mart.	X			
Primulaceae				
Cybianthus cuneifolius Mart.		X		
Putranjivaceae				
Drypetes amazonica Steyerm.		X		
Rhabdodendraceae				
Rhabdodendron gardneranum (Benth.) Sandwith	X			

Continued...

Family/ species	**Cerrado Endemic**	**New records (Cerrado)**	**New records (states)**	**IUCN category**
Rhamnaceae				
Rhamnidium glabrum Reissek				VU
Rubiaceae				
Borreria crispata (K.Schum.) E.L.Cabral & Bacigalupo	X			
Calycophyllum spruceanum (Benth.) K.Schum.		X		
Cordiera myrciifolia (K.Schum.) C.H.Perss. & Delprete			PI	
Guettarda pohliana Müll.Arg.	X			
Palicourea guianensis Aubl.			TO	
Palicourea justiciifolia (Rudge) Delprete & J.H.Kirkbr.		X		
Psychotria guianensis (Aubl.) Rusby		X		
Rutaceae				
Pilocarpus trachylophus Holmes				EN
Salicaceae				
Casearia altiplanensis Sleumer	X			
Casearia rufescens Cambess.	X			
Casearia rupestris Eichler	X			
Xylosma benthamii (Tul.) Triana & Planch.			TO	
Xylosma venosa N.E.Br.			MA	
Sapindaceae				
Cupania castaneaefolia Mart.			MA, TO	
Dilodendron bipinnatum Radlk.			PI	
Matayba peruviana Radlk.		X		
Talisia subalbens (Mart.) Radlk.				VU
Sapotaceae				
Chrysophyllum lucentifolium Cronquist		X		
Ecclinusa ramiflora Mart.		X		
Elaeoluma schomburgkiana (Miq.) Baill.		X		
Micropholis egensis (A.DC.) Pierre		X		
Micropholis emarginata T.D.Penn.			GO, PI	EN
Pouteria anomala (Pires) T.D.Penn.		X		
Pouteria bangii (Rusby) T.D.Penn.		X		
Pouteria cladantha Sandwith		X		
Pouteria furcata T.D.Penn.			TO	EN
Pouteria macrocarpa (Mart.) D.Dietr.		X		VU
Pouteria procera (Mart.) K.Hammer		X		
Pouteria subcaerulea Pierre ex Dubard	X			
Pouteria torta (Mart.) Radlk.	X			
Pradosia granulosa Pires & T.D.Penn.				VU
Schoepfiaceae				
Schoepfia lucida Pulle	X		MA	
Schoepfia velutina Sandwith	X		MA	
Simaroubaceae				
Homalolepis ferruginea (A.St.-Hil.) Devecchi & Pirani	X			
Homalolepis warmingiana (Engl.) Devecchi & Pirani			BA	EN

Continued...

Family/ species	Cerrado Endemic	New records (Cerrado)	New records (states)	IUCN category
Solanaceae				
Solanum falciforme Farruggia	X			
Styracaceae				
Styrax ferrugineus Nees & Mart.	X			
Symplocaceae				
Symplocos nitens (Pohl) Benth.	X			
Symplocos rhamnifolia A.DC.				EN
Turneraceae				
Piriqueta breviseminata Arbo	X			
Turnera lamiifolia Cambess.	X			
Turnera melochioides Cambess.	X			
Verbenaceae				
Citharexylum poeppigii Walp.		X		
Lippia eupatorium Schauer	X			
Vochysiaceae				
Callisthene major Mart.	X		SP	
Callisthene mollissima Warm.	X			
Qualea cordata Spreng.	X			
Qualea dichotoma (Mart.) Warm.	X			
Qualea hannekesaskiarum Marc.-Berti	X			
Qualea selloi Warm.	X		GO	
Vochysia cinnamomea Pohl	X			
Vochysia discolor Warm.	X			
Vochysia gardneri Warm.	X			
Vochysia herbacea Pohl	X			
Vochysia palmirana F.França & Proença	X			
Vochysia pruinosa Pohl	X			
Vochysia pumila Pohl	X			
Vochysia rufa Mart.	X			
Vochysia sessilifolia Warm.	X			
Vochysia thyrsoidea Pohl	X			

Copaifera langsdorffii, Coussarea hydrangeifolia, Dendropanax cuneatus, Eugenia florida, Hirtella glandulosa, Protium heptaphyllum, P. spruceanum, Tapura amazonica, Vochysia haenkeana and *Xylopia emarginata*. These species are widely distributed in Brazilian wet forests, and their shared occurrence between central Brazil riparian forests, Amazon and Atlantic Forest reinforces the floristic links among these domains (Oliveira-Filho & Ratter 1995, Oliveira-Filho & Fontes 2000, Miranda et al. 2018).

Seasonally dry forests occur as scattered patches within the Cerrado, often associated with limestone outcrops and high-fertility soils, which stand in contrast to the acidic and nutrient-poor soils that predominate in the Cerrado region. As a consequence, central Brazil dry forests, which are mostly deciduous during the dry season, differ markedly in species composition compared to adjacent savannas and wet forests (Pennington et al. 2000, Bueno et al. 2018). The characteristic dry forest species *Aspidosperma subincanum* and *Tabebuia roseoalba* were among those highly collected species in the IFN Cerrado, which also recorded other typical dry forest representatives mentioned in the literature, such as *Aspidosperma pyrifolium, Commiphora leptophloeos, Machaerium scleroxylon* and *Schinopsis brasiliensis* (Scariot & Sevilha 2005, Pereira et al. 2011). Although occurring in discontinuous patches, Cerrado dry forests share several species in common with the Caatinga, highlighting the floristic link between these seasonally dry vegetation nuclei (Prado & Gibbs 1993, Neves et al. 2015). In addition, a large sample of sites located at the Cerrado/Caatinga boundary, particularly in Bahia, contributed to increased numbers of typical dry forests and shrublands in our list.

It is worth highlighting that the systematic sampling methodology of the IFN generated new plant occurrence records in areas little sampled in the Cerrado (Sousa-Baena et al. 2014), such as Piauí, Maranhão (the second state in number of IFN specimens), Tocantins, Mato Grosso and southwestern Goiás, thereby filling some important gaps in species distributions. Another positive aspect of the IFN Cerrado methodology was the sampling in different physiognomies, which included habitats that are not frequently surveyed by botanists, such as riparian forests, swamps and seasonally dry forests, including remote areas difficult to access. Botanical exploration of these areas contributed to a number of new species occurrence records for the Cerrado and also for individual states, contributing floristic knowledge, particularly to some states, such as Maranhão, Piauí and Tocantins, for which floristic information is limited (BFG 2015). The contribution of IFN collections towards new state occurrence records has also been highlighted elsewhere for Rio Grande do Norte where 71 new angiosperm records were reported (Versieux et al. 2017).

A large number of new occurrence records for the Cerrado, such as *Aniba hostmaniana, Calycophyllum spruceanum, Elvasia calophyllea, Eugenia cupulata, Jacaranda copaia, Matayba peruviana, Miconia eugenioides, Pouteria anomala* and *Theobroma speciosum*, were mostly collected in wet forests located in northern Mato Grosso and Tocantins along the Cerrado/Amazonia boundary. This ecotone between the Cerrado and Amazonia, a complex transition zone, is composed of interdigitating patches of savannas and various forest types (Marques et al. 2020), and it has certainly incremented the number of species sampled by the IFN Cerrado. Coinciding with an area that lacks botanical records overall (Souza-Baena et al. 2014), this ecotone also helps to explain the large number of new occurrences in the Cerrado for species previously reported only to Amazonia. New occurrences for the Cerrado also comprised typical elements from the Caatinga (e.g., *Cratylia mollis, Pouteria furcata*) and Atlantic Forest (e.g., *Dendropanax denticulatus, Myrocarpus frondosus*), albeit to a lesser extent compared to Amazonia. These results highlight the contribution of marginal/ecotonal areas between the Cerrado and other biomes to the species richness of the Cerrado's tree flora, as reported in previous studies (Oliveira-Filho & Ratter 1995, Françoso et al. 2016, Miranda et al. 2018).

It is important to mention that new occurrence records should be viewed with caution since most were based on sterile specimens identified by non-specialists. Consequently, they may not represent accurate records. This means that potentially new occurrence records should be further confirmed, preferably based on fertile specimens determined by experts. Among the 214 species collected by the IFN Cerrado cited in the CNCFlora's red list, 36 are classified to some degree of threat, including 2 critically endangered (CR), 14 endangered (EN) and 20 vulnerable (VU). Another 179 species are classified as least concern (LC) or near threatened (NT). A total of 232 Cerrado endemic species were collected, representing 12.4% of 1,858 endemic woody species registered in the Flora do Brasil 2020 for this biome. New occurrence records for threatened, as well as rare and endemic species provide crucial data expanding knowledge of geographic ranges and, hence, enabling updates of species threat status and furthering efforts to subsidise conservation initiatives. In addition to new distribution records, specimens collected by the IFN Cerrado supported the description of a new *Harpalyce* (Fabaceae) species from western Bahia (São-Mateus et al. 2019). It is likely that ongoing taxonomic work by specialists based on IFN Cerrado collections deposited in herbaria will reveal more new species to science. Also, with progress in specimen identification in herbaria, new species occurrences for the Cerrado and states, as well as records for endemic and threatened species, are expected.

Conclusion

Although initially developed to assess land coverage, forest structure and wood production, national forest inventories have also played a role as a major source of data for monitoring forest biodiversity (e.g., Chirici et al. 2012). In the present study, focusing specifically on the biodiversity dimension, we showed that the IFN Cerrado has provided a useful source of occurrence data for woody species, spanning wide geographic and habitat coverage. The large number of woody species reported here reflects the floristic variation found in diverse vegetation types found in the Cerrado region, which includes savannas, seasonally dry forests, and wet forests. Our analyses, based on the latest dataset available, showed that about a third of the floristic diversity of the Cerrado woody plants was sampled in the IFN. Our results also show that the typical floristic composition of the different vegetation types of the Cerrado was captured by that survey, and that species cited in the literature as common appear in high numbers among IFN collections. However, differential sampling efforts between field teams and difficulties in naming specimens are expected to influence the floristic diversity reported here. The fact that 35% of conglomerates sampled were located in anthropic areas, which are likely to be less diverse than well-preserved areas, may have also influenced the great variation found in sampling units in terms of species richness. The floristic richness compiled by the IFN Cerrado, which included a number of potential new occurrence records, was greatly influenced by typical woody elements from surrounding biomes, such as Amazonia, Caatinga and Atlantic Forest. Species collected in these marginal/ecotonal zones greatly contributed to the overall number of species recorded here.

Although considered a biodiversity hotspot, it is estimated that the Cerrado will lose 31-34% of its remaining native vegetation by 2050, mostly from agricultural expansion and limited protected areas (Strassburg et al. 2017). Therefore, increasing Cerrado floristic knowledge is critical to support conservation planning in this threatened biome. We expect that the occurrence records derived from IFN collections, particularly those from rare, endemic and endangered species, will contribute to the identification of priority areas for further surveys and conservation of the rich Cerrado flora.

Supplementary Material

The following online material is available for this article:

Table S1 - Specialists that contributed with the identification of selected families, and generalist botanists that identified miscellaneous families (bottom of the table).

Table S2 - Herbarium specimens of woody species collected by the IFN Cerrado. Only records belonging to native species identified at the species level are listed.

Table S3 - List of woody species collected by the IFN Cerrado, including information on states of occurrence, number of specimens collected, habit, vegetation type, and selected voucher specimen. A voucher specimen, either sterile or fertile, was chosen to represent each species.

Associate Editor

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Author Contributions

Fernanda K. Kiataqui: Conceptualization, Formal analysis, Writing – original draft-, Writing – review & editing. Sérgio Eustáquio de Noronha: Formal analysis, Writing – review & editing. Marcelo F. Simon: Data curation, Formal analysis, Writing – review & editing.

Conflicts of Interest

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

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Thematic Review

Zooplankton diversity in Acre state, Amazon, Brazil: an overview of previous studies

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Abstract: This work assesses current knowledge of zooplankton biodiversity in aquatic ecosystems of the state of Acre (Brazil). A bibliographic survey was undertaken, showing that most of the reported studies have focused on zooplankton diversity and composition. Fifteen locations have been studied. There is still a scarcity of information concerning zooplankton populations in the Amazon region. To date, 170 species of planktonic rotifers have been recorded, distributed in 26 families and 38 genera, together with 18 species of cladocerans, distributed in 8 families and 18 genera, and 4 species of copepods in 2 families and 6 genera. The results indicate the need for further research concerning the biodiversity of this group of organisms at locations in the basins of the Purus and Juruá Rivers, especially in lotic ecosystems and littoral zones, given the present lack of information and the socioeconomic importance they play in the region.

Keywords: *Amazon; bibliographic survey; list of species; Rotifera; Cladocera; Copepoda.*

Diversidade do zooplâncton no estado do Acre, Brasil: uma revisão de estudos anteriores

Resumo: Este trabalho apresenta uma avaliação do estado da arte da biodiversidade do zooplâncton em ecossistemas aquáticos do estado do Acre (Brasil). Um levantamento bibliográfico foi realizado e a maior parte das pesquisas tiveram enfoque na composição e diversidade do zooplâncton. 15 locais foram estudados. A Amazônia ainda é uma região incipiente quanto aos estudos de zooplâncton. Foram registradas 170 espécies de rotíferos planctônicos, distribuídos em 26 famílias e 38 gêneros, 18 espécies de cladóceros distribuídos em 8 famílias e 18 gêneros, e 4 espécies de copépodes distribuídos em 2 famílias e 6 gêneros. Os resultados sugerem ainda a necessidade de mais pesquisas sobre a biodiversidade destes grupos em locais na bacia do Purus e Juruá, bem como em ecossistemas lóticos e nas zonas litorâneas, tendo em vista a carência de informações e a importância socioeconômica que exercem na região.

Palavras-chave: *Amazônia; levantamento bibliográfico; lista de espécies; Rotifera; Cladocera; Copepoda.*

Introduction

Biodiversity information is still incomplete for the majority of the aquatic systems in Brazilian Amazonia. There are areas of the Amazon that have never been visited or documented, with many of the species present not having been identified or analyzed in detail (Souza et al. 2019). Although the state of Acre lies in the Amazon basin and is rich in limnic environments, to date there have been only limited studies concerning the aquatic invertebrates present, with emphasis on the phylum Rotifera (Keppeler & Hardy 2004, Keppeler et al. 2010, Santos Nascimento & Keppeler 2017), followed by the groups Cladocera and Copepoda (Keppeler 2003a, b, Nascimento & Keppeler 2017).

The vast majority of taxonomic and ecological studies of the zooplankton community in Acre have been performed in lakes, from the 1990s onwards (Sendacz & Melo-Costa 1991, Keppeler 2003a, b, Keppeler & Hardy 2004, Oliveira et al. 2010, Santos et al. 2013). However, only a few studies have investigated the zooplankton of rivers and streams (creeks) (Keppeler et al. 2010, Silva et al. 2014, Nascimento & Keppeler 2017, Santos Nascimento & Keppeler 2017).

It is important to emphasize that studies on the composition of zooplankton communities can assist in the environmental management of hydrographic basins, because the presence of groups can vary according to the studied environment. For example, in lentic environments, Cladocera, Rotifera and Copepoda represent about 90% of the total biomass of zooplankton (Ghidini & Santos-Silva 2009, Setubal et al. 2020). On the other hand, despite presenting low abundance in relation to other groups in lentic environments, in lotic environments testaceous amoebae can act as protagonists in the environment, especially in streams (Araujo et al. 2019). These groups are the main source of food for species, including fish, acting as a link between producers and consumers at higher levels in the food chain (Iskın et al. 2020). These organisms, which play a fundamental role in nutrient cycling, are sensitive to environmental changes and have rapid responses, which can be used as indicators of the quality and trophic status of aquatic systems (Cardoso et al. 2008, Pinheiro et al. 2019, Karpowicz et al. 2020).

There is limited information available concerning the zooplankton communities in aquatic environments in the state of Acre compared to other regions of Brazil, and there is a growing threat of biodiversity loss in the region due to anthropic activities (Collier et al. 2019). In Brazil, urban development almost invariably results in the unplanned expansion of the urban periphery and the pollution of aquatic ecosystems through the discharge of untreated domestic waste into these environments (IBGE 2011). Agriculture also impacts these environments; sources of pollution derived from those activities are generally related to the disposal of organic materials, such as phosphates and nitrogenated compounds, as well as pesticides and other chemical products (Araújo et al. 2009, García et al. 2016). Therefore, the aim of this work is to summarize current knowledge about the biodiversity of species of the zooplankton community in Acre. This is the first bibliographic survey of zooplankton species found in freshwater environments of this region. Various works concerning biodiversity have highlighted the need for biological databases, as part of efforts to improve programs for the conservation of biota and ecosystems (Groombridge 1992, Heywood 1995). Undertaking a bibliographic survey can contribute to collective knowledge and indicate areas where further work is needed. It can assist with understanding the issues or, when appropriate, reusing and replicating research at different scales and in different contexts (Galvão 2010).

Material and Methods

This study contemplated published papers regarding studies performed in two of the most important water basins of the State of Acre (Purus and Juruá rivers). The Purus River basin, located in southwestern Amazonia, is the fourth largest (370,000 km²) among the seven tributary river basins on the right bank of the Amazon River. Its area within Brazil covers around 354,000 km², distributed in 32 municipalities in the states of Acre, Amazonas, and Rondônia (ANA 2011).

The hydrographic basin of the Juruá River is shared between Brazil (states of Acre and Amazonas) and Peru (Ucayali Department). Within Acre, there are eight municipalities in the basins of the Juruá River and its main tributaries, the Tarauacá and Envira Rivers, covering an area of 74,950 km². This area corresponds to approximately 49% of the state of Acre, 19.9% of the area of the Solimões/Juruá/Japurá basin (considering its entire course within the Amazon region), and 1.9% of the Brazilian part of the Amazon basin (ACRE 2012).

The zooplankton inventory for limnic aquatic ecosystems in Acre was produced based on a review of the information available in scientific journals up to 2017, given that there are no other publications for the state of Acre on zooplankton after this year. For this search, we used the keywords "zooplankton," "rotifera," "cladocera," "copepoda," "testacea," and "acre", in Google Scholar (http://scholar.google.com). All results were then filtered considering the following criteria: i) it was published in a scientific journal (indexed or not); ii) it was performed in natural environments (lakes, streams, rivers); and iii) it contained a composition table. Unpublished works (dissertations and theses) and conference abstracts were not considered.

Table 1 provides information about each study (where available), including the geographic coordinates, basin, and hydrological system. It is known that the basins of the Purus and Juruá Rivers are found in other regions; therefore, only water bodies located within the territorial limit of the state of Acre were considered, that is, areas outside that limit were discarded.

We compiled the taxa composition data based on the articles' tables, without performing any calculations, corrections, and processing, considering the purpose of this article was to group the information already published regarding the zooplankton community.

In the present review, the names of the species are as recorded by the authors, but, when necessary, the currently valid names according to taxonomic reviews are also included. After the review, the species are listed according to the classification separated by family, genus, and species proposed by Segers (2007). Some species have been cited more than once for the same environment but have been considered only once for each environment. It should be noted that in these procedures, citations only of morphotypes (for example, Rotatoria sp. or Rotifera sp. 1) were excluded if not in the generic level.

Results

A total of 11 studies were found, distributed between two basins. In the Purus basin, studies were found in the Acre river and the lakes Amapá and Pirapora. Regarding the Juruá basin, the Sacado, Pedernal, Anil, Preto and Jesumira streams and the Canela Fina, Cigana, Novo, Verde, Monju, St. Elias and Miritizal lakes were studied (Table 1 and Figure 1). The main sampling method was vertical and horizontal pulls using zooplankton nets. In most of these studies, 50–55 µm plankton

Table 1. List of water bodies in which zooplankton studies were performed, with their respective geographic coordinates and studies list.

Basin	System	Coordinates	Studies
Purus	Amapá Lake	10°02'36"S e 67°50'24"W	Keppeler E., 2003b
			Keppeler E., 2003a
			Sendacz & Melo-Costa, 1991
			Keppeler & Hardy, 2004
	Pirapora Lake	9°27'21"S e 67°31'30"W	Keppeler E., 2003b
			Keppeler E., 2003a
	Acre River	7 e 12° S e 66 e 74° W	Sendacz & Melo-Costa, 1991
Juruá	Sacado Stream	7°33'0"S e 72°36'0"W	Nascimento & Keppeler, 2017
	Pedernal Stream	7°30'23,7"S e 73°42'05,2"W	Silva et al., 2014
	Anil Stream	7°27'0124"S e 73°37'30,8"W	Silva et al., 2014
	Preto Stream	7°35"38,46"S e 72°4316,46"W	Oliveira et al., 2010
	Jesumira Stream	7°28'10,2"S; 73°33'54,6"	Keppeler et al., 2010
	Canela Fina Lake	7°33'40,63"S e 72°42'55,95"W	Oliveira et al., 2010
	Cigana Lake	7°34'15"S; 72°37'57"W	Santos et al., 2013
	Novo Lake	7°44'01"S; 72°37'56"W	Santos et al., 2013
	Verde Lake	7°50'02"S; 72°38'21"W	Santos et al., 2013
	Monju Lake	7°48'51"S; 72°36'37"W	Santos et al., 2013
	Santo . Elias Lake	7°46'19"S; 72°36'37"W	Santos et al., 2013
	Miritizal Lake	Not available	Nascimento & Keppeler, 2017

nets were used, the 50-µm size net being used in the studies by Sendacz & Melo-Costa (1991), Keppeler et al. (2010) and Silva et al. (2014). In the studies by Keppeler (2003a, b), Keppeler & Hardy (2004), Santos et al. (2013), Nascimento & Keppeler (2017), and Santos Nascimento & Keppeler (2017), 55 µm nets were used. It was not possible to determine, based on what the authors described, the total number of samples or the water volume that was filtered/taken in all the studies.

Up to now, studies in Acre have described 170 species of Rotifera (Table 2 and Figure 2), 18 species of Cladocera, 2 of Copepoda Cyclopoida, and 2 of Copepoda Calanoida (Table 3 and Figure 3). Planktonic fauna populations have been studied in 15 freshwater environments, 3 of them at more than one time, mainly in the southwestern Amazon region (Figure 1).

When considering all zooplankton groups, a total of 164 species were recorded in the Juruá River basin, while 73 species were found in the Purus River basin. The most studied periods were summer and winter; in the Amazon, these are known periods of drought and flood, respectively.

Research concerning plankton from limnic environments in Acre began in the southwestern region of the state, with Sendacz & Melo-Costa (1991) recording 32 species and 14 organisms at the genus level for Rotifera, 6 species and 1 organism at the genus level for Cladocera, and 2 species and 3 organisms at the genus level for Copepoda Cyclopoida. Moreover, larval and juvenile forms of copepods (nauplii and copepodites) were also recorded for the two water bodies. The first major species surveys in lakes and streams were undertaken from 2000 onwards, as reported by Keppeler (2003a, b), Keppeler & Hardy (2004a), Keppeler et al. (2010), Oliveira et al. (2010), Santos et al. (2013), Silva et al. (2014), Nascimento & Keppeler (2017), and Santos Nascimento & Keppeler (2017).

In this data compilation, 26 families of rotifers were recorded, among which the most representative families were Lecanidae (41 species), Brachionidae (38), Lepadellidae (17), and Filinidae (9). The overall richness in Acre was 170 rotifers identified at the species level and 34 identified only at the genus level, totaling 204 taxa. A total of 149 species of Rotifera were recorded for the Juruá River basin, while 58 were recorded for the Purus River basin, with 37 species being common to the two basins. Among the species studied, 21 were reported only in the Purus River basin and 112 only in the Juruá River basin.

In the Cladocera group, the species identified were distributed in 8 families, with the Chydoridae family presenting the greatest richness (5 species), followed by the families Sididae (3), Daphnidae (3), Bosminidae (2), Moinidae (2), and Ilyocryptidae (2). The dominant species, occurring in more than 70% of the environments and therefore classified as very frequent, were *Bosminopsis deitersi*, *Moina minuta*, and *Moina reticulata*. In the Juruá River basin, 15 species were registered, and in the Purus basin, 9 were registered; 6 species were common between the two basins. Among the species studied, 3 were described only in the Purus River basin and 9 occurred only in the Juruá River basin.

The Copepoda group showed a difference in terms of its distribution, because it was only found in white water environments (with records only for the Purus River basin). Eight taxa were recorded, of which 4 were identified at the species level and 4 at the genus level, distributed in the families Diaptomidae and Cyclopidae, with a total of 6 genera. The family with the greatest representation was Cyclopidae, with 5 taxa recorded, while 3 species were recorded for Diaptomidae. The larval and juvenile phases (such as copepodites and nauplii) were found in the two basins studied. For the Juruá basin, only the juvenile forms of the copepods (nauplii and copepodites) were registered.

For the environments and basins studied, the Juruá River basin presented greater richness and species diversity compared with the Purus River basin. In the case of the water bodies, Amapá Lake showed the greatest Rotifera richness, followed by Anil Stream and Verde Lake. Preto and Jesumira streams presented the lowest richness and diversity. The findings indicate that Rotifera diversity is higher in lentic environments and lower in lotic environments.

Discussion

Given that the basins of the Juruá and Purus Rivers cover vast areas and are of great importance for the Amazon basin, it can be considered

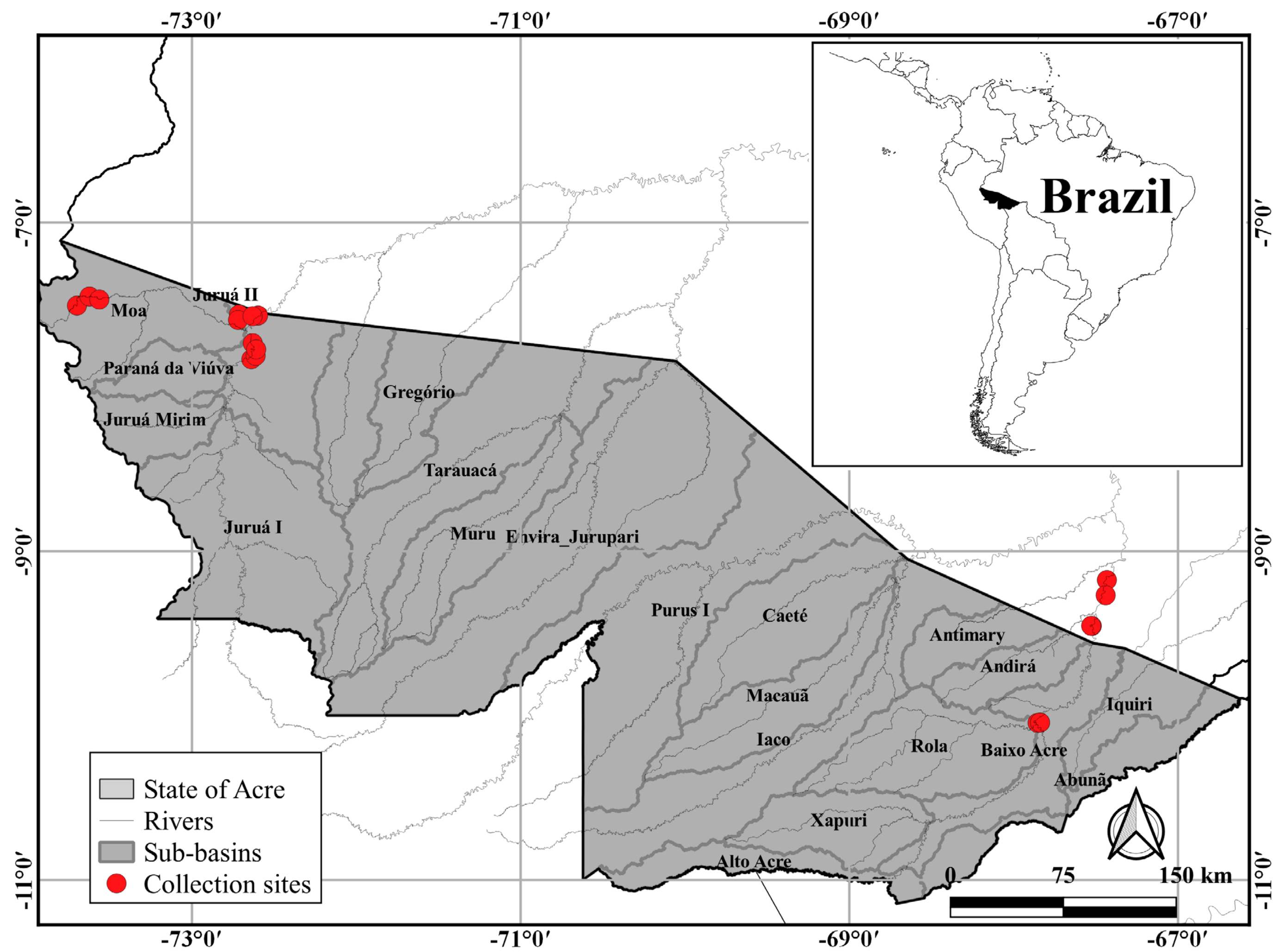

Figure 1. Map with study sites in which previous published papers have been performed.

that studies of the aquatic invertebrate community are still scarce. There has been greater emphasis on studies concerning lakes and the Rotifera phylum in Acre, as seen in the studies of Keppeler & Hardy (2004), Keppeler et al. (2010), Do Nascimento & Keppeler (2017), and Santos Nascimento & Keppeler (2017). Compared with other communities of organisms, the numbers are quite low and are mostly related to a specific group rich in Rotifera. This scarcity of information may be due to a lack of specialists dedicated to the different zooplankton taxonomic groups, as well as the difficulties in access and the limited resources and infrastructure available to researchers. Nonetheless, the environments studied showed high species diversity, mainly due to the great heterogeneity of habitats. This richness and diversity of zooplankton in Acre can be compared with data for the floodplain lakes in the Itacoatiara region of Amazonas (Ghidini et al. 2018). Given the high richness of 43 species in the 4 studied lakes, the most abundant rotifers with 21 species, cladocerans and copepods with quantities and species similar to those found in the state of Acre. Nauplii and copepodites were also found.

Based on the collected studies, Rotifera was the most diverse group, with the greatest number of species recorded, following the same pattern for all the water bodies. This pattern is common in tropical environments (Rocha et al. 1995) and can be attributed to three factors, namely the high population growth rates of this group (Allan 1976) the fact most species of this group inhabits freshwater environments and the emphasis given to the group in these particular published studies (Keppeler & Hardy 2004, Keppeler et al. 2010). Due to the hydrological conditions of the aquatic ecosystems in the Amazon, communities tend to present recurrent variations in each type of environments in which they are present, which explains the greater wealth and diversity in the lakes and less wealth and diversity in the lotic environments studied (Esteves 1998, Lair 2006), as lakes constitutes a more stable environment for Rotifers to develop. In addition, regional climate patterns affect riverside and lake floodplains in similar ways, despite general differences in their linkages with basins (Wantzen et al. 2008). The sampling effort can be one of the reasons for such diversity of Rotifera, being registered in the studies of Sendacz & Melo-Costa (1991), Keppeler (2003a), Keppeler (2003b), Keppeler & Hardy (2004), Oliveira et al. (2010), Keppeler et al. (2010), Santos et al. (2013), Silva et al. (2014), Nascimento and Keppeler (2017) and Santos Nascimento & Keppeler (2017).

In the first study, performed by Sendacz & Melo-Costa (1991), rotifers showed the highest species diversity among the groups studied, with a total of 46 species, including 11 belonging to the genus

Table 2. Rotifera species list in limnic ecosystems of Acre State, indicating species location, considering studies published so far.

		Purus								**Juruá**					
	Amapá Lake	Pirapora Lake	Acre River	Sacado Stream	Pedernal Stream	Anil Stream	Preto Stream	Jesumira Stream	Canela Fina Lake	Cigana Lake	Novo Lake	Verde Lake	Moju Lake	Santo Elias Lake	Miritizal Lake
Rotifera															
Adineta barbata (Janson, 1893)				X											X
Epiphanes spp.	X	X													
Keratella híspida (Lauterborni, 1900)											X		X	X	
Keratella lenzi (Hauer, 1953)	X							X							
Keratella lenzi heliaca (Berzins, 1955)											X				
Keratella lenzi lenzi (Hauer ,1953)	X							X							
Keratella sp.											X				
Lecane acus (Harring, 1913)					X								X		
Lecane decipiens (Murray, 1913)						X						X	X	X	
Ascomorpha spp.	X	X													
Adineta gracilis (Janson, 1893)				X											
Adineta sp.				X											
Anuraeopsis coelata (De Beauchamp, 1932)											X	X			
Anuraeopsis fissa (Gosse, 1851)															X
Anuraeopsis navicula (Rousselet, 1911)											X	X			X
Anuraeopsis sp.	X	X									X		X	X	
Ascomorpha ecaudis (Perty, 1850)					X	X									X
Ascomorpha ovalis (Bergendal, 1892)				X	X						X	X		X	X
Ascomorpha saltans (Bartsch, 1870)															X
Ascomorpha sp.	X			X	X					X		X			X
Asplanchna brightwellii (Gosse, 1850)	X	X										X			

Asplanchna cf. (Herrickii De Gueme, 1850)									X				
Asplanchna sieboldi (Leydig, 1854)	X	X							X	X			
Asplanchna sp.	X		X					X	X	X			X
Beauchampiella eudactylota eudactylota (Gosse, 1886)					X	X							
Brachionus angularis (Gosse, 1851)								X		X			
Brachionus bidentatus (Anderson, 1889)													X
Brachionus bidentatusbidentatus (Anderson, 1889)			X										
*Brachionus bidentatus*f.inermis(Rousselet, 1906)	X												
Brachionus budapestenensis (Daday, 1885)									X				
Brachionus calyciflorus (Pallas, 1766)	X	X											
Brachionus calicyflorus anuraeformis (Brehm, 1903)	X	X											
Brachionus caudatus (Barrois & Daday, 1884)	X	X	X	X				X	X	X	X	X	X
Brachionus diversicornis (Daday, 1883)													X
Brachionus dolabratus (Harring, 1914)	X	X	X					X	X	X	X	X	
Brachionus falcatus (Zacharias, 1898)	X	X						X	X	X	X	X	X
Brachionus havanaensis (Rousselet, 1911)	X	X						X	X	X	X		
Brachionus mirus (Daday, 1905)													X
Brachionus mirus f.laticaudatus (Paggi, 1973)			X										
*Brachionus mirus*var.*reductus*(Koste, 1972)	X	X											
Brachionus patulus var. *macrocanthus* (Jakubski, 1912)	X		X										
Brachionus plicatilis (Müller, 1786)	X								X	X			
Brachionus quadridentatus mirabilis(Daday, 1897)	X												
Brachionus quadridentatus(Hermann, 1783)	X	X	X										X
Brachionus sp.													X
Brachionus tridentata (Anderson, 1889)				X									
Brachionus urceolaris (Müller, 1773)						X		X		X		X	
Brachionus zahniseri (Ahlstrom, 1934)													X
Cephalodella gibba (Ehrenberg, 1830)	X	X				X	X	X		X		X	X

Cephalodella hollowdayi (Koste, 1986)	X														
Cephalodella intuta (Myers, 1924)															X
Cephalodella mira (Myers, 1934)											X	X			
Cephalodella sp.			X		X						X	X			X
Cephalodella spp.						X									
Colurella anodonta (Carlin, 1939)					X										
Colurella obtusa clausa (Hauer, 1936)						X									
Colurella sp.				X		X									X
Colurella uncinata (Müller, 1773)						X									
Dipleuchlanis propatula (Gosse, 1886)				X											
Dipleuchlanis propatula macrodactyla (Gosse, 1886)	X					X									
Dissotrocha aculeata (Ehrenberg, 1832)									X						
Dissotrocha spp.									X						
Epiphanes macrourus (Barrois & Daday, 1894)	X	X					X								
Epiphanes pelagica (Jennings, 1900)	X									X			X	X	
Euchlanis dilatata (Ehrenberg, 1832)	X														
Euchlanis incisa f. *mucronata* (Ahlstrom, 1934)															X
Euchlanis lyra (Hudson, 1886)															X
Euchlanis sp.				X											
Euchlanis spp.					X										
Euchlanis triquetra (Ehrenberg, 1834)															X
Filinia cf. *terminalis* (Forsyth & James, 1991)	X	X								X	X	X	X	X	
Filinia limnetica (Zacharias, 1893)	X	X													
Filinia longiseta (Ehrenberg, 1834)	X	X		X							X			X	X
Filinia longiseta var. *limnetica* (Ehrenberg, 1834)				X											
Filinia novaezealandiae (Shiel and Sanoamuang 1993)					X	X									
Filinia opoliensis (Zacharias, 1898)	X	X	X							X	X	X	X	X	
*Filinia pejleri (*Hutchinson, 1964)	X	X	X							X	X	X	X	X	

Filinia saltator (Gosse, 1886)	X											
Filinia sp.					X							
Filinia terminalis (Plate, 1886)	X											X
Floscularia sp.					X		X	X	X			
Gastropus stylifer (Imohof, 1891)				X					X			
Hexarthra intermedia braziliensis (Hauer, 1953)	X	X	X									
Hexarthra intermedia (Wiszniewski, 1929)							X		X		X	
Hexarthra mira (Hudson, 1871)												X
Hexarthra sp.	X	X					X	X	X	X	X	X
Keratella americana (Carlin, 1943)	X	X	X					X		X	X	
Keratella cochlearis cochlearis (Plate, 1886)	X						X	X	X	X	X	
Keratella cochlearis (Gosse, 1851)	X	X		X								X
Keratella cochlearis hispida (Lauterborn, 1900)	X	X										
Keratella tropica tropica(Apstein, 1907)						X	X					
*Keratella tropica(*Apstein, 1907*)*	X											
Lecane aculeata (Jakubski, 1912)												X
*Lecane acus (*Harring, 1913)					X					X		
Lecane arcuata cf. (Bryce, 1891)					X							
*Lecane benjamini brasiliensis (*Koste, 1972)						X						
Lecane bulla (Gosse, 1851)	X			X		X						X
Lecane bulla goniata (Harring & Meyers, 1896)						X						
Lecane cf. *furcata (*Murray, 1913)					X							
Lecane cf. *murrayi (*Hauer, 1965)						X						
Lecane cf. *stichaea verecunda* (Harring & Myers, 1926)								X				
*Lecane clara (*Bryce, 1892)				X								X
*Lecane closterocerca amazonica (*Koste, 1972)					X	X		X				
Lecane closterocerca(Schmarda, 1859)	X					X	X	X		X		
Lecane cornuta (Müller, 1786)												X

Lecane curvicornis(Murray, 1913)	X	X		X											X
Lecane decipiens (Murray, 1913)						X						X	X	X	
Lecane doryssa cf. (Harring, 1914)						X									
*Lecane elegans (*Harring, 1914)						X									
Lecane elsa (Hauer, 1931)	X	X											X	X	X
*Lecane flexilis (*Hudson & Gosse, 1886)						X									
Lecane grandis (Murray, 1913)															X
*Lecane imbricata (*Carlin, 1939)					X								X		
Lecane inermis (Bryce, 1892)															X
Lecane kutikova (Koste, 1978)									X						
Lecane lauterborni (Hauer, 1924)					X	X					X				X
Lecane leontina (Turner, 1892)	X			X					X						X
Lecane ludwigii (Eckstein, 1883)	X			X	X										
*Lecane luna (*Müller, 1776)	X					X							X		X
*Lecane lunaris constricta (*Murray, 1930)					X					X	X	X	X	X	
*Lecane lunaris crenata (*Harring, 1930)					X										
*Lecane lunaris (*Ehrenberg, 1838)	X	X		X					X						X
Lecane lunaris lunaris (Ehrenberg, 1832)						X				X	X	X	X	X	
*Lecane lunaris perplexa (*Alstrom, 1938)					X										
Lecane murrayi (Koste, 1978)															X
*Lecane monostyla (*Daday, 1897)						X		X			X	X			
*Lecane papuana (*Murray, 1913)						X							X		X
Lecane pauliane cf. (Berzins, 1960)					X										
*Lecane pyriformis (*Daday, 1905)				X	X	X				X	X	X	X	X	X
*Lecane quadridentata (*Ehrenberg, 1832)				X	X		X				X		X	X	X
*Lecane sola (*Hauer, 1926)				X											
Lecane sp.									X	X	X	X			X
Lecane sp. *(Hernimonostyla)*	X														

Lecane spp.					X	X									
Lecane stichaea cf. *verecunda* (Harring & Myers, 1926)					X										
Lecane stichaea (Harring, 1913)				X											
Lepadella akrobeles (Myers, 1934)												X			
Lepadella acuminata (Ehrenberg, 1834)															X
Lepadella astacicola (Hauer, 1926)				X											
Lepadella benjamini benjamini (Harring, 1916)						X									
Lepadella cf. *benjamini* (Harring, 1916)										X		X	X	X	
Lepadella cf. *princisi* (Berzins, 1943)					X	X									
Lepadella costatoides (Segers, 1992)															X
Lepadella favorita (Klemet, 1962)				X											
Lepadella imbricata (Harring, 1914)				X											X
Lepadella minuta (Weber & Montet, 1918)				X											
Lepadella oblonga (Ehrenberg, 1834)															X
Lepadella ovalis (Müler, 1786)	X			X	X	X							X		X
Lepadella patella (Müller, 1786)	X			X	X	X	X		X						X
Lepadella patella similis (Remane, 1929)					X	X				X			X	X	
Lepadella princisi (Berzins, 1943)				X									X	X	
Lepadella rhomboides (Gosse, 1886)															X
Lepadella sp.	X	X	X		X	X					X	X			X
Lepadella triptera triptera (Ehrenberg, 1930)						X									
Paracolurella logima (Myers, 1924)															X
Lindia fulva (Harring & Myers, 1922)															X
Macrochaetus collinsi (Gosse, 1867)				X											
Macrochaetus sericus (Thorpe, 1893)				X											
Macrochaetus sp.									X						
Beauchampiella eudactylota (Gosse, 1886)	X														

Monommata sp.							X								
Mytilina bisulcata (Lucks, 1912)				X											
Mytilina sp.					X										
Mytilina ventralis macracantha(Gosse, 1886)	X														
Notommata sp.										X		X	X	X	X
Paranuraeopsis sp.						X									
Platyias leloupi (Gillard, 1957)															X
Platyias quadricornis brevispinus (Daday, 1905)						X									
Platyias quadricornis (Ehrenberg, 1832)	X		X	X								X			X
Platyias quadricornis quadricornis (Ehrenberg, 1832)					X										
Platyionus patulus fa. *Macrodactyla* (Hauer*, 1965)*	X	X													
Platyionus patulus macrachanthus (Daday, 1905)	X														
Platyionus patulus (Muller, 1786)										X		X			X
Platyionus patulus patulus (Muller, 1786)						X									
Polyarthra bicerca (Wulfert, 1956)						X					X	X			
Polyarthra dolichoptera (Idelson, 1925)											X	X		X	X
Polyarthra remata (Skorikov, 1896)				X											X
Polyarthra sp.	X	X	X			X									
Polyarthra vulgaris (Carlin, 1943)	X	X							X	X	X	X	X	X	
Proales doliaris (Rousselet, 1895)															X
Proales sp.					X	X				X	X	X		X	X
Sinantherina sp.	X														
Synchaeta sp.			X												
Testudinella cf. *aspis* (Carlin, 1939)												X			
Testudinella mucronata (Gosse, 1887)															X

Testudinella mucronata hauerensis (Gillard, 1967)						X				X					
Testudinella parva (Ternetz, 1892)				X											X
Testudinella patina (Hermann, 1783)	X	X				X				X	X	X	X	X	
Testudinella patina f. trilobata (Shephard, 1892)	X		X												
Testudinella patina intermedia Anderson, 1889					X										
Testudinella sp.	X	X								X	X			X	X
Testudinella tridentata (Smirnov, 1931)				X	X	X				X	X	X	X	X	
Trichocerca bicristata Gosse, 1887	X	X								X	X	X	X		X
Trichocerca bidens (Lucks, 1912)													X		
Trichocerca capucina (Wierzejski & Zacharias, 1893)															X
Trichocerca chattoni(Beauchamp, 1907)	X											X			
Trichocerca montana cf. (Hauer, 1956)					X	X									
Trichocerca myersi (Hauer, 1931)						X							X		X
Trichocerca similis (Wierzejski, 1893)	X	X			X	X				X	X	X	X	X	X
Trichocerca sp.	X					X			X	X	X	X	X		X
Trichocerca spp.	X	X			X										X
Trichocerca tenuior (Gosse, 1886)					X	X					X	X	X		
Trichotria tetractis (Ehrenberg, 1830)						X									X
Trochosphaera aequatorialis (Semper, 1872)	X	X													
Wierzejskiella sabulosa (Wiszniewski, 1932)					X										

Brachionus, which has a high degree of endemism in South America and Australia (Ruttner-Kolisko 1972, Pejler et al. 1977 *apud* Brandorff et al. 1982, Dumont 1983, Rocha et al. 1995, Sendacz et al. 2006, Santos et al. 2014). The species that occurred in only one of the environments studied were *Brachionus mirus voigti*, *Brachionus zahniseri gessneri*, and *Trichocerca chattoni* (Lua Nova Lake); *Brachionus mirabilis*, *Brachionus bidentatus inermis*, and *Mytilina* sp. (Amapá Lake); and *Brachionus bidentatus*, *Brachionus mirus laticaudatus*, and *Brachionus quadridentatus* (Acre River). Robertson & Hardy (1984) analyzed the zooplanktonic composition in Central Amazonia, comparing several várzea lakes, and found that the rotifers were not restricted to any particular type of water in the region.

Studies were only continued in 2003, when Keppeler (2003a) investigated two ecosystems (Pirapora and Amapá Lakes) to identify differences in the abundances of invertebrates in the pelagic and littoral zones and the diurnal variation. The pelagic and littoral zones of those two studied lakes did not show any statistically significant difference and the vertical distribution of Rotifera was found to be uniform in the lakes, in contrast to the other groups analyzed. The author related these results with the sampling period, as cycles of inundation could exert a homogenizing effect, but also indicates the need for further studies.

Later in the same year, Keppeler (2003b) compared the zooplanktonic compositions in Pirapora and Amapá Lakes. Rotifera was the most abundant group, with 38 species and the greatest abundance of the family Brachionidae, followed by Filinidae. The Rotifera group

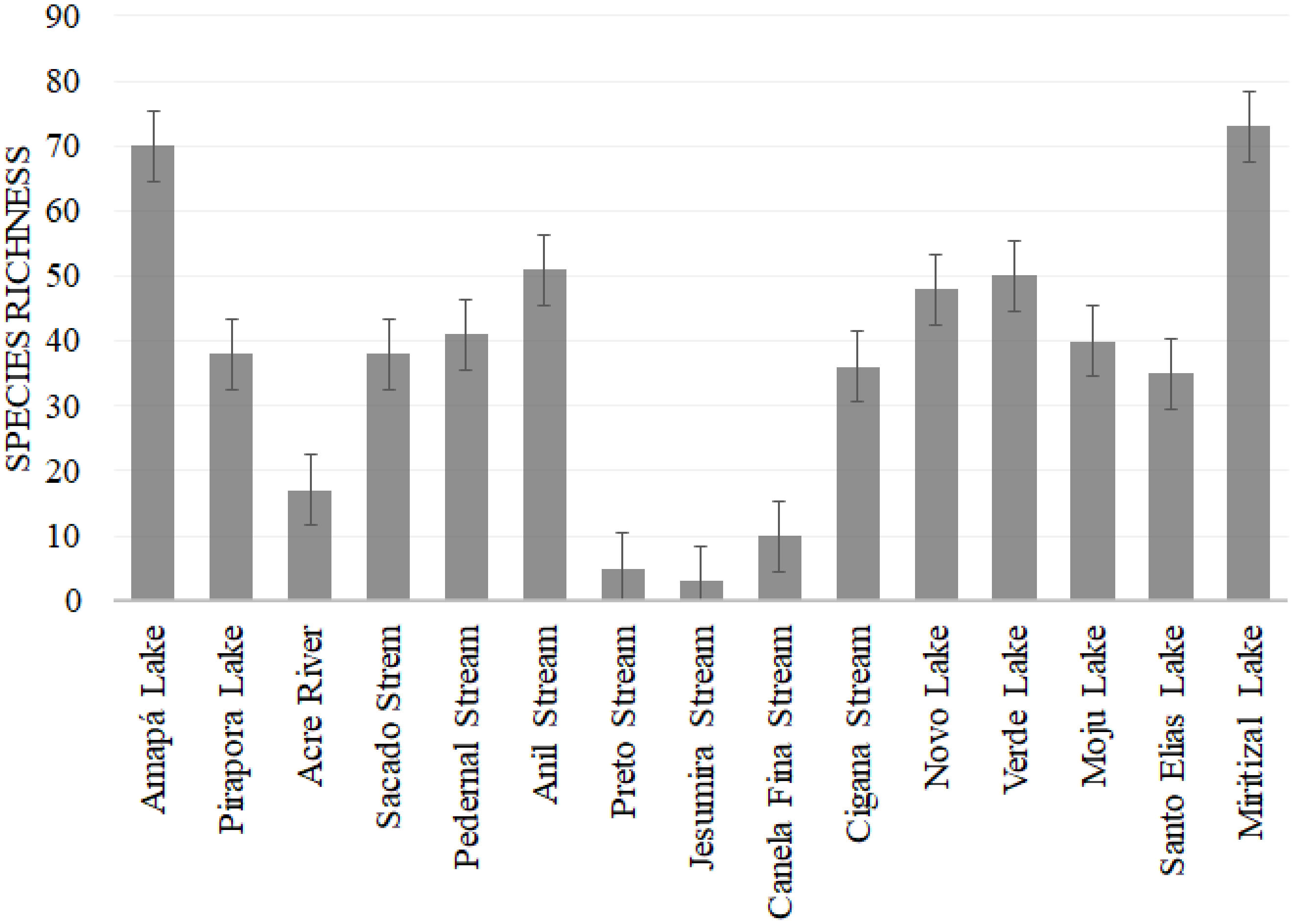

Figure 2. Rotifera species richness in each environment in previously published papers of Acre State. Brazil.

had more species identified in different aquatic environments in this part of the Amazon basin. In the same basin, this group of organisms showed the highest species diversity in the studies by Hardy (1980), Koste & Robertson (1983), Hardy et al. (1984), Robertson & Hardy (1984), Sendacz & Melo-Costa (1991), and Bozelli (1992). Similar findings were reported for other environments (Sharma & Sharma 2001, Lansac-Tôha et al. 2009). Birky and Gilbert (1971) suggested that the reproductive systems of rotifers have several advantages that favor the activity of most of these animals as opportunists, due to their rapid reproduction and adaptation.

In later work, Keppeler & Hardy (2004) undertook a survey of the abundance and composition of rotifers in Amapá Lake during low water and flood periods. The Rotifera composition included 48 species, 20 of which were newly recorded for the Acre River floodplain, while some species were common. The following genera occurred in the dry period: *Asplanchna*, *Brachionus*, *Epiphanes*, *Filinia*, *Keratella*, and *Polyarthra*. During the flood period, the following genera predominated: *Brachionus*, *Filinia*, *Keratella*, *Trichocerca*, and *Polyarthra*.

The rotifers showed greater occurrence during the dry period, especially the following species: *Asplanchna brightwelli* (Gosse, 1850), *Asplanchna sieboldi* (Leydig, 1854), *Brachionus calicyflorus anuraeformis*, *Filinia longiseta* (Ehrenberg, 1834), *Filinia terminalis* (Ehrenberg, 1834), *Filinia opoliensis* (Zacharias, 1898), *Keratella cochlearis* (Plate, 1886), and *Keratella cochlearis hispida* (Lauterborn 1900). The use of Pearson's correlation coefficients showed significant correlations ($p < 0.05$) between environmental variables and the density of rotifers. Specifically, electrical conductivity was negatively correlated with the density of rotifers in the low-water phase ($r = -0.8824$, $p < 0.05$), and was negatively correlated with depth in the high-water phase ($r = -0.7513$, $p < 0.05$). Seasonal changes, caused by water level fluctuations, and low niche diversification influenced the composition and abundance of the studied group (Keppeler & Hardy 2004).

Based on the reviewed studies, the most frequently observed family was Brachionidae, followed by the family Lecanidae, which is predominant in tropical environments. Carvalho (1983) also reported the dominant occurrence of these families, found in abundance in Amazon aquatic environments. The number of species of the zooplankton community in Amapá Lake could be attributed to the variety of niches available during the phases of the hydrological cycle (drought and flood) and to the absence of aquatic plants. The number was low compared with the diversity reported for other basins and várzea lakes.

Oliveira et al. (2010) found a predominant occurrence of rotifers in Preto stream, representing the greatest diversity of organisms, with 18 individuals. Newly recorded species of rotifers in Acre included *Lecane lunaris*, *Lecane quadridentata*, *Lecane kutikova*, *Dissotrocha aculeata*, and the genera *Dissotrocha* and *Macrochaetus*. Aoyagui & Bonecker (2004) reported

Table 3. Cladocera and Copepoda species list and Copepodites and Nauplii in limnic ecosystems of Acre State, indicating species location, considering studies published so far.

	Purus				**Juruá**										
	Amapá Lake	Pirapora Lake	Acre River	Sacado Stream	Pedernal Stream	Anil Stream	Preto Stream	Jesumira Stream	Canela Fina Lake	Cigana Lake	Novo Lake	Verde Lake	Moju Lake	Santo Elias Lake	Miritizal Lake
Cladocera															
Alona cambouei (Guerne & Richard, 1893)						X									
Alona glabra (Brehm, 1909)				X											
Alona sp. (Bourd, 1843)				X											
Alona sp. cf.							X								
Alona sp.															X
Alonella sp.				X											X
Bosmina tubicen (Brehm, 1953)	X						X								
Bosminopsis deitersi (Richard, 1895)	X	X	X								X	X		X	
Ceriodaphnia cornuta (Sars, 1885)	X	X	X												
Chydorus sp. (Stebling, 1902)				X											
Chydorus sp.															X
Daphnia gessneri (Herbst, 1967)	X	X					X								
Daphnia sp.															X
Diaphanosoma brachyurum (Liévin, 1848)	X	X													
Diaphanosoma brevireme(Sars, 1901)			X												X
Diaphanosoma sp.															X
Diaphanosoma sp. (Fischer, 1859)				X			X								
Diaphanosoma spinulosum Herbst, 1975	X	X													
Disparalona dadayi (Birge, 1910)				X											

Disparalona sp.				X											
Ephemeroporus sp.				X											
Eurycercus lamellatus (O. F. Müller, 1776)															X
Ilyocryptus spinifer (Herrick, 1882)															X
Kurzia polyspina (Hudec, 2000)					X										
Latonopsis sp.			X												
Macrothrix sp.					X		X								X
Moina minuta (Hansen, 1899)	X	X	X							X	X	X	X	X	
Moina reticulata (Daday, 1905)	X	X													X
Moina sp.				X			X			X		X	X	X	X
Pleuroxus sp.				X											
Simocephalus latirostris cf. (Stingelin, 1906)							X								
Simocephalus vetulus (O. F. Müller, 1776)															X
Copepoda															
Calanoida															
Calodiaptomus perelegans(Wright S, 1927)	X	X													
Calodiaptomus spp.	X	X													
Notodiaptomus coniferoides(Wright S, 1927)	X	X													
Cyclopoida															
Mesocyclops meridianus(Kiefer, 1926)	X	X													
Mesocyclops sp.	X														
Microcyclops sp.	X	X													
Neutrocyclops brevifurca (Lowndes, 1934)	X	X													
Thermocyclops sp.	X	X	X												
Copepodites	X		X		X		X		X	X	X	X	X	X	
Nauplii	X		X				X		X	X	X	X	X	X	

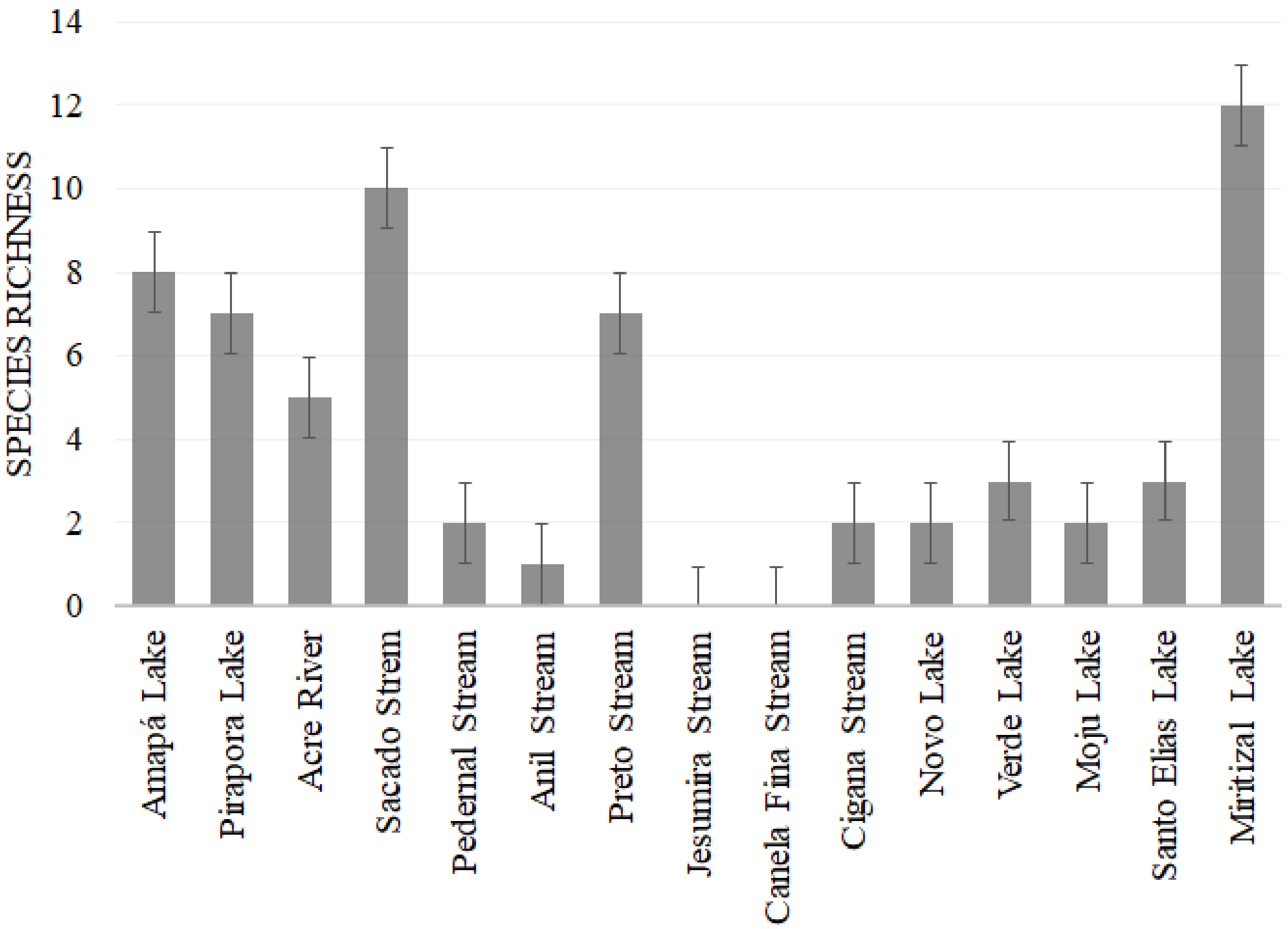

Figure 3. Cladocera and Copepoda species richness in each environment in previously published papers of Acre State. Brazil.

that some families were important contributors to rotifer populations in floodplain environments, including Lecanidae and Brachionidae among them, also observed in previous studies. Only half of the species were common to the surveys undertaken in the Acre River and its tributaries (Sendacz & Melo-Costa 1991), Amapá Lake (known to be a polluted environment), and Pirapora Lake (Keppeler 2003a, b, Keppeler & Hardy 2004a), excluding the species *Colurella* sp., *Lecane lunaris*, *Lecane quadridentata*, *Lecane kutikova*, *Monomotta* sp., *Dissotrocha aculeata*, *Dissotrocha* sp1, *Dissotrocha* sp2, and *Macrochaetus* sp., which were recent occurrences in Acre.

Keppeler et al. (2010) recorded the occurrence of two new species of rotifers in Acre: *Keratella lenzi* and *Lecane monostyla*. The first occurrence of *Keratella lenzi* in Acre was recorded at Jesumira stream, in the Serra do Divisor National Park. The genus *Lecane* of the family Lecanidae (Segers, 2004) is common in shallow and littoral waters, as well as in eutrophic environments such as ports, meandering lakes, and river mouths. In terms of geographic distribution, these organisms are considered hot stenothermal species with pantropical distributions (Ridder 1981).

In studies of zooplankton composition in Alto Juruá lakes, the rotifer class was found predominantly, distributed in 15 families (Santos et al. 2013), this same pattern was observed for Acre and other states in recent studies, by Nascimento and Keppeler (2017) in the Juruá River sub-basin; by Hardy and Keppeler (2004) at Amapá lake and by Santos et al., 2014 at Reserva Cuniã. The most common genera of Rotifera were Lecane and Brachionus, the most frequent families were Lecanidae, Lepadellidae, Keratellidae, Testudinellidae and Trichocercidae (Table 2). Some species had restricted occurrence to only one of the lakes that followed the study, as is the case of *Kerantella lenzi helíaca* (Berzins, 1995), *Lecane acus* (Harring, 1993). The study found five new occurrences for the state of Acre: Asplanchna cf. herricki (Novo Lake), Anuraeopsis coelata (Novo and Verde lakes), Testudinella cf. aspis (Verde Lake), Floscularia sp. (Novo, Verde and Cigana lakes) and Lepadella acrobeles (Moju lakes). The studied lakes, in general, presented different compositions, only Moju and Cigana lakes showing high similarity. Novo lake presented a different pattern in relation to the species found, as there is a division that separates Moju and Cigana lakes from Santo Elias and Verde lakes. This separation was also evident in the analysis of main components considering the first two components, managing to retain 77.5% of the original variation. This difference in lakes is possibly associated with the migration of species from the river to the lakes or vice versa.

The most frequent species in Novo Lake were *Brachionus falcatus*, followed by *Notommata* sp., while the most frequent species in Verde Lake was *Brachionus caudatus*. In Cigana Lake, the most frequent species were *Notommata* sp., *Trichocerca similis*, and *Polyarthra vulgaris*. In Moju Lake, the most frequent species were *Brachionus* *caudatus*, *Brachionus dolabratus*, and *Brachionus falcatus*. In Santo Elias Lake, *Brachionus caudatus*, *Brachionus dolabratus*, *Notommata* sp., *Polyarthra vulgaris*, and *Brachionus. falcatus* were most common. The species with the greatest abundance were the rotifers *Keratella cochlearis*, *Polyarthra vulgaris*, and *Trichocerca similis*, which were also numerous in Pirapora and Amapá lake (Keppeler 2003, Keppeler & Hardy 2004). The species were typically not restricted to any single lake, occurring commonly in meandering lakes, such as Pirapora and Amapá lakes (Keppeler 2003, Keppeler & Hardy 2004). The richness and abundance of the zooplankton species in the lakes studied were similar to observations made in other tropical and subtropical systems, with Rotifera showing a wide geographical distribution.

Silva et al. (2014) studied the zooplanktonic composition in Pedernal and Anil streams. For both water bodies, the most common rotifer families were Lecanidae (18 species) and Lepadellidae (7 species). There were 21 species newly recorded for the state of Acre. The species were generally found at low frequency, with their seasonal distributions not revealing any clear trends for the rainy and dry periods. Even the most frequent species showed similar values for the different seasons, in contrast to the results of other investigations in Lakes Amapá and Pirapora (Keppeler 2003a, b, Keppeler & Hardy 2004a, b). This difference could be explained by the lower frequencies observed for the populations.

Nascimento and Keppeler (2017) studied the zooplankton population with a focus on rotifers in the southeastern basin. The most prevalent family was Lecanidae, characterized as typically being non-planktonic (Joko et al. 2008). Also significant was the family Lepadellidae, which is common in environments where Lecanidae occurs, as reported by Pradham et al. (2011) and Lima et al. (2012). The eight occurrences for the state of Acre were *Pleuroxus* sp. and *Ephemeroporus* sp., at the genus level, while at the species level, the organisms present were *Adineta gracilis*, *Mytilina bisulcata*, *Gastropus stylifer*, *Lecane sola*, *Macrochaetus sericus*, and *Macrochaetus collinsi*.

Richness and diversity of rotifers were observed in the Juruá River floodplain by Santos Nascimento & Keppeler (2017). In total, 85 species were found throughout the study, 73 of Rotifera and 12 of Cladocera. Lecanidae, Brachionidae, and Lepadellidae were the most represented families, with 17, 14, and 10 species, respectively. In this study, eight new species were also reported for the state of Acre. Silva et al. (2014) registered 45 species of Rotifera, 21 being new occurrences for the state. Lecanidae and Lepadellidae are common in the tropics according to Lucinda et al. (2004).

Cladocerans and copepods were reported in the Jurua and Purus basins. The cyclopoid copepods were mainly represented by *Thermocyclops* sp., present in both the river and the lakes, notably in Lakes Lua Nova and Amapá. *Mesocyclops meriadinus brevifurca* was only recorded in the second phase of collection (in February 1988), principally in Lake Amapá. It should be noted that copepodites and nauplii were found in all the water bodies studied.

Keppeler (2003a) observed that at the bottom of lakes, cladocerans and copepods were more frequent than rotifers. The use of Pearson's correlation coefficient revealed that the cladocerans *Moina* spp. (*Moina minuta* and *Moina reticulata*) were correlated with physical and chemical parameters (water temperature [°C], transparency and depth of the water column [m], electrical conductivity [µS cm^{-1}], turbidity [NTU], and dissolved oxygen [mg L^{-1}]), while *Ceriodaphnia cornuta* and *Daphnia gessneri* were correlated with Chaoboridae. The cladoceran populations were larger during the nighttime period, acting as a strategy against predation and explaining the positive correlation between Chaoboridae and *Daphnia gessneri*. Food may be the main factor acting to suppress zooplankton (Arcifa et al. 1992). The flood period results in greater dilution of food, making it less accessible to the zooplankton community. In addition, adequate oxygen concentrations are crucial for aquatic invertebrates (Hardy 1992), as described in several studies of lakes and reservoirs in the Amazon (Junk 1973, Brandorff 1977, Fisher et al. 1983).

Copepods constituted the most representative species in all studies. According to Robertson & Hardy (1984), zooplankton communities in the Amazon are characterized by rotifers presenting the greatest diversity of species, while copepods occurred more, due to the presence of the juvenile stages (nauplii and copepodites). Keppeler (2003b) recorded the cladoceran species *Bosminopsis deitersi*, *Ceriodaphnia cornuta*, *Diaphanosoma spinulosum*, and *Moina minuta*. Keppeler (2003b) saw that Lakes Amapá and Pirapora both showed a high prevalence of *Moina minuta* throughout the study, while there was low representation of microcrustaceans (principally calanoids). Cyclopoida was represented by four genera: *Thermocyclops*, *Mesocyclops*, *Microcyclops*, and *Neutrocyclops*.

In the reservoir of the Canela Fina dam, Oliveira et al. (2010) found 7 species of cladocerans and 1 copepod, with predominance of Cladocera. The genus *Alona* sp. and the species *Simocephalus latirostris* were new discoveries in Acre. The families identified were Chydoridae, Daphnidae, Bosminidae, Macrothricidae, Moinidae, Ilyocryptidae, and Sididae, which are commonly found in most continental water environments (Sendacz 1993), including in the Amazon (Robertson & Hardy 1984, Keppeler & Hardy 2004b) and elsewhere in South America (Korovchinsky 2006). In the case of copepods, both environments showed the frequent presence of nauplii and copepodites. The existence of environmental stress in aquatic systems may hinder the development of these organisms up to the adult stage (Keppeler & Hardy 2004b).

Santos et al. (2013) showed three species of cladocerans and various copepods were found. The cladocerans were represented only by Bosminidae and Moinidae and occurred in all the lakes, with the exceptions of *Moina* spp. in Lake Novo and *B. deitersi* in Lake Cigana and Lake Moju. *Moina* cf. *minuta* was the only species present in all the lakes. In the case of copepods, only the larval forms (nauplii and copepodites) were frequent in the five environments studied.

In the study by Nascimento & Keppeler (2017), the Cladocera genus *Alona* sp. was highlighted, contributing 44.4% and 29% in two different months. Two new genera for Acre were observed: *Pleuroxus* and *Ephemeroporus*, which are both common in Brazil (Elmoor Loureiro 2014). There was a greater presence of cladocerans than observed in other ecosystems of Acre, such as Lake Amapá in Rio Branco (Keppeler & Hardy 2004a), which could have been due to the presence of aquatic macrophytes. The family Chydoridae, also found in this study, was observed elsewhere (in the Alto Paraná), where it was associated with aquatic macrophytes (Serafim-Júnior et al. 2006).

Santos Nascimento & Keppeler (2017) found 12 species of Cladocera, with the species *Alonella brasiliensis* as a new occurrence in the state of Acre. There was a greater species quantity found compared to the previously mentioned studies. The authors reported that their greatest abundance was during the flood. In 2004, Yamamoto found that the abundance of zooplankton increased during the flood because of the availability of more resources.

Despite the large number of variables analyzed among the studies, few have considered the seasonal changes in the zooplankton populations during the entire annual cycle, generally being registered only in some dry (June to September) and rainy (December to March) months. In general, reports in the literature have addressed spatial variability, specific diversity indices, equitability, diurnal variation, the coastal zone, and vertical distribution. Among these aspects, the diurnal variation, the fauna associated with macrophytes, and collections in the coastal and limnetic zones were mentioned in only 15% of the consulted reports. Thus, it is important to emphasize that in addition to the most studied aspects, seasonality is also a relevant factor for examining zooplankton species, as shown by the studies by Araujo et al. (2019) and Cabral et al. (2020), in which the authors took into consideration this aspect and obtained greater species richness in the studied environments for the state of Acre.

It is also important to consider the sampling effort and its effects when analyzing the species richness and composition and this analysis was not possible due to the lack of information on total number of samples or volume filtered in the previous studies. Bottrel et al. (1976) and Ghidini & Santos-Silva (2018) discussed that different sampling gear, effort, and other environmental conditions can affect results regarding species composition, especially when considering rarer species.

Knowledge of zooplankton in the state of Acre is limited by several factors, notably the inherent taxonomic difficulties and problems with accessing the wide range of ecosystems, many of which may be temporary. The scarcity of studies covering entire hydrological (annual) cycles, together with the spatial limitations (both horizontal and vertical) of studies, are major challenges for researchers endeavoring to understand the real biodiversity of this group of organisms in Acre. Therefore, these gaps in knowledge remain to be addressed in future studies.

The present study provides an important synthesis of the current understanding of zooplankton biodiversity in the state of Acre, serving as a basis for future monitoring studies of basins and/or the implementation of programs for the conservation of species biodiversity and ecosystems. It is suggested that intensified research concerning this group of organisms should be carried out for other environments within Acre, including lotic ecosystems. Other issues to be considered in future studies of zooplankton biodiversity in this region are the characterization of limnetic zones of ecosystems and the compilation of inventories for species typical of temporary limnic environments. The results obtained in this bibliographic survey contribute to understanding the biodiversity and species distributions of zooplankton in different biomes in Brazil.

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Author Contrubutions

Larissa Araújo dos Santos: Contribution in the concept of the study; data collection; data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

Andressa Crystine Souza da Silva: Contribution in the concept of the study; data collection; data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

Pedro Paulo Pereira: Contribution in the concept of the study; data collection; data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

Ronnilda Maria Gonçalves Araujo: Contribution in data analysis and interpretation; manuscript preparation; contribution to critical revision, adding intellectual content.

André Ricardo Ghidini: Contribution in the concept of the study; data collection; data analysis and interpretation; 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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